

**SNOWMELT AFFECTS APHID (*APHIS ASCLEPIADIS*) POPULATIONS
THROUGH SOIL MOISTURE AND HOST PLANT PHENOLOGY**

by

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Thesis directed by Assistant Professor Emily H Mooney

ABSTRACT

Plant-insect interactions are ecologically important relationships that are greatly affected by climate change. Previous research concerning an aphid species (*Aphis asclepiadis*) and its host plant (*Ligusticum porteri*) has shown that aphid abundances correlate significantly with snowmelt date in the subalpine zone of the Rocky Mountains. The mechanisms underlying this phenomenon have not been previously identified. Our construction of a structural equational model identified soil moisture and plant phenology as two major pathways by which snowmelt indirectly affects aphids. By comparing aphid and host plant populations at different elevations and experimentally manipulating soil moisture we observed significant responses in aphid abundance, ant abundance, aphid predators, and some measures plant quality. While bottom-up processes may be playing a role in determining aphid abundances, top-down pressures appear to have much more control over this aphid dominated ecosystem.

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CHAPTER I

INTRODUCTION

The impacts of climate change are being observed around the world (Walther *et al.*, 2002). Ecosystems from ocean depths to desert sands are being affected, but few changes are happening as rapidly as those in alpine and subalpine ecosystems. Previous research reveals alpine flora and fauna to be particularly vulnerable to climate change (Guisan & Theurillat, 2000; Gobbi *et al.*, 2006). Some species benefit from these changes (Hodkinson & Bird, 1998) yet others experience negative effects and even population collapses (Johnson *et al.*, 2010). While species in these communities may disperse to higher elevations in order to adjust to climactic challenges, communities are unlikely to remain intact (Theurillat & Guisan, 2001). Biotic and abiotic processes in alpine, subalpine, and arctic ecosystems are particularly driven by snow cover (Chapin *et al.*, 2005; Sturm *et al.*, 2005; Neuner *et al.*, 1999). Accordingly, changes in the timing of snow cover is a particularly significant mechanism by which climate change is altering these ecosystems (Baptist *et al.*, 2010; Wipf *et al.*, 2009).

Early snowmelt dates increase alpine plant vulnerability to frosts (Wheeler *et al.*, 2014), advance phenology (Inouye, 2008), invoke species specific population level responses (Baptist *et al.*, 2010), and alter microbial processes (Edwards *et al.*, 2007). With climate change altering snowmelt dates through modifications to snow-rain ratios and increasing winter temperatures these snow-dominated ecosystems are experiencing marked changes. Often times these changes are not brought about directly by altered snowmelt dates but rather mediated indirectly through mechanisms (Price & Waser, 1998). Soil moisture and phenology are two such mechanisms known to be greatly

affected by snowmelt and in turn affect natural communities (Petraglia *et al.*, 2014; Iler *et al.*, 2013; Cooper *et al.*, 2011; McNamara *et al.*, 2005; Robinson *et al.*, 2017). Soil moisture is intricately linked with plant productivity in alpine environments (Taylor & Seastedt, 1994). Likewise, phenology greatly affects alpine communities through influencing plant fitness and insect herbivore establishment (Collinge & Louda, 1989; Price & Waser, 1998).

These effects of snowmelt date mediated through soil moisture and phenology are not limited to affecting organisms directly. Snowmelt date and its effects also alter the way organisms interact with each other (Gillespie *et al.*, 2016; Gallagher & Campbell, 2017; CaraDonna *et al.*, 2014). In these times of change it is imperative to study and understand not only how organisms themselves may be changing, but also how interactions between organisms are changing. Some of the most diverse and abundant taxa (plants and insects) are already altering the way they interact in response to climate change (De Lucia *et al.*, 2012; Hegland *et al.*, 2009). For example, Huberty and Denno (2004) and Simpson *et al.* (2012) show how soil moisture not only affects the health of plant populations, but the population dynamics of insects through bottom-up processes. Likewise, plant phenology affects insects through altering the timing of when insects can feed on their hosts (Crawley & Akhteruzzaman, 1988; Hegland *et al.*, 2009). Our research concentrates on studying these changing plant-insect interactions due to climate change, and particularly snowmelt date, through the lens of an ecologically important subalpine interaction between aphids (*Aphis asclepiadis*) and their host plant (*Ligusticum porteri*).

Aphids are keystone species responsible for simultaneously exerting herbivorous pressure over host plant populations and being host to a diverse array of predators and mutualists (Snyder *et al.*, 2008; Smith & Chuang, 2014; Diehl *et al.*, 2013). *Aphis asclepiadis* abundances have also been shown to positively correlate with snowmelt date (Robinson *et al.*, 2017) with later spring snowmelts indicating higher aphid abundances the following summer. Accordingly, they provide an exemplary opportunity to study how plant-insect interactions are changing in alpine environments due to altered snowmelt dates. Due to their previously described influences on plant and insect ecosystems through snowmelt, soil moisture and phenology are our major suspected mechanisms underlying this association between snowmelt date and aphid abundance.

Our first objective was to determine the relative roles of soil moisture and host plant phenology in determining how snowmelt timing affects aphid abundance, or if there are other mechanisms at work. We used observations of snowmelt dates, plant and insect phenology, and plant and insect abundances from an elevational gradient to evaluate the mechanisms determining aphid abundance. Elevation gradients provide for natural experiments to approximate future shifts in temperature and other cues subject to climate change effects (Dunne *et al.* 2004). Using these data, we tested hypothesized relationships between variables using a structural equation model. Our second objective was to experimentally test how soil moisture and host plant phenology affect this aphid dominated system. Utilizing a natural elevation gradient and experimentally manipulating soil moisture, we were able to parse the relative effects of soil moisture and host plant phenology on aphid abundance and the multitrophic interactions that govern aphid abundance. We observed changes to aphid population dynamics, interactions

between aphids and ants, the prevalence of aphid predators, and changes in host plant quality to test for the effects of soil moisture and host plant phenology on this system.

CHAPTER II

METHODS AND MATERIALS

Study site and organisms

We conducted our research at the Rocky Mountain Biological Laboratory near Crested Butte, Colorado (RMBL; 39°01'38.77"N, 107°03'10.50"W). The station is located at 2,890 meters of elevation where the dominant vegetation is aspen stands interspersed by subalpine meadows (Langenheim 1962). *Ligusticum porteri*, commonly known as oshá, is a perennial herb of the Rocky Mountains that reaches high abundances in both of these communities (Weber and Witman, 1996). *Aphis asclepiadis* (syn. *Aphis helianthi*), also known as the sunflower aphid, is a common insect herbivore of *L. porteri* among other plants in both agricultural and natural systems (Addicott, 1978; Addicott, 1981). These aphids colonize the flowering stalks of *L. porteri* as early as June or July in this system (Addicott, 1981) and form mutualistic relationships with various ant species including *Formica fusca*, *Formica rufa*, and *Tapinoma sessile* (Addicott, 1979). These relationships have been well documented and are extremely powerful in determining the success or failure of aphid colonization in this system (Mooney *et al.*, 2016). Mirids, specifically lygus bugs (*Lygus hesperus*), also play a significant role in this system. Lygus bugs are intraguild predators of aphids, a role that has shown to exert substantial control over population dynamics (Rosenheim *et al.*, 1993)(Hodge, 1999). Various predators and parasitoids attack *A. asclepiadis* including wasps, beetles, and lacewing flies (Kummel *et al.*, 2013).

Identifying Influential Mechanisms of Snowmelt Date from an Elevation Gradient *SEM Model*

During the 2018 field season we monitored 200 *L. porteri* plants across 20 populations. We selected populations along an elevational gradient ranging from 3120m to 2770m. Weekly surveys at each plant recorded the number of arthropods such as aphids, ants, and mirids located on flowering stalks of *L. porteri*. We also recorded plant information such as number of flowering stalks per plant, umbels per flowering stalk, seeds produced, and phenological stage in addition to soil moisture measurements. We scored flowering phenology using methods as described by Robinson *et al* (2017) for both terminal and primary umbels. We took soil moisture measurements using a FieldScout TDR Soil Moisture Meter (Specmeters, Aurora, IL).

We estimated the snowmelt date of each population by placing data loggers (HOBO, Onset Technology, Bourne, MA, USA) at each site at ground level (covered in reflective tape) the previous fall and recorded temperatures throughout the winter. To estimate snow melt timing at each site for 2018, we determined the day of year when the loggers began to show diurnal fluctuations in temperature (Lundquist & Lott, 2008). Two temperature loggers malfunctioned, and one was likely uncovered by human disturbance. To interpolate values for those sites, we used a model-selection approach to choose the site variables or combinations of site variables most predictive of snow melt date. We compared regression models using QAICc scores computed using the ‘aictab’ function in the *AICcmodavg* package (Mazerolle 2016). Models with AICc of 2 or less were considered to be similarly supported by the data. Based on this approach, we used an additive model of site elevation and night-time temperature to interpolate snowmelt date for the three sites with missing values.

Using these data, we utilized a structural equation model (SEM) approach to identify how snowmelt was affecting each variable throughout the system. This approach followed methods described by Mortensen *et al.* (2016) and allowed us to evaluate hypotheses about the direct and indirect effects of snowmelt timing on insect abundance and phenology. As a result, we could then study significant mechanisms in further detail. The original hypothesis model represented a multitrophic system with the timing of snow melt affecting both soil moisture and phenology, and the effects of snow melt timing on phenology determining abundance of both host plants and insects (Figure 1). We hypothesized that timing of snow melt will have direct effects on the phenology of host plants and mirids, but indirect effects on the phenology of ants and aphids.

We assessed host plant phenology using the day of year when umbels emerged from enclosing bracts. Long-term monitoring at RMBL shows that flowering phenology of *L. porteri*, *A. asclepiadis*' host plant, has advanced in response to earlier snow melt dates (Iler et al., 2013). Mirids overwinter as either adults or eggs in fallen leaves or stems and emerge following snowmelt (Khattat & Stewart, 1980; Ralphs & Jones, 2000). Mirids then multiply through successive generations on host plants. We used the day of year that mirids were first observed on the host plant flowering stalk as our indicator of mirid phenology. Mirid bugs are attracted to floral resources (Pan et al., 2013), thus we also modeled host plant flowering phenology determining mirid abundance. In the model, both host plant and mirid phenology are influenced by snowmelt timing.

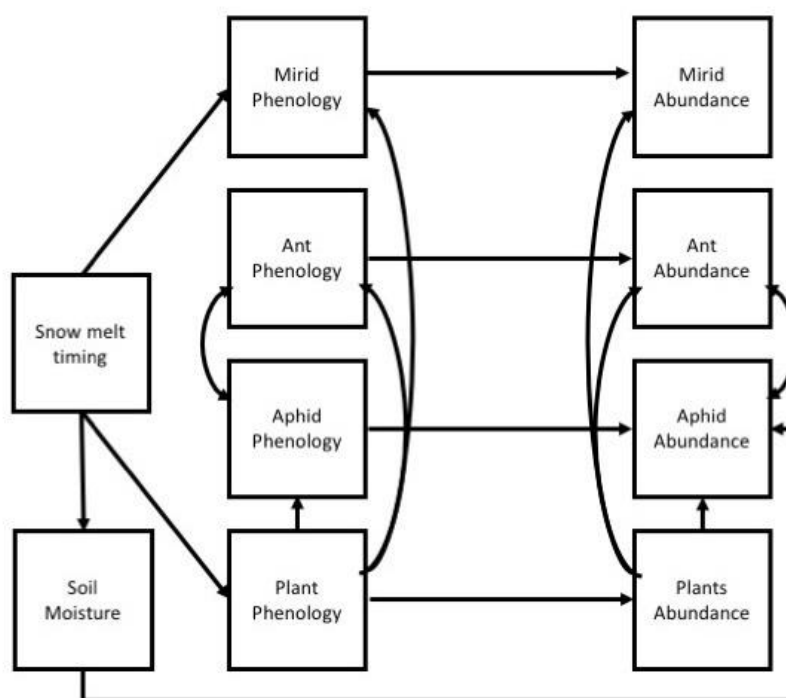


Figure 1: Hypothesized structural equation model highlighting our expected connections within and between phenology and abundances.

Because their seasonal population dynamics, we used day of year at maximum abundance to assess ant and aphid phenology. *Aphis asclepiadis* arrive on host plants in June through early July, and successful colonies achieve a single peak in population size before declining to extinction (Addicott, 1978). Ant phenology on host plant flowering stalks follow a similar pattern. We hypothesized that snowmelt timing indirectly affects ant and aphid phenology through its influence on host plant flowering phenology. For ants, their phenology would be driven by host plant flowering phenology because they are attracted to floral nectaries on umbels. For aphids, flowering stage likely determines phloem sap sugars and amino acids (Weibull, 1987), key determinants of host plant quality (Karley *et al.*, 2002). Because of the ant-aphid protection mutualism, we included

a correlation between ant and aphid phenology. All hypotheses regarding ants are referring to their abundance and phenology on flowering stalks, opposed to ambient levels surrounding *L. porteri* host plants.

We hypothesized that abundance across trophic levels would be driven by the effects of snowmelt timing on phenology. We included a direct effect of host plant phenology on host plant abundance: early snow melt can expose fragile flower buds to frost damage (Inouye, 2008). We likewise included direct effects of insect phenology on insect abundance. For mirids, population sizes can rapidly increase when earlier onset of activity allows for multiple generations (Steinbauer *et al.*, 2004). For ants and aphids, we used their peak abundances on host plants to indicate their abundance. These insect abundances are notably determined by trophic interactions, e.g. top-down and bottom-up control (Hairston *et al.*, 1960; Vidal & Murphy, 2018). We represented bottom-up effects in the model with plant abundance influencing the abundance of all insects. Because they are omnivores, we modeled mirid abundance as being dependent on both plant and aphid abundance. Ants engage in a protection mutualism with aphids; thus, we modeled that their abundances would be correlated with aphid abundance. Finally, we also predicted an effect of snow melt timing on aphid abundance arising through soil moisture. Drought stress generally—but not always—reduces host plant quality for aphids (Huberty & Denno, 2004). Although other many other factors have been demonstrated to impact aphid abundance (Snyder & Ives, 2003; Sarwar *et al.*, 2011) we did not include these in the model for simplicity.

Structural equation model evaluation

We specified the model, estimated parameters and tested model fit using the package *lavaan* in R (Rosseel, 2012). Prior to model estimation, we transformed all variables to make scales of measurement similar among all variables. We square root transformed all phenology measurements and abundance were transformed through z-score scaling to have a mean of zero and standard deviation of one. We used a model evaluation approach to refine the model. We determined if any important links were missing using modification indices found using the ‘modindices()’ function in *lavaan*; any links with a modification index greater than three were sequentially added to the model. We then reevaluated the model fit and removed the added links if model fit was not significantly (P-values > 0.05) improved. We then examined the model summary and sequentially removed the weakest links (P-values > 0.05) to create simpler but sufficient models. This process resulted in four alternative models, and we used the ‘aictab.lavaan()’ function to compare the fit among these alternative models. We considered models to be equally supported by the data if QAICc values were less than two.

Structural equation model analysis:

The resultant model consists of our original tri-tiered model consisting of abiotic variables, measures of plant and arthropod phenology, and abundances of plants and arthropods (Figure 2). Snowmelt date and volumetric soil moisture are abiotic factors in our model that drive subsequent biotic relationships with phenology and abundances. These relationships are solidly supported by previous studies (Wells & Nugent, 1980; Cooper *et al.*, 2011; Wang & Camp, 2000). However, as our model suggests snowmelt timing is the ultimate driver in this system particularly through soil moisture and plant

phenology. Neither soil moisture or plant phenology held significant relationships with any other factors. A weak link was maintained between plant phenology and mirid phenology and plant abundance. Links were also maintained between soil moisture and ant phenology, yet they remained insignificant. Arthropod phenological factors are measured as the ordinal day of year that populations were observed to reach maximum abundances. Ant phenology had a significant interaction with aphid phenology and the connection between ant phenology and abundance was also significant. We preserved links between all phenological measures and their corresponding abundance due to the logical necessity of the connections between these factors, however none of these links were significant. Our model did not provide any significant relationships affecting plant abundance (measured as umbel count) aside from our preserved link from plant phenology. Yet plant abundance significantly affected all arthropod abundances. Mirid abundance insignificantly affected aphid abundance. This is likely due to their role as intraguild predators of aphids (Rosenheim *et al.*, 1993). Snowmelt maintained insignificant links to both mirid and aphid abundances.

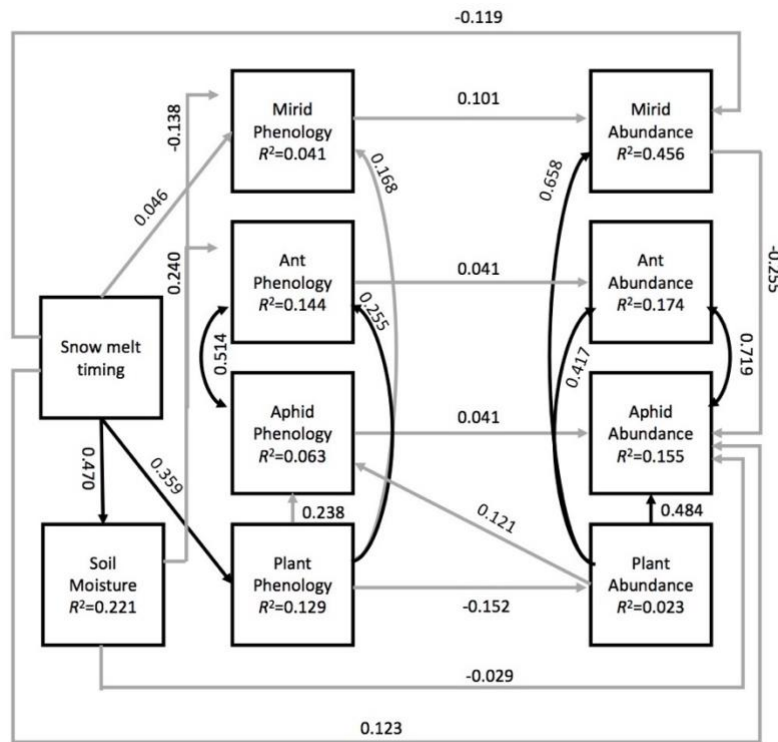


Figure 2: Structural equation model of the study system in subalpine meadows of Colorado. Significant interactions ($p > 0.05$) are indicated by solid black arrows in the direction of effect. Relationships that were not significant are indicated by gray arrows. Each arrow is paired with the standardized parameter estimates. The hypothesized model predicts snowmelt date affects aphid abundance through many mediating factors.

Responses to experimental soil moisture manipulations and host plant phenology manipulations

Experimental design

We combined two approaches to understand how both soil moisture and host plant phenology affects herbivore abundance in this system: (1) monitoring plants at differing elevations provides a natural experiment to test the effects of host plant phenology and (2) monitoring plants where we manipulated soil moisture at low elevation allowed us to parse the effects of soil moisture (Figure 3). The comparisons this

design allows highlight how these two mechanisms separately affect aphids and plants within this system.

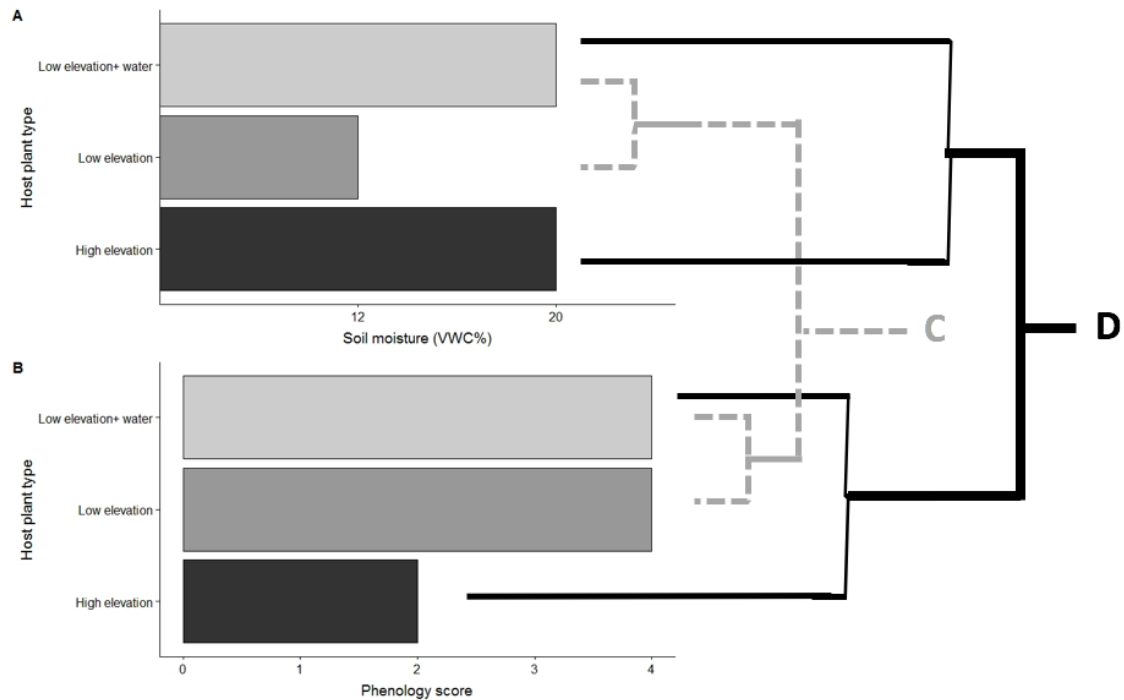


Figure 3: Experimental schematic showing expected differences in soil moisture (A) and host plant phenology (B) among three host plant types created by combining an elevation gradient with experimental water addition. Contrasts between low elevation watered and unwatered plants (C) compare insect performance and host plant physiology between host plants differing in soil moisture status. Contrasts between low elevation watered plants and high elevation plants (D) compare insect performance and host plant physiology between host plant differing in phenological stage.

We expected high elevation sites (3000m) to have increased soil moisture compared with low elevation (2700m) sites due to later snowmelt dates. Effects of snowmelt date on soil moisture can last throughout a season (McNamara et al, 2005). As a result, we controlled for differences in soil moisture between elevations by watering the soil surrounding select low elevation plants. This allows comparisons between high elevation plants and low elevation watered plants to concentrate on differences other than soil moisture; particularly flowering phenology, temperature, and community

composition (Dunne *et al.*, 2003). Because host plants at low elevation sites would have similar phenology (Liu *et al.*, 2014), comparisons between low elevation watered and unwatered plants parse differences in host plant quality, aphid abundance and multitrophic interactions driven by soil moisture. Soil moisture does play a role in phenology in many ecosystems (Jolly & Running, 2004). In order to account for this, we will not manipulate soil moisture until flowering stalks have already emerged from the ground and started their phenological trajectory for the growing season. Phenological alterations caused by differences in soil moisture during the growing season are an unavoidable confounding variable and we will monitor for this by tracking phenological stages throughout our observational period.

We randomly selected host plants in replicate *L. porteri* populations in open meadows with little to no canopy cover. By using a compass and random number generator (<https://www.random.org/>) we established two 30m transect tapes at three low elevation sites and one 30m tape at three high elevation sites with random orientation. We selected plants by using random numbers along these transects and placing a pin flag next to the closest plant. For each low elevation site, one transect was used to select plants to receive additional soil moisture while the other did not. We assigned these plants unique identification and monitored these plants throughout the growing season starting on 6-5-18.

In order to determine the amount of water to add to the soil at low elevation plants we experimented with adding various amounts of water to 3 separate populations of 8 *L. porteri* plants near the low elevation sites where watered and unwatered plants would be located. This test started on 6-8-18 and completed on 6-11-18. We tested the effects of

adding 1.5 liters of water, 2.25 liters of water, and 3 liters of water on soil volumetric water content surrounding *L. porteri* plants using a probe (Field Scout, TDR 150 Soil Moisture Meter, Spectrum Technology, Aurora, IL US). Each volume of water was added to a 36cm radius from the center of the plant. There were 2 plants of each treatment plus 2 control plants at each of the 3 populations. We measured the soil VWC before adding any water, 15 minutes after watering, 1 hour after watering, 3 hours after watering, 24 hours after watering, 48 hours after watering, and 72 hours after watering. All measurements were taken approximately 20 cm below the soil surface. Watering treatments were not significantly different from each other but all significantly higher than unwatered plants (Table 1). Accordingly, for the watered plants in our low elevation treatment we added 1.5L of water over a 36cm radius circle twice weekly until monsoon rains arrived (7-9-18).

Table 1: Summary statistics of the soil VWC at the experimental low elevation sites. These values were used to justify 1.5L being sufficient to raise soil moisture levels to that of the high elevation sites.

1.5L	2.25L	3L	Control
Min. :-6.400	Min. :-3.60	Min. :-4.800	Min. :-2.4000
1st Qu.: 1.400	1st Qu.: 2.70	1st Qu.: 1.950	1st Qu.: -0.9750
Median : 2.750	Median : 6.15	Median : 5.500	Median : -0.5000
Mean : 3.612	Mean : 5.90	Mean : 4.667	Mean : -0.5292
3rd Qu.: 5.825	3rd Qu.: 8.25	3rd Qu.: 6.950	3rd Qu.: 0.1000
Max. :10.300	Max. :16.40	Max. :13.400	Max. : 2.9000

Once we found the amount of water needed to add to low elevation soils we began watering the selected transects at low elevation (6-11-18). We watered each plant twice a week in the same manner as our original test (emptying water on soil within a 36cm radius of the center of the plant). A circle of black tubing was used as a guide to ensure accurate measurement. We monitored soil moisture twice a week at the low elevation sites and once a week at the high elevation sites. We analyzed this data using a repeated measures approach implement in the *lme4* package in R (Bates *et al.*, 2015). We used R version 3.4.3 for all statistical analysis performed (R Development Core Team, 2018). We used population site and individual plant identification as random effects with day of year as a fixed effect along with treatment. We then used the ‘lmer()’ function to specify the model and the ‘Anova()’ function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values. We used a normal distribution for this data. We performed *post-hoc* contrasts using the *emmeans* package (Lenth, 2018); all post-hoc contrasts throughout this study were between high elevation plants vs low elevation unwatered plants, high elevation plants vs low elevation watered plants, and low elevation unwatered plants vs low elevation watered plants.

Aphid population dynamics

Each week from 6-5-18 until 7-23-18 we conducted surveys for all observable insects. This included a count of aphids and alates on each plant’s flowering stalks. We varied the order of the survey in order to account for time of day (8am-6pm). Starting 7-10-19, half of the plants used for surveys were converted into plants used to assess the growth rates of aphid populations, indirectly measuring plant quality for aphids but halting our observational period for those plants.

To test for differences in aphid abundances over the monitoring period we used a repeated measures approach implemented in the package *lme4* (Bates *et al.*, 2015) in R. This package allows us to use both linear and generalized linear mixed effect models. By using these models, we can account for differences between our populations and repeated measurements of the same plant throughout the season (Zuur *et al.*, 2009). Accordingly, we treated both population site and individual plants as random effects. Treatment was a fixed effect. Day of year was also included as a fixed effect due to our multiple measurements throughout the growing season. We used a Poisson distribution to account for our count data and flowering stalks as a covariate. We used the ‘*glmer()*’ function to specify the model and the ‘*Anova()*’ function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values. We then performed *post-hoc* contrasts using the *emmeans* package (Lenth, 2018). This allowed us to determine how the treatments varied according to the day of the year.

We collected aphids from our treatment plants at the end of the growing season (8-1-18) by using small paintbrushes on colonies protected from predation. These colonies were enclosed with a mesh bag and twist tie. Predators were also prevented from crawling into the bag using masking tape and insect barrier (Tree Tanglefoot, Contech Enterprises, Marysville, OH) on the flowering stalk below the bag. Predators were excluded for thirteen days; this approach allows us to test for differences in plant quality among host plants (i.e. bottom-up effects) without the influence of site-to-site variation in predation (Mooney *et al.* 2016). We froze these aphids until analysis. To quantify an aphid colony’s population structure, we organized these aphid collections from each plant independently and separated them into instars 1-4, mature aphids, and

alates. We took pictures of the organized aphids with a ruler and measured them using ImageJ software version 1.8.0 (National Institutes of Health, Bethesda, MD) to confirm size classes. Matures were measured as greater than 1.49mm, 4th instars between 1.09mm and 1.49mm, 3rd between 0.89mm and 1.09mm, 2nd between 0.69mm and 0.89mm, and 1st smaller than 0.69mm. Alates were separated by the presence of wings.

To get a direct measure of aphid fecundity, mature aphids and alates moved onto dissections where we counted all the embryos yet to be born following the methods of Kaiser and Heimpel (2016). We used a stereo dissection microscope for organizing aphids by size, taking pictures, and dissecting mature aphids and alates (EZ4 HD with LED, Leica Microsystems).

To analyze how our treatments may have resulted in our observed differences in population structure and fecundity, we used a mixed effect analysis of variance implemented in the *lmer* package (Bates *et al.*, 2015) in R. We treated individual plants as a random effect and treatment as a fixed effect. We used ‘*lmer()*’ to specify the model and the ‘*Anova()*’ function from the *car* package to find test statistics and P-values. We used a Poisson distribution for the analysis of our fecundity traits as we used count data of embryos and the distribution of our resultant residuals was non-normal (Cameron & Trivedi, 2013). For all count data, we checked the distribution of the residuals for normality by using the ‘*ggdensity*’ and ‘*ggqqplot*’ functions from the *ggpubr* package (Kassambara, 2017) and the ‘*shapiro.test*’ function from the *stats* package (R Development Core Team, 2018). We performed *post-hoc* contrasts using the *emmeans* package (Lenth, 2018).

Effects on ant behavior

Ants and aphids have a strong mutualistic relationship in this ecosystem (Robinson *et al.* 2017). Ants are attracted to honeydew as a food source while aphids receive protection from predators by their ant mutualists (Völkl *et al.*, 1999). To understand changes to aphid tending behavior we took weekly counts of ants on the flowering stalks of our study plants. We used ant abundance on these flowering stalks to represent attraction to aphids as most ants observed on these flowering stalks were interacting with aphids in some capacity. To test for differences in ant abundances over the monitoring period we used a repeated measures approach implemented in the package *lme4* (Bates *et al.*, 2015). We treated both population site and individual plants as random effects. Treatment was a fixed effect. Day of year was also included as a fixed effect due to our multiple measurements throughout the growing season. We used a Poisson distribution to account for our count data and flowering stalks as a covariate. We again used the ‘*glmer()*’ function to specify the model and the ‘*Anova()*’ function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values. Subsequently, we performed *post-hoc* contrasts using the *emmeans* package (Lenth, 2018).

To test if aphid colonies were similarly attractive to ants as colony size grew among our different treatments, we looked at the proportion of ants to aphids at colonies among our different treatments. We used data collected each week to see how many ants were active on the flowering stalks of *L. porteri* that had an aphid colony, and related that to how large the aphid colony was. We separated these observations into our different treatments and created a model to compare the rate at which aphid colonies attracted ants as they got larger. The model we used was a generalized linear mixed model using the *lme4* package (Bates *et al.*, 2015) in R. We identified individual plants as random effects

while aphid abundance was a fixed effect along with treatment. Our data fit a Poisson distribution. We used the 'glmer()' function to specify the model and the 'Anova()' function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values. We then performed *post-hoc* contrasts using the *emmeans* package (Lenth, 2018).

To understand the impacts of host-plant phenology and altered soil moistures on interactions with ants via honeydew we placed squares of aluminum foil under the terminal umbels of plants with aphid colonies to collect honeydew dropped by aphids for 5 hours. We excluded ants from feeding on this honeydew using an insect barrier created from masking tape and insect barrier (Tree Tanglefoot, Contech Enterprises, Marysville, OH) surrounding the flowering stalk below the colony and collecting foil. We froze honeydew foils until analysis.

Using the same ImageJ software and microscope as our population structure analysis we analyzed drop count and percent coverage of the honeydew collected on our foils. We removed the honeydews from foils by immersing them in ultrapure water (Resistivity >18.18 M Ω ·cm) at 80°C in a 50mL centrifuge tube. We then put the tubes in a sonicator for 15 minutes at 60°C in degas mode. We transferred 1 mL of each sample in an autosampler vial for direct analysis with high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). By using hydrophilic interaction chromatography (HILIC) for LC-MS/MS we could measure the amounts of various sugars in search of differences in honeydew composition between treatments. We analyzed the samples for six different sugars: fructose, glucose, sucrose, trehalose, melezitose and raffinose. These sugars are commonly found in honeydew (Fischer *et al.*, 2002; Byrne *et al.*, 2003; Pringle *et al.*, 2014). We used sugars standards (SigmaAldrich,

Merck KGaA, Darmstadt, Germany) to make eight calibration curve standards varying from 0.05 ppm to 10 ppm. For samples with a concentration of sugars out of calibration curve, we also did 1:20 and 1:400 dilution of honeydew samples in a mixture of methanol and water (10/90 in v/v). We used multiple analysis of variance (MANOVA) using the ‘manova()’ function in R to simultaneously analyze variation in each of these sugars between temperature treatments. We used counts of honeydew droplets as a covariate in these analyses to account for variation in the amount of honeydew in each extract. We performed univariate tests for individual sugar responses using the ‘summary.aov()’ function.

Prevalence of aphid predators

During our weekly surveys of our study plants we also recorded the presence of aphid predators including lygus bugs (Hemiptera: Miridae), syrphid flies (Diptera: Syrphidae), parasitoid wasps (Hymenoptera: Aphidiinae), ladybugs (Coleoptera: Coccinellidae), ant mimicking mirids (Hemiptera: *Miridae*), and red spider mites (Sarcoptiformes: Acaridae). The most common of these are lygus bugs, which feed on both plant tissue (including *L. porteri*) and aphids (Backus *et al.*, 2007; Agusti & Cohen, 2000). We also found ladybugs and ant mimicking mirids in sufficient quantities for analysis. The presence of other predators on host plants was rare (32 observed during 630 observations); thus, we chose to only analyze how the abundances of lygus bugs, ladybugs, and ant mimicking mirids were affected by our experimental manipulations. During our previously described dissection of aphids we also counted endoparasites inside our collected aphids as a measure of parasitoid attack rates in each of our treatments. We only found one parasite in all 117 mature aphids and alates collected,

insufficient for statistical analysis. We analyzed lygus nymphs, lygus adults, ladybugs, and ant mimicking mirid abundance separately using the ‘glmer()’ function to specify the model and the ‘Anova()’ function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values. We then performed *post-hoc* contrasts using the *emmeans* package (Lenth, 2018). We utilized a Poisson distribution for our models.

Effects of plant quality and abiotic variables

Each week we observed flowering stalk count, umbel count, and phenological stage of flowering stalks at each study plant. We assessed flowering phenology of both terminal and primary umbels by scoring phenological stage on a scale between 0 and 7 following the methods of Robinson *et al* (2017).

In an effort to quantify the influence of abiotic factors we placed temperature loggers in the center of each site (HOBO, Onset Technology, Bourne, MA). We also measured light as photosynthetically active radiation using a light meter and line quantum sensor (LI-250A and LI-191R, LiCor Corporation, Lincoln, NE, USA) above each plant in the morning (10:00-12:00AM) and afternoon (12:00-2:00) as this system’s insect abundance has been shown to be greatly affected by light (Mooney *et al.*, 2016). We measured plant quality using six different techniques; Leaf temperature taken by an infrared sensor (IR400 Extech Instruments, Nashua, NH), chlorophyll content using a chlorophyll meter (SPAD 502, Spectrum Technologies, Aurora, IL), stomatal conductance using a steady-state leaf porometer (SC-1, Meter Group, Pullman, WA), leaf water content using a dehydrator and scale, and photosystem efficiency measurements using a chlorophyll fluorometer (miniPPM, EARS, Kanaalweg,

Netherlands). The sixth technique involved obtaining an estimate for plant quality from an aphid's perspective using aphid colony growth rate.

In order to assess these growth rates, we cleared plants of all insects by shaking the flowering stalk and using paintbrushes, introduced 5 mature and 5 immature aphids to the terminal umbel, enclosed the umbel with a mesh bag and twist tie, and excluded insects from crawling into the bag using masking tape and insect barrier (Tree Tanglefoot, Contech Enterprises, Marysville, OH). We surveyed aphids and alates within these enclosures every 3-4 days. We calculated aphid colony relative growth rate (RGR) as $\ln(n_t) - \ln(n_0)/t$. I refer to this design as an insectometer.

To analyze the resulting RGR values we utilized a repeated measures approach implemented in the package *lme4* (Bates *et al.*, 2015). We treated both population site and individual plants as random effects. This allowed us to include our multiple measurements collected throughout the observational period even when they were from the same plant. It also corrected for differences between our sites even if they were in the same treatment group. Treatment was treated as a fixed effect. To analyze our umbel counts, we used counts per flowering stalk. For both umbel counts and flowering stalk counts we utilized Poisson distributions due to their non-normal fits. To analyze this count data as well as our measurements of plant quality we used the 'lmer()' function to specify the model using the *lme4* package (Bates *et al.*, 2015) and the 'Anova()' function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values. Again, the models created had population site and individual plants as random effects and treatment as a fixed effect. If there were multiple measurements throughout the growing season we added day of year as a fixed effect. We then performed *post-hoc* contrasts using the

emmeans package (Lenth, 2018). Analysis of stomatal conductance data was the exception to this method. We collected conductance data from our porometer along with the time of observation. To account for differences in conductance due to time of day, we calculated a linear model of our conductance measures and time of day using the ‘lm()’ function. Then, using the ‘residuals()’, ‘write.table()’, and ‘lmer’ functions, we analyzed our time corrected data with treatment as a fixed effect and both population and plant id were random effects. We again used the ‘Anova()’ function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values.

Responses to Plant Trait Measures

To find if plant quality had a significant effect on aphid populations at all, we analyzed our collected data using plant traits as predictor variables, and aphid colonies on those plants as response variables. We analyzed flowering stalk abundance, photosystem II efficiency, leaf water content, leaf temperature, stomatal conductance, and umbel abundance per flowering stalk for correlations with aphid colony sizes (i.e. aphid counts per flowering stalk). Data among all of our treatments was pooled in order to increase statistical power. For our initial analysis, we used the ‘glmer()’ function to specify a model using a plant trait and the ‘Anova()’ function from the *car* package (Fox *et al.*, 2012) to find test statistics and P-values to find that trait’s relationship with aphid counts. In these models, we used population and plant id as random effects. In the instance of stomatal conductance, we again analyzed that data according to the previously described methods. We also utilized Poisson distributions in order to correct for our count data.

Following this, we took all significant relationships and investigated the slopes of the resultant regression lines, as these indicate the strength of association between that

plant trait and aphid colony size. In other words, the degree to which our plant trait predict aphid colony size is conveyed by the parameter estimates or slope terms. To get slopes, we first standardized the data by subtracting the mean value of the variable and dividing by the standard deviation. We then used the “lm” function from the *stats* package (R Development Core Team, 2018) and the “summary()” function from the *base* package (R Development Core Team, 2018).

CHAPTER III

RESULTS

Identifying Influential Mechanisms of Snowmelt Date

SEM model

The initially hypothesized SEM contained 2 latent factors, 9 regressions, and 1 covariance. Through stepwise pruning of the model we developed a more efficient model (Figure 1). The resultant model fit our observed data well ($\chi^2 = 31.64$, $P = 0.34$) and signaled a dominance of snowmelt affecting aphid populations through both soil moisture and flowering phenology. Host plant phenology ($z = 3.98$, $P < 0.01$) and soil moisture ($z = 4.64$, $P < 0.01$) were the only significant regressions with snowmelt date in the model. We preserved regressions between snowmelt date and both mirid abundance and aphid abundance despite their insignificant relationships (P -values > 0.05). Previous research by Robinson *et al.* (2017), along with our methods for simplifying our model, argue for their logical inclusion in our model. Despite significant relationships between snowmelt and both soil moisture and host plant phenology, no further significant links between these mechanisms and other components of our model were identified.

Responses to experimental soil moisture manipulations and host plant phenology manipulations

Aphid population dynamics

Aphid abundance was significantly higher at high elevation plants compared to watered or unwatered plants at low elevation ($\chi^2 = 11.69$, $P < 0.01$; Figure 4). High

elevation plants had on average 1972% more aphids per flowering stalk than the combined average of low elevation plants (N=630). Low elevation watered plants had on average 216% more aphids than low elevation unwatered plants (N=421).

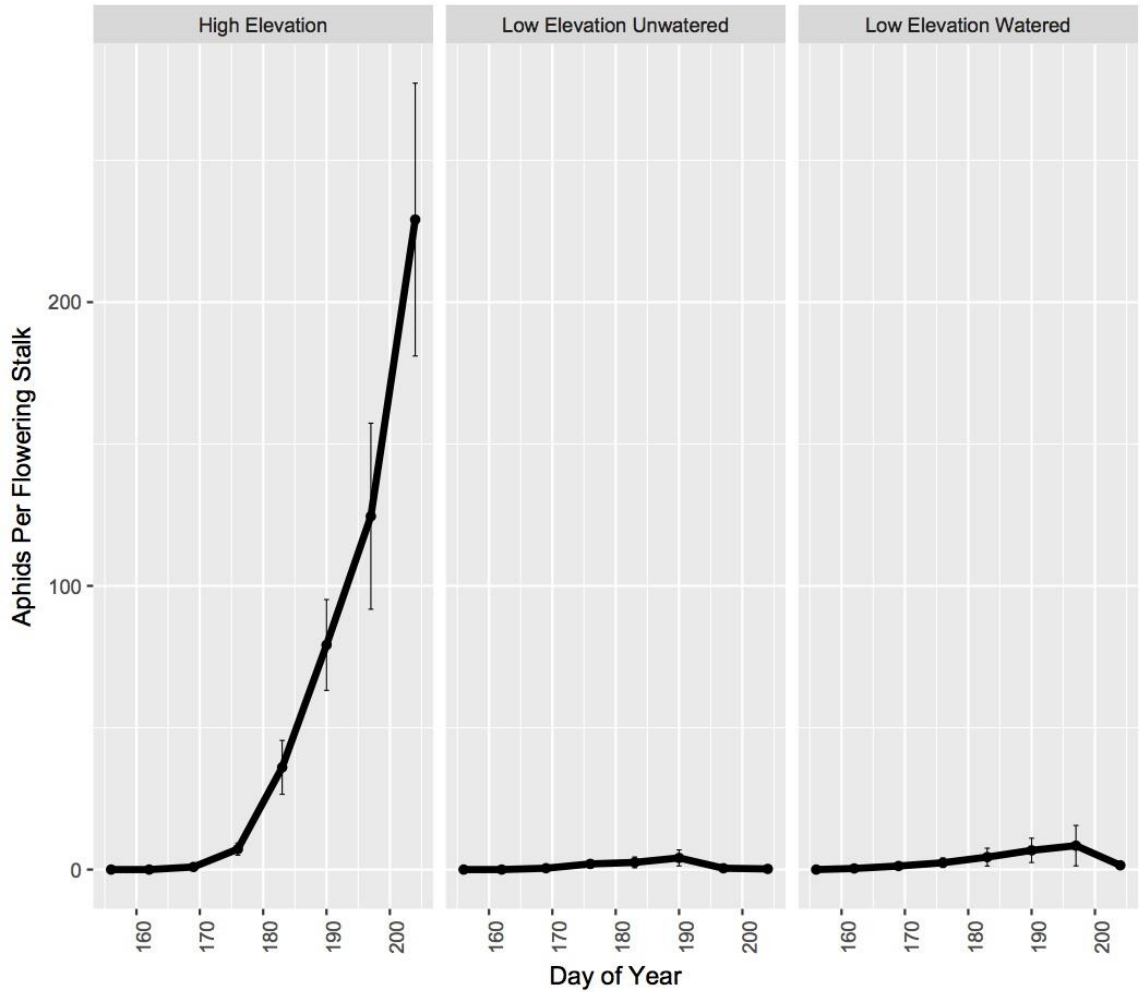


Figure 4: Differential response of average aphid abundance per flowering stalk throughout the growing season among our different treatments.

We found a trend for aphid population structure to differ among host plant treatments ($\chi^2 = 5.80$, $P = 0.05$; Figure 5). However, *post-hoc* analysis revealed no statistically significant or trending differences between the comparisons of our study groups. Our dissections of aphids revealed a significant difference in aphid fecundity

among our treatments ($\chi^2 = 13.83$, $P < 0.01$). Comparisons showed us aphids from high elevation sites were less fecund than aphids from either low elevation watered ($z = -3.69$, $P < 0.01$) or unwatered sites ($z = -2.35$, $P = 0.04$). Aphids from low elevation watered and unwatered sites were not significantly different ($z = -1.09$, $P = 0.52$).

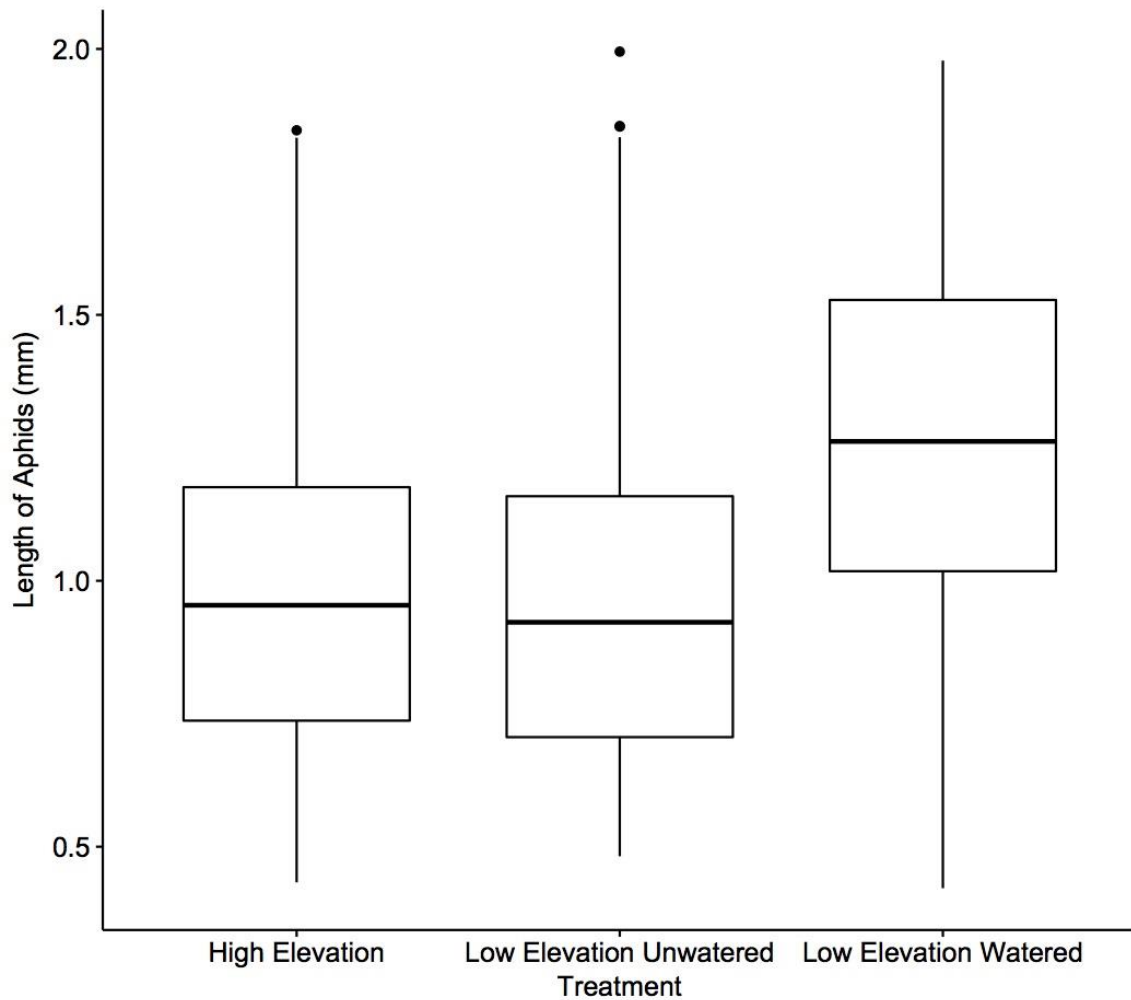


Figure 5: Length of aphids collected from plants among different treatments.

Effects on ant behavior

Ant species found tending aphids also differed between elevations. We found *Formica fusca* at significantly higher abundances at high elevation sites compared to both low elevation sites ($\chi^2 = 13.48$, $P < 0.01$; Figure 6A). Flowering stalks on plants at low

elevations had only 1% of the *F. fusca* found on higher elevation flowering stalks (N=630). We did not find a significant difference of *Tapinoma sessile* abundances between our sites ($\chi^2 = 0.35$, $P = 0.84$; Figure 6B), however, we did notice some obvious trends. For every thousand *T. sessile* ants at low elevations we found six *T. sessile* individuals on high elevation flowering stalks (N=630). Overall ant abundance, disregarding ant species, was also significantly different between our treatments ($\chi^2 = 65.52$, $P < 0.01$). Low elevation plants had 19% of the ant abundance levels per flowering stalk than high elevation plants (N=630).

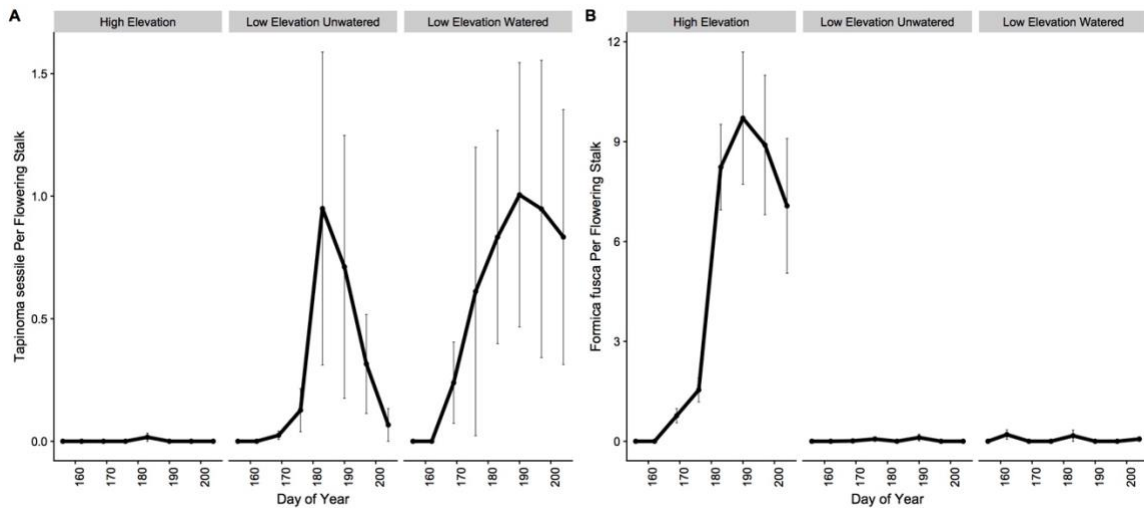


Figure 6: (A) Differential response of average *Formica fusca* found per flowering stalk throughout the growing season among our different treatments. (B) Differential response of average *Tapinoma sessile* found per flowering stalk throughout the growing season among our different treatments.

Not only did we find ant species to be different among host plant treatments, we also found their relationship with the aphids they associated with did not follow the same patterns between treatments. Ants at high elevation sites were less responsive to changes in aphid population size than plants at low elevations ($\chi^2 < 27.32$, $P < .01$; Figure 7). As aphid populations increased on plants, ant numbers increased as well. However, the rate

at which aphids attracted ants was reduced at high elevation sites compared to low elevation sites as aphid colonies got larger regardless of watering treatment (Watered: $z=3.13$, $P = 0.01$; Unwatered: $z=3.63$, $P < 0.01$).

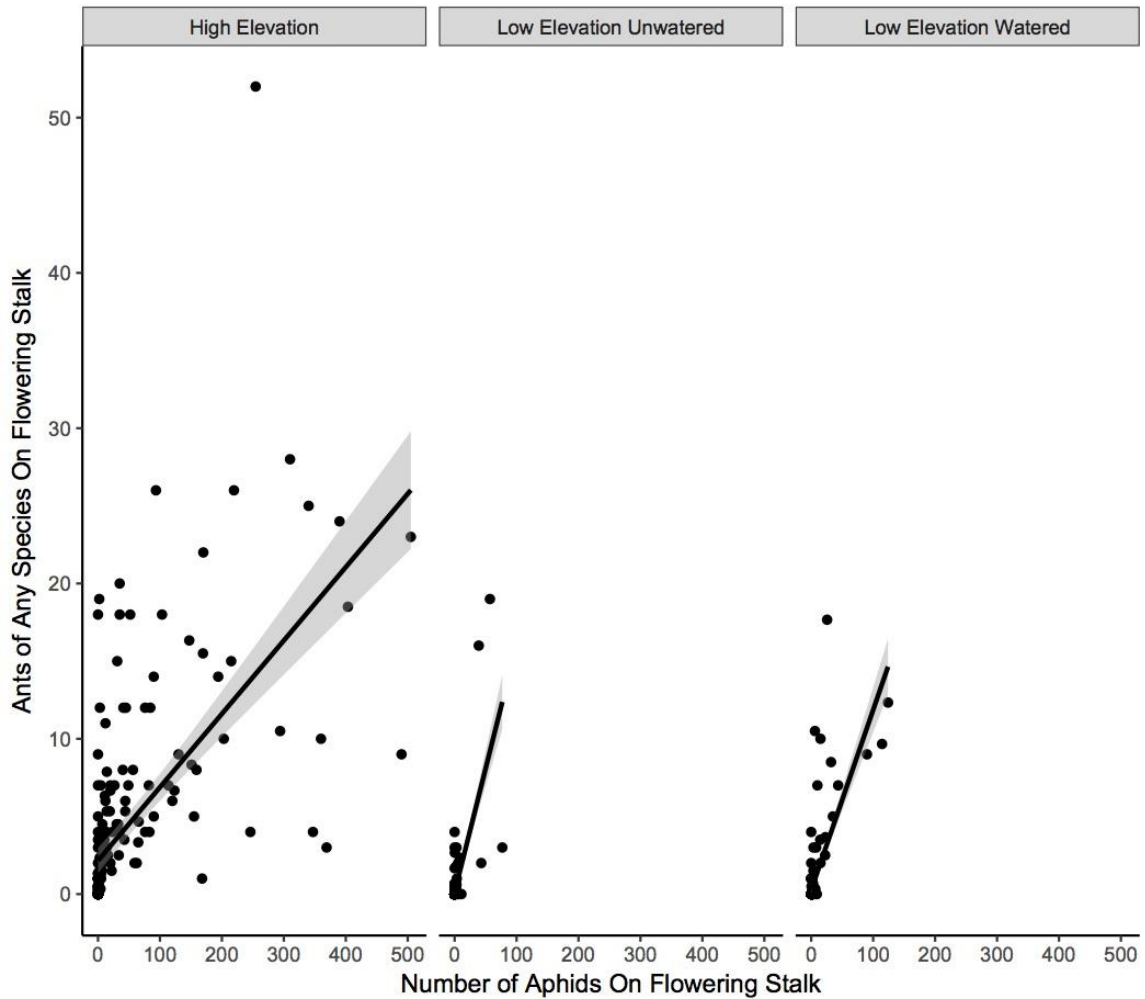


Figure 7: Total number of ants found on flowering stalk in relation to the abundance of aphids on that flowering stalk.

Laboratory analysis of honeydew showed effects of host plant treatment.

Treatment significantly altered the number of honeydew drops per aphid ($\chi^2 = 169.13$, $P < 0.01$). *Post-hoc* analysis showed a trend for deposition to vary between high elevation and both low elevation watered ($z=5.60$, $P < 0.01$) and unwatered plants ($z=5.80$, $P <$

0.01). Aphids from high elevation plants produced on average 733% more drops of honeydew than aphids from unwatered low elevation plants (N=630; Figure 8). However, analysis of the sugar composition of honeydew from each treatment did not show significant differences between each other ($F_{12,20}=1.41$, $P=0.24$). Concentrations of individual sugars also did not show differences between treatments: fructose ($\chi^2 = 0.99$, $P = 0.61$), glucose ($\chi^2 = 0.22$, $P = 0.89$), sucrose ($\chi^2 = 1.72$, $P = 0.42$), trehalose ($\chi^2 = 0.33$, $P = 0.85$), melezitose ($\chi^2 = 1.23$, $P = 0.54$), and raffinose ($\chi^2 = 1.36$, $P = 0.50$).

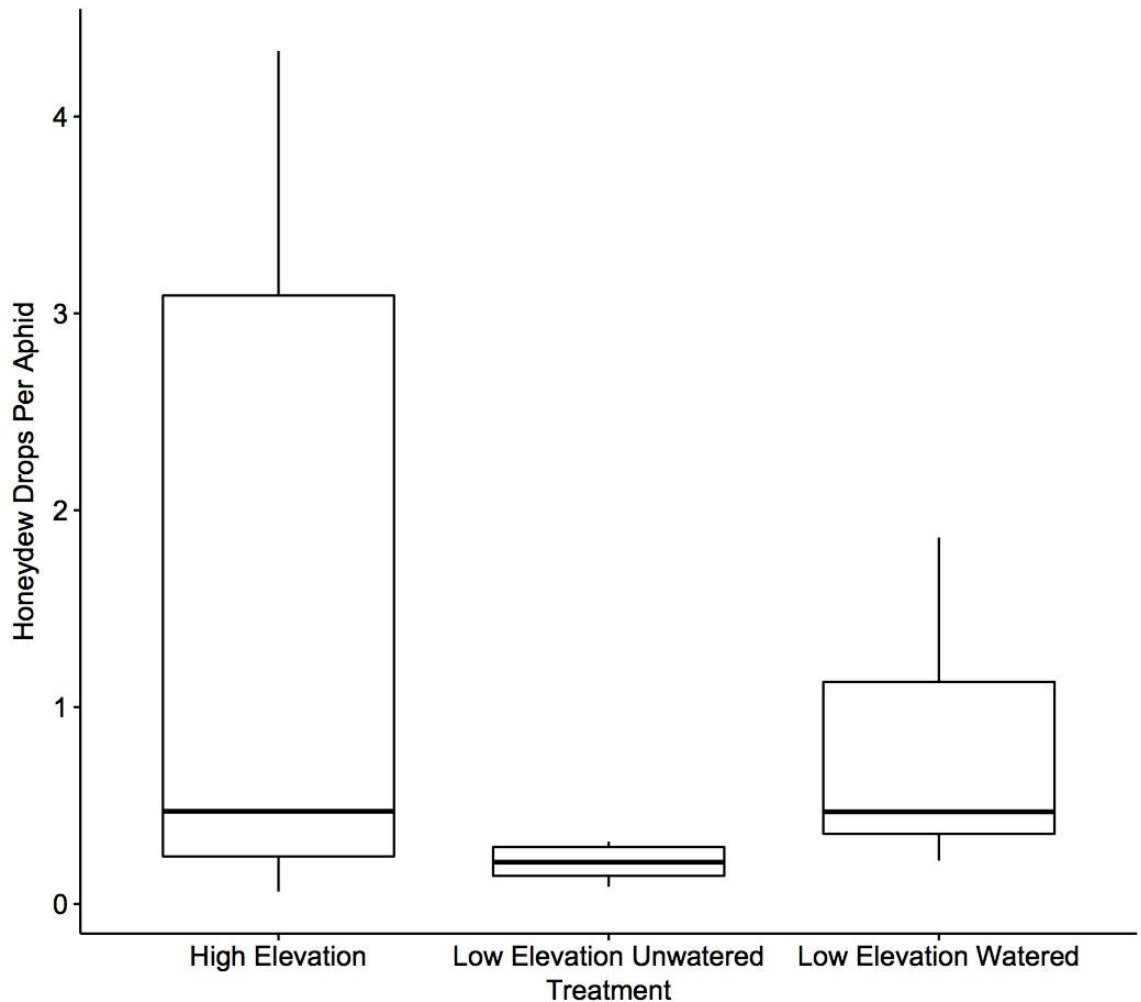


Figure 8: Honeydew drops per aphid collected on foil squares over five hours below terminal umbels of plants.

Prevalence of aphid predators

Lygus bug nymphs (chiefly *Lygus hesperus*) were significantly fewer on plants at high elevation sites when compared to unwatered host plants at low elevation ($\chi^2 = 8.11$, $P = 0.02$; Figure 9). Watered host plants at low elevation sites were not significantly different from either high elevation host plants or unwatered host plants at low elevation. On average low elevation plants had 442% more lygus nymphs per flowering stalk than high elevations (N=630). The number of adult lygus bugs on host plants was also significantly different among treatments ($\chi^2 = 6.62$, $P = 0.04$) with more adult lygus at low elevation sites independent of soil moisture levels and fewer adult lygus at high elevations. Low elevations had on average 1345% more lygus adults per flowering stalk than high elevations (N=630).

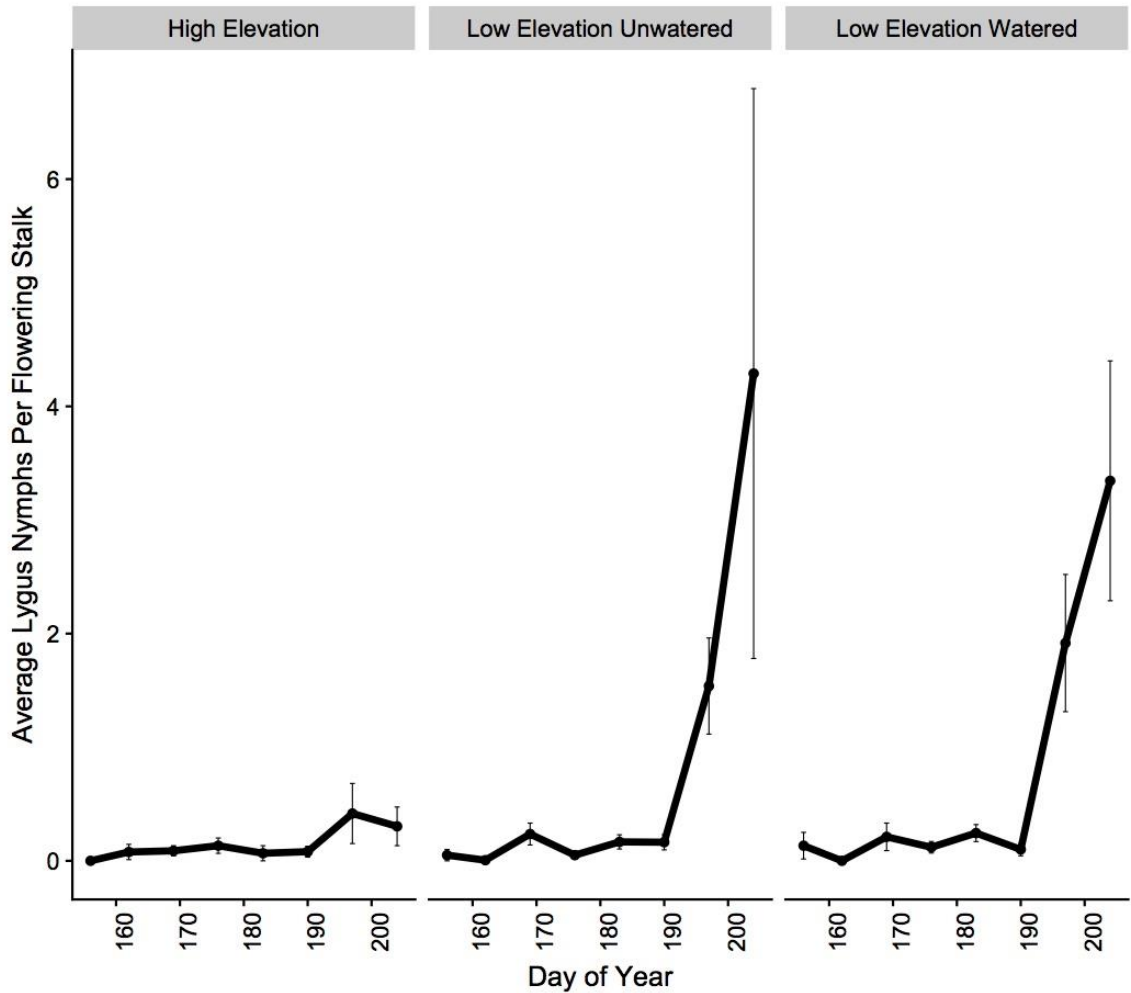


Figure 9: Differential response of average lygus nymph abundance per flowering stalk throughout the growing season among our different treatments

We made 29 observations of ladybugs (adults and larvae) over 630 observations of study plants throughout the growing season. While we observed more ladybugs on unwatered plants at low elevations than plants in other treatments our analysis revealed this to be insignificant ($\chi^2 = 3.42$, $P = 0.18$). Despite 36 observations of ant-mimicking mirids on our study plants, we also observed no pattern in their occurrence among different host plants ($\chi^2 = 0.87$, $P = 0.35$).

Effects of plant quality and abiotic variables

Temperature was significantly different between elevations ($\chi^2 = 5.99$, $P = 0.01$). On average, higher elevations experienced 1.8°C higher temperatures throughout the growing season. Most of this difference was accounted for in daytime temperatures (8am-6pm) with the average difference between high and low elevation being 2.71°C. Nighttime (8pm-6am) temperatures were almost identical (0.003°C difference on average). Nighttime temperatures were statistically the same ($\chi^2 < 0.01$, $P = 0.99$). Photosynthetically active radiation was not significantly different between treatments ($F_{2,267} = 0.98$, $P = 0.38$).

Unwatered plants at low elevation had significantly more flowering stalks per plant than either of the other study groups ($\chi^2 = 54.15$, $P < 0.01$; Figure 10B). Plants that received no added soil moisture had on average 1.31 times more flowering stalks ($N=630$). We also found elevation to significantly delay plant phenology ($\chi^2 = 37.82$, $P < 0.01$; Figure 10A). On average, we scored the terminal umbels of plants at high elevation 4 points below the score of low elevation umbels during each observational visit ($N=630$), which indicates that high elevation host plants had markedly delayed phenology relative to low elevation host plants. The number of umbels per flowering stalk was not significantly different between treatments ($\chi^2 = 0.03$, $P = 0.99$).

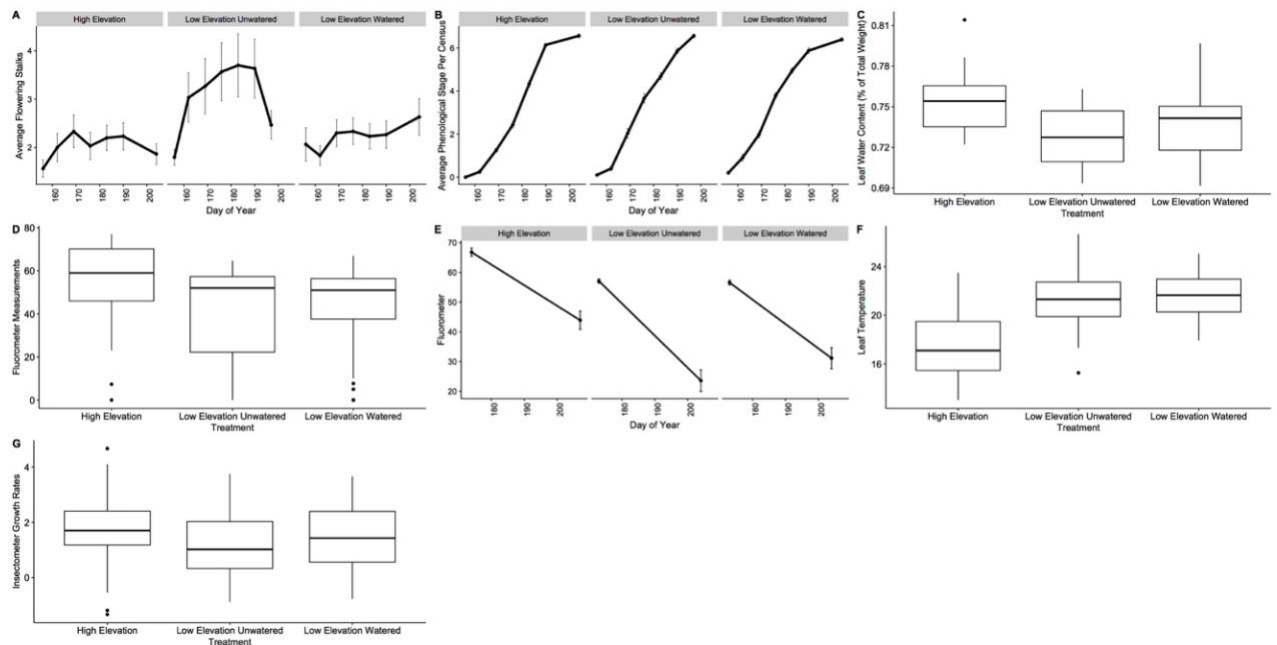


Figure 10: Measures of plant health between our treatments. (A) Average phenological score of terminal umbels at each census date throughout the growing season among our different treatments. (B) Average number of flowering stalks at a plant at each census date throughout the growing season among our different treatments. (C) Leaf water content expressed as a percentage of total leaf weight among our different treatments. (D) Photosystem efficiency measures of host plants by fluorometer measurements averaged between two separate measurements throughout the growing season among our different treatments. (E) Differential rate of change of fluorometer measurements throughout the growing season among our treatments. (F) Differential response of leaf temperature among our different treatments. (G) Relative growth rates of aphid colonies in our insectometer treatments among our treatments averaged throughout thirteen days.

While our analysis showed leaf temperature to be marginally significant ($\chi^2 = 4.63$, $P = 0.09$; Figure 11B), our *post-hoc* comparisons did not reveal any significant association with our treatments. However, high elevation plant leaves had a temperature 3.9°C cooler than low elevation watered plants on average ($N=630$). We found leaf water content to be marginally significantly different between high elevation and low elevation unwatered plants ($z = 2.51$, $P = 0.08$; Figure 11A) however the comparison between plants from high elevation and plants from low elevation watered treatments was not significant ($z = 1.38$, $P = 0.40$). Additionally, our comparison between watered and unwatered plants from low elevation was not significant ($z = -1.23$, $P = 0.44$). Our measurements of chlorophyll content change throughout the growing season were significantly different between our treatments ($\chi^2=8.85$, $P = 0.01$). However, none of our *post-hoc* comparisons showed significant results. Our stomatal conductance data did not reveal any significant differences among host plant treatments ($\chi^2 = 0.30$, $P = 0.86$).

We also observed photosynthetic physiology responses by host plants to our treatments. Photosystem II efficiency (F_v/F_m) also significantly differed between our treatments ($\chi^2 = 6.72$, $P = 0.04$; Figure 11C). However, *post-hoc* contrasts indicate a very weak trend for differences between high elevation and low elevation unwatered plants only ($\chi^2 = -2.58$, $P = 0.11$) while watered plants at low elevation were not significantly different from either high elevation or unwatered low elevation plants ($P > 0.05$). When we looked at how photosystem II efficiency (F_v/F_m) changed over time (6-22-18 to 7-23-18), we found significant differences among host plant treatments ($\chi^2 = 7.02$, $P = 0.03$; Figure 11D). *Post-hoc* contrasts showed marginal significant differences in F_v/F_m

between high elevation and low elevation unwatered plants ($z = 2.81$, $P = 0.09$). Low elevation unwatered plants lost photosystem II efficiency at a more rapid rate throughout the growing season than high elevation plants. Insectometer growth rates did not significantly differ among host plant treatments ($\chi^2 = 2.41$, $P = 0.30$; Figure 12).

Table 2: Test statistic and P-values for post-hoc tests between treatments. All tests were performed with the lmerTest package in R

	<i>Post-hoc test results</i>		
Response variable	Effect of soil moisture: Watered vs. Unwatered at low elevation	Effect of host plant phenology: High elevation vs. low-elevation watered	Combined effect of host plant phenology and soil moisture: High elevation vs. low-elevation unwatered
Aphid Abundance	z=-0.60 P=0.820	z=2.64 P=0.023	z=2.89 P=0.011
Lygus Nymph Abundance	z=-1.50 P=0.292	z=5.60 P<0.001	z=5.80 P<0.001
Lygus Adult Abundance	z=-0.61 P=0.812	z=-8.11 P<0.001	z=-7.77 P<0.001
<i>Formica fusca</i> Abundance	z=-1.45 P=0.292	z=5.60 P<0.001	z=5.80 P<0.001
<i>Tapinoma sessile</i> Abundance	z=-0.57 P=0.836	z=-1.524 P=0.280	z=-1.208 P=0.449
All Ant Species Abundances	z=-0.83 P=0.684	z=3.47 P=0.002	z=3.93 P<0.001
Recruitment of Ants by Aphid Colonies of Differing Sizes	z=-1.10 P=0.518	z=3.13 P=0.005	z=3.63 P<0.001
Flowering Stalks Per Plant	z=2.26 P=0.062	z=-0.24 P=0.969	t=-2.50 P=0.034
Terminal Umbel Phenological Score	z=-1.72 P=0.198	z=-3.23 P=0.004	t=-2.30 P=0.057
Leaf Temperature	z=-0.36 P=0.930	z=-2.15 P=0.191	z=-2.05 P=0.212
Leaf Water Content	z=-1.23 P=0.442	z=1.38 P=0.396	z=2.51 P=0.081
Fluorometer Measurements	z=-1.02 P=0.566	z=1.98 P=0.214	z=2.58 P=0.107
Changes of Fluorometer Measurements Over Time	z=-1.58 P=0.261	z=2.18 P=0.180	z=2.81 P=0.093
Aphid Size	z=2.08 P= 0.079	z=-2.00 P= 0.251	z=-0.28 P= 0.958
Honeydew Drops Per Aphid	z=-1.50 P= 0.292	z=5.60 P< 0.001	z=5.80 P< 0.001

Table 2 cont.

Embryo Count	z=-2.35 P=0.049	z=-3.69 P<0.001	z=0.52 P=0.521
Insectometer Growth Rates	z=-0.45 P=0.897	z=1.06 P= 0.573	z=1.51 P= 0.354
Changes of Chlorophyll Content Over Time	z=0.97 P= 0.597	z=1.84 P= 0.239	z=1.14 P= 0.529

Responses to Plant Trait Measures

While our treatments induced only some effects on host plant phenology and physiology, aphids did respond to differences in plant quality regardless of treatment. Aphids had a significant positive relationship with photosystem II efficiency ($\chi^2=5.67$, $P = 0.017$). The higher the photosystem II efficiency of a plant, the more aphids we observed on those plants. Aphids relationship with leaf water content was not significant ($\chi^2=0.79$, $P = 0.37$). Leaf temperature did have a significant effect on aphid abundance ($\chi^2=13.24$, $P < 0.01$) with more aphids on plants with lower leaf temperatures. Aphid response to chlorophyll content was also significantly different between treatments ($\chi^2=215.28$, $P < 0.01$). Plants with more chlorophyll content had lower aphid abundances. Aphid abundance showed no response to stomatal conductance variation ($\chi^2<0.01$, $P = 0.94$).

The significant relationships between aphids and plant traits therefore only include photosystem II efficiency, leaf temperature, and chlorophyll content. To look at these relationships further we analyzed the slopes of the resultant regression lines. Leaf temperature had the largest slope ($m=8.844$), indicating it was the best predictor of aphid

populations of all our plant quality traits. Photosystem II efficiency had the next largest slope ($m=8.568$), and chlorophyll content had the smallest slope ($m=-4.53$).

CHAPTER IV

DISCUSSION

Our results revealed the multitrophic mechanisms by which the timing of snow cover affects herbivore abundance. Our SEM model indicated soil moisture and phenology are significantly affected by snowmelt date, although the variation in snowmelt date provided by the elevation gradient failed to reproduce the associations between snowmelt date and insect abundance seen in interannual studies in this system (Robinson et al. 2017). Thus, we rely on our experimental study to expose possible mechanisms by which snowmelt date is altering this biological system. During our experimental test of these mechanisms, we successfully matched the higher soil moisture found at our high elevation sites to select lower elevation plants. Our elevational difference between our sites also successfully delayed phenology at high elevations. We attempt to use the resultant phenological and soil moisture comparisons between these treatments to explain increased abundance of aphid herbivore in late snowmelt years.

Identifying Influential Mechanisms of Snowmelt Date from an Elevation Gradient

Our structural equation model identified soil moisture and *L. porteri* phenology to be the only two significant variables correlated with snowmelt date. This confirmed our hypothesis that these were the main mechanisms by which snowmelt date could affect aphid abundances. In doing so our model suggests that bottom-up processes are exerting significant control over this system. Plant phenology has been strongly connected to plant-insect interactions in many different ecosystems around the world (Hegland *et al.*, 2009; Elzinga *et al.*, 2007) while soil moisture is a significant determinant of plant health (Taylor & Seastedt, 1994; Mooney *et al.* 1965). In this way snowmelt may be changing

plant quality, quantity, or timing and thereby affecting aphid abundance. However, our model cannot describe exactly how snowmelt date or plant phenology influences aphid population dynamics, thereby justifying our experimental and observational study.

We did not observe any significant links between snowmelt timing, or its effects on host plant phenology and soil moisture, on either aphid or lygus bug abundance in sites along the elevation gradient. This is in contrast to the pattern seen from year-to-year variation in this system, where lygus bugs achieve high abundance in early snow melt years and aphids achieve high abundance in late snowmelt years (Robinson et al. 2017). The snow pack for the Gunnison River Basin where RMBL is located was less than 60% of the 30-year (1981-2010) median snow water equivalents (SWE) in 2018 (Natural Resource Conservation Service 2019). As a result, even the high elevation sites in the elevation gradient may have had reduced snow cover and early dates of bare ground relative to previous years. Snowmelt dates observed in the year-to-year study ranged from 114 to as late as 158 (Robinson et al. 2017). The latest snowmelt date in this current study was 127. Our study highlights the inevitable limitations of elevation gradients in perfectly replicating the year-to-year changes in climate seen in long-term studies.

Our research is not alone in experiencing the difficulties of recreating variations caused by year-to-year variations through experimental means. For example, Hoffmann *et al.* (2010) found interannual variations created larger phenological variations than could be created through the experimental warming of plots of subalpine plants. Giménez-Benavides *et al.* (2005) recorded the germination rates of an alpine plant species varied more according to year-to-year variation than any recorded variations

along their elevational gradient. Research in the mountains of Patagonia has also shown that interannual climactic variations can account for spatial alterations of major feeding guilds that may be crucial to predicting insect herbivory along environmental gradients (Noemi Mazia *et al.*, 2004). Despite evidence that combining experimental manipulations with elevational gradients can be a beneficial method for studying community-oriented research (Dunne *et al.*, 2004) these studies suggest ample room for improvement in replicating year-to-year variations in temporally constrained studies.

Significant links among the abundances of host plants and insects underscore the importance of trophic interactions in this system. Sites with high abundances of host plant flowering stalks had correspondingly greater amounts of both lygus bugs and aphids. These relationships are expected given that sites with more host plant flowering stalks have increased the likelihood of either insects being observed. Unexpectedly, we did not observe significant links between lygus bug and aphid abundance. As intraguild predators, lygus bugs can facultatively switch between feeding on plant tissues (such as *L. porteri*) to feeding on insects (such as *A. asclepiadis*) (Hagler *et al.*, 2010). Instead, we found aphid abundance to be closely associated with ant abundance, likely as a result of their mutualism (Mooney *et al.* 2016). Ant abundance was also closely associated with flowering stalk abundance. Altogether, this suggests the primacy of flowering stalk abundance in directing insect abundance at a particular site, but neither soil moisture nor snowmelt date govern flowering stalk abundance.

Responses to experimental soil moisture manipulations and host plant phenology manipulations

We saw significantly more aphids on host plants at high elevation sites than on host plants at low elevation, regardless of watering treatment. This suggests the effects of snowmelt timing on aphid abundance are independent of its effects on soil moisture. Snowmelt timing can have a variety of other effects on processes that govern insect abundance. For example, multivoltine species—such as lygus bugs— can benefit from a longer growing season (Connor *et al.*, 1994; Tobin *et al.*, 2008, Logan *et al.*, 2003). Thus, aphid populations at low elevation sites may experience greater top-down control than at high elevation sites. Indeed, lygus bugs were significantly less abundant on host plants at high elevation in comparison to host plants at low elevation, regardless of moisture status. Previous research revealed lygus densities were highest where phenology was the most advanced (Butts & Lamb, 1991). As a multivoltine species, lygus bugs that establish early on plants have the best chance to produce the most generations in a growing season (Tobin *et al.*, 2008). The increased abundance of mirids at low elevations, and likewise their relative absence at high elevations, may be underlying the differences in aphid abundances between our treatments through their potential to both compete for plant resources and feeding directly on aphids (Hagler *et al.*, 2010).

Snowmelt timing will also have an effect on host plant phenology, as seen with our SEM model results. In other systems, host plant phenology is a key factor in determining the abundances of insect herbivores (Hunter, 1990; Liu *et al.*, 2011), and phenological mismatches occur as a result of unequal responses to phenological cues (Visser & Both, 2005). Phenological mismatches have been shown to have a great effect upon insect abundances (Russell & Louda, 2004; Schwartzberg *et al.*, 2014; Martemyanov *et al.*, 2016). While some studies show insect herbivore abundances

increasing under advancing host plant phenology (de Sassi *et al.*, 2012; Floate *et al.*, 1993), other studies show their abundance decreasing (Hunter & Elkinton, 2000; Stoyenoff *et al.*, 1994). In our experimental study, high elevation host plants were phenologically delayed relative to low elevation plants when we applied aphid colonies to test growth rates. However, we failed to observe differences in aphid population growth rates among these colonies. The timing of experimental colonies in late July overlapped with early host plant senescence driven by a weak monsoon season (Munné-Bosch *et al.*, 2004). Senescence likely overrode any differences in plant quality such that aphid population growth rates did not differ. The experimental colonies were protected from lygus bug predation, thus, differences in growth rates—had they occurred—would be driven by differences in host plant quality alone. The absence of significant differences between these groups suggests predation may be a significant factor in determining our observed differences of aphid abundances between our treatments.

Accelerated phenology has been shown to alter measures of plant quality including reproductive success, transpiration rates, and plant growth (Nord & Lynch, 2009; Lewis *et al.*, 2003; McKown *et al.*, 2013). In our system, the only host plant quality indicator we observed to be driven by phenology alone was fecundity. No other comparisons of variables between high elevation plants and low elevation watered plants were significant. Previous literature also shows a connection between plant quality, measured through productivity, and soil moisture (Taylor & Seastedt, 1994). Contrastingly, our study found no significant differences in host plant quality as a result of manipulating soil moisture alone, as indicated by our post-hoc comparisons between low elevation watered and unwatered plants. There was often a significant effect of the

combination of both soil moisture and host plant phenology on plant health, seen through our *post-hoc* comparisons of high elevation plants to low elevation unwatered plants. The combination of both mechanisms significantly affected leaf water content, photosystem II efficiency, and chlorophyll content. However, these comparisons also took into account many confounding variables associated with differences in elevation. Neither soil moisture or phenology alone could account for the majority of these differences. Altogether, our results suggest the primary mechanism by which snowmelt timing affects aphid abundance is through top-down control either by affecting aphid predators (such as lygus bugs) or protectors (such as ants), with little evidence of an effect of phenology or soil moisture on host plant quality.

We observed differences in ant species composition and abundance between high elevation and low elevation watered host plants. Overall, we counted more ants on flowering stalks at high elevation than at low elevation while *Formica fusca* was more abundant at high elevation plants. Because these ants were counted on either host plant flowering stalks alone or tending aphid colonies, differences in species composition and abundance reflect both differences in ant communities at these sites and differences in recruitment of ants by host plants and aphids. Previous studies have repeatedly shown elevation to be a major factor in ant distribution (Human *et al.*, 1998; Glaser, 2006). Pitfall traps utilized in concurrent research at our elevational sites confirms this suspicion. At our study sites ant abundance declined with increasing elevation (Hewett, 2018). Given the many more aphid colonies on high elevation host plants, we are likely seeing the product of recruitment to flowering stalks with aphid colonies rather than differences in abundance of ants in the sites. *F. fusca*, which we found in higher

abundances at high elevations, has also proven itself to be both more aggressive and more effective at protecting aphids than *T. sessile*, which we generally found in high abundances at low elevations despite our insignificant analysis (Buckley & Gullan, 1991). The increased abundance of *F. fusca* at high elevations likely contributes to the increased abundance of aphids given their relatively high aggressive behaviors and thereby reducing predation on aphids. The mutual interdependence of ant and aphid abundance is also supported by our SEM results.

While larger aphid colonies attract more ants as seen in previous studies (Mooney *et al.*, 2008; Zhang *et al.*, 2015), the rate of ant recruitment by aphids differed between high and low elevation host plants. As aphid colonies grew at high elevation sites they attracted fewer ants than low elevation colonies given the same aphid colony growth rates. Underlying this effect may be ants changing their behavior at different elevations. Research has shown ant behavior is indeed elevation dependent (Nelson *et al.*, 2018; Keeler, 1979; Trimble & Sagers, 2004). For example, Nelson *et al.* (2018) showed that ants in this study area show a behavioral shift as altitude increases, relying less on the carbohydrates that would be provided by the honeydew of aphids at high elevations. Our analysis of honeydew composition suggests no differences in honeydew quality or attractiveness between elevations. Thus, behavioral shifts resulting from increased elevation may explain our observation of lower ant recruitment by aphids at high elevations. Additionally, our analysis of honeydew excretion rates (a 733% increase at high elevations) suggests ants may be influencing the quantity of honeydew produced. Previous research has shown aphids that are tended by ants excrete up to seven times more honeydew than untended aphids (Takeda *et al.*, 1982; Banks & Nixon, 1958). As a

result of more ants at high elevations, and presumably more tending as a consequence, these increased excretion levels may be a remnant of the effects of ant tending even though ants were excluded during honeydew collection.

The population structures of aphid colonies from low elevation watered and unwatered host plants also had a marginally significant difference, indicating that increasing soil moisture may be associated with more late-instar aphids. Populations composed of more late-instar aphids indicate altered aphid development rates, higher predation rates, or both (Aslam *et al.*, 2013). Studies have shown aphid fecundity and growth rates increasing at moderate levels of plant drought stress, and decreasing for aphids from both unstressed and highly drought stressed plants (Tariq *et al.*, 2012). Further, Aslam *et al.* (2013) has shown that altered aphid development rates as a result of increased drought stress on host plants has the potential to decrease parasitoid attack rates.

Our observed differences in population structure between low elevation watered and unwatered plants suggests we may find differences in insect abundances, particularly predators, between our low elevation watered and unwatered treatments. Additionally, research concerning both the plant stress and plant vigor hypotheses expect drought stressed plants to attract or deter insect populations respectively, and provide more support to our expectation of differing insect abundances between treatments (Price, 1991; White, 1984). However, our study saw statistically similar insect abundances between watered and unwatered low elevation plants, suggesting our treatments did not significantly affect plant health enough to have reverberating bottom-up effects through the ecosystem as expected by either the plant stress or plant vigor hypotheses. This absence of predator differentiation suggests our differences in population structure may be due to altered aphid development

rates opposed to any difference in predation rate. Additionally, despite these insignificant *post-hoc* comparisons of insects, we found marginally significant differences both of photosystem II efficiency throughout the growing season and aphid length when comparing these low elevation watered and unwatered plants. Perhaps the differences between our watered and unwatered treatments were not sufficient to provide statistical power to an underlying bottom-up effect of soil moisture. However, these statistically trending differences suggest more extreme variations in soil moisture may be worthy of further investigation.

Further evidence for this theory is provided by significant relationships between aphid abundances and photosystem II efficiency, leaf temperature, and leaf chlorophyll content. While we cannot assume aphid abundances were directly due to these changes in plant quality as our data highlights the importance of community composition on this system, these relationships do provide support for the idea that aphid populations are also affected by bottom-up processes. Of these plant quality measures, aphid populations were the most responsible to photosystem II efficiency and leaf temperature. These factors showed aphid populations increase in response to increasing plant quality. While chlorophyll content may also play a role in determining aphid populations, the relationship was less strong and indicated increased plant quality negatively affected aphids, unlike our other two factors. Perhaps our treatments were not significant enough to produce differential plant qualities sufficient to result in altered aphid populations, but our results do imply plant quality has some role to play in aphid abundance levels.

Greater temperatures at high elevation likely contributed to increased aphid abundance, and in this way, our elevation gradient replicated year-to-year variation

between warmer late snowmelt years and early snowmelt cooler years. Previous research has shown variable responses of aphids to increased temperatures. Some studies show higher temperatures decrease the abundance of aphids while increasing their growth rates (Barton and Ives, 2014; McCornack *et al.*, 2004) while others show temperature does not affect aphid growth rates (Leather & Dixon, 1984; Desneux *et al.*, 2006), and perhaps most applicable, aphids in arctic and alpine environments benefit greatly in both growth rates and abundance under increased temperatures (Hodkinson & Bird, 1998; Hodkinson *et al.*, 1998). Our study system in the sub-alpine zone of the Rocky Mountains is more akin to these snow-dominated ecosystems of the arctic and alpine zones. However, these studies do not separate the effects of daytime temperature and nighttime temperature.

Our results show that temperature differences between our elevational treatments can be almost completely accounted for by daytime temperatures. Nighttime temperatures were nearly identical. Recent research shows nighttime temperatures are of particular importance for ecosystem functioning (Barton & Schmitz, 2018; Mohammed & Tarpley, 2009), and the effects of day and night temperatures can produce distinct results (Speights *et al.*, 2018)(Yang & Stamp, 1995). Regardless, warmer temperatures at high elevation may have contributed to patterns we observed. For example, research by Fitzpatrick *et al.* (2014) reveals that some ant species reduce resource tending and protection during times of increased temperatures. Previous research has also found reduced abundances of aphid predators among aphid colonies experiencing warmed conditions (Robinson *et al.*, 2017; Khan & Khan, 2010; Marquis *et al.*, 2014).

Pulsed stress events may also play a role in this ecosystem. Huberty and Denno (2004) showed phloem feeding insects benefit from plants experiencing intermittent

stress compared to continuous stress. The wider range of temperatures observed at our high elevation sites may have provided these short-term stress events that benefited aphids at those locations. Additionally, Marchand *et al.* (2005) has shown high temperature pulses can greatly affect arctic plant performance. Accordingly, future experiments should more accurately assess daytime temperatures as well as daily extremes and record the different effects of each on both plants and aphids.

Taken together, while differences in plant quality and abiotic conditions between our treatments do not seem to be the major determinants in aphid abundances, their role may still prove important. Our experiment's design to control for intra-annual variations likely does not span the extremes of conditions that inter-annual variations may induce. More intense experimentation may be required to see these bottom-up effects reverberate through this complex ecosystem.

Conclusions

Climate change is altering plant-insect interactions around the world (DeLucia *et al.*, 2012). Changes in arctic and alpine ecosystems are particularly dominated by alterations to snowmelt date (Baptist *et al.*, 2010; Wipf *et al.*, 2009). *Aphis asclepiadis* is a common aphid whose abundance is greatly affected by snowmelt date (Robinson *et al.*, 2017). By studying this aphid and its interactions with its host plant and associated insects we have described mechanisms by which snowmelt is altering this important plant-insect interaction. Our results suggest soil moisture and host plant phenology are exerting control over aphid abundance through their combined effects on ant abundance and aphid predators.

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