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Snowmelt timing determines aphid abundance through multitrophic interactions

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ABSTRACT

Ecologically important relationships are being altered by climate change. It is important to understand these relationships in order to predict future changes in species distribution and abundance. Previous research has shown that aphid (*Aphis asclepiadis*) abundances correlate significantly with snowmelt date in subalpine Rocky Mountain ecosystems, with late snowmelt years correlating with larger aphid populations in summer. Although consistent across years, the mechanisms underlying this phenomenon have not been previously identified. We suspected early snowmelt decreases aphid abundance by inducing early season host plant drought stress. In this study, we used an elevation gradient to mimic year-to-year variation in snowmelt date. We experimentally added soil moisture to separate the effects of early season drought stress on aphid populations from other effects of elevation and snowmelt date. Our manipulations resulted in significant responses in aphid abundance, ant abundance, aphid predators, and plant quality yet revealed host plant drought stress was a minor mechanism by which snowmelt determined aphid abundance. Instead, host plant phenology and mutualist behavior appeared to greatly influence aphid abundance. This research highlights the importance of considering multi-trophic interactions in determining the effects of climate change and how changes of snowmelt dates may be affecting insect communities.

1. Introduction

Early loss of snow cover in spring is a key signal of climate change. Early snowmelt advances both animal and plant phenology (Inouye et al., 2000) and decreases soil moisture, especially important in ecosystems where snow is a major precipitation input (Harpold, 2016). Snowmelt timing also alters the way organisms interact with each other (Gallagher and Campbell, 2017; Gillespie et al., 2016; Rafferty et al., 2013). For example, phenology shifts can affect both plant and herbivore populations by altering the timing of when insects can feed on their hosts, i.e. trophic mismatch (Renner and Zohner, 2018). Reduced early season soil moisture can also induce drought stress of host plants and affect the population dynamics of insect herbivores as a result (Huberty and Denno, 2004; Simpson et al., 2012). How herbivore populations react to changes in host plant quality can also be mediated by other interactions. Notably, a variety of herbivores depend on protection mutualisms with ants (Ness et al., 2010), and many aspects of these mutualisms are mediated by host plant quality (Fischer and Shingleton, 2001; Mooney, 2011; Mooney and Agrawal, 2008).

Predicting how species interactions will be altered by climate change remains a significant challenge in ecological research (Penczykowski et al., 2017). One of the key limitations is scale: studies that alter temperature, precipitation or other climate variables do so most frequently across relatively small plots, e.g. < 36 m^2 (Ettinger et al., 2019). However, species interactions occur over much larger spatial scales than can be practically addressed by experimental manipulation. Likewise, species interactions are often diffuse (Bakker et al., 2014), although manipulative studies often focus on how climate variation will alter the interactions between a set of focal species (Pelletier et al., 2009; Russell et al., 2012). To overcome these limitations, ecologists have long used elevation gradients to study how climate variation will impact natural communities (Dunne et al., 2004). In these studies, high elevation populations are proxies for cooler and wetter climatic conditions, and low elevation sites represent warmer and drier future climates (Moreira et al., 2018). The community at low elevation would likewise be a stand-in for future biotic interactions (Rafferty et al., 2013). This approach has been used to study a variety of species interactions including herbivory (Leckey et al., 2014), seed

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predation (Hillyer and Silman, 2010), pollination (Benadi et al., 2014) and ant-aphid mutualism (Nelson et al., 2019). One of the strengths—and caveats— to this approach is that multiple factors will vary with elevation. For example, plant resistance to herbivores often increases along with elevation (Hahn and Maron, 2016; Rasmann et al., 2014). Other reviews suggest opposite elevation gradients of plant defense (Moreira et al., 2018), Elevational gradients in herbivory are thus influenced by multiple factors and also idiosyncratic to specific herbivore-host plant interactions.

In this study, we combined an elevation gradient approach (165 m) with an experimental manipulation of early season soil moisture to determine aphid responses to advanced snowmelt date. Through their interactions with other species, aphids are keystone herbivores in many natural communities (Eubanks and Styrsky, 2007). They can exert influence on host plant fitness and population growth (Snow and Stanton, 1988), host a diverse array of predators (Styrsky and Eubanks, 2007) and provision mutualist ants, who can be keystone species themselves (Mills et al., 1993). Like other such interactions, the ant-aphid mutualism is variously altered by climate change (Blanchard et al., 2019). Most experimental studies to date have focused on how elevated temperature and CO_2 will alter aphid population dynamics, ant behavior, honeydew production and chemical communication (Blanchard et al., 2019). In our study system, aphid (*Aphis asclepiadis*) abundances on host plants (*Ligusticum porteri*) positively correlate with snowmelt date; years with later spring snowmelt dates have higher aphid abundances in the summer (Robinson et al., 2017). The mechanisms leading to this association are unknown. These aphids exclusively colonize the flowering stalks of the host plant, and *L. porteri* flowering phenology is driven by snowmelt timing (Iler et al., 2013). Thus, early snowmelt years may present colonizing aphids with a later, lower quality phenological stages of flowering stalks. At the same time, early snowmelt years may also induce drought stress in host plants in this area by lengthening the period between snowmelt and summer monsoons, a critical period of growth for this host species. While water stress can sometimes improve host plant quality (Hale et al., 2003), aphids are unable to tap into phloem when turgor pressure is too low (Huberty and Denno, 2004). The timing of drought has also been shown to have complex and long-lasting effects on aphid populations (Banfield-Zanin and Leather, 2015). In addition to such bottom up effects, abundance of this aphid species, like many others, is governed by a protection mutualisms with ants: without such protection, predation drives colonies to extinction (Mooney et al., 2016).

To understand the abiotic and biotic impacts of snowmelt date, we monitored eight host plant populations along an elevation gradient over two years (2018–2019). Elevation gradients have been effectively used to study the effects of environmental variation on other aphid species in our study area (Nelson et al., 2019). Along this gradient, we found effects of snowmelt date on early season soil moisture, host plant phenology, and aphid colonization of host plants. Aphid colonies were more abundant in sites with later snowmelt dates. Concurrent changes in host plant phenology and early season soil moisture make determining the underlying mechanism(s) of this change in aphid abundance challenging with observational data alone. Therefore, in 2018, we combined this elevation gradient with experimental water addition in order to identify these mechanisms. We used replicate host plant populations at low (2964 m.a.s.l.) and high (3109 m.a.s.l) elevation sites as a 'natural experiment' to manipulate snowmelt date. We then added water to half of the low elevation sites until monsoon rains arrived in July, effectively matching soil moisture at high elevation. This experimental design allowed us to make three important contrasts: (1) comparing high elevation to low elevation non-watered plants assessed differences driven by drought stress *plus* other biotic or abiotic factors that could vary due to snowmelt date, (2) comparing high elevation to low elevation watered plants isolated differences due to factors *other than* early season drought stress (e.g. phenology), and lastly, (3) comparing watered and non-watered plants at low elevation isolated the effects of early season drought stress from other factors that would vary due to snowmelt date. We measured host plant phenology, physiology, and quality in response to these conditions. For host plant quality, we tracked the growth of experimental aphid colonies protected from predators. We also tested for these effects on the ant-aphid mutualism.

2. Methods and materials

2.1. Study site and species

We conducted our research near the Rocky Mountain Biological Laboratory (RMBL) in Crested Butte, Colorado (RMBL; 39°01′38.77″N, 107°03′10.50″W). The host plant *Ligusticum porteri*, commonly known as oshá, is a perennial herb of the Rocky Mountains that reaches high abundances in subalpine meadows and aspen stands (Weber and Wittmann, 2001). *Aphis asclepiadis* (syn. *Aphis helianthi*) is a common insect herbivore of *L. porteri* among other host plants in both agricultural and natural systems (Addicott, 1981; Lagos-Kutz et al., 2016). In our study area, these aphids overwinter on *Cornus* host plants before colonizing the flowering stalks of *L. porteri* as early as June. While feeding on *L. porteri* they form mutualistic relationships with various ant species including *Formica fusca*, *Formica rufa*, and *Tapinoma sessile*. These mutualisms with ants determine aphid colony abundance and distribution on *L. porteri* (Mooney et al., 2016). A variety of predators and parasitoids attack *A. asclepiadis*, chiefly parasitic chalcid and braconid wasps, coccinellid beetles and adult mirids (Kummel et al., 2013; Wheeler, 2001).

2.2. Observational study

To observe responses to snowmelt timing, we monitored 8 host plant populations along an elevation gradient (2964–3109 m a.s.l) within 15 km of RMBL in 2018 and 2019. We anchored the temperature loggers at the soil surface at each site in October 2017 and 2018 to estimate snowmelt timing for 2018 and 2019. The day of snow cover loss at each site occurred when loggers showed diurnal fluctuations in temperature (Lundquist and Lott, 2010). Two temperature loggers malfunctioned in 2018–2019, and another was lost during the winter of 2017–2018. We interpolated snowmelt dates for those 3 sites using topographic variables (e.g. elevation, slope etc.) selected by stepwise regression in the 'stepAIC()' function from the MASS package in R (Venables and Ripley, 2002). In June of each study year, we randomly selected ten focal host plants with flowering stalks in each population. We then performed weekly counts of aphids and ants on host plant flowering stalks in each population until August. We also recorded the flowering phenology of terminal umbels of each plant as described in Robinson et al. (2017). We measured volumetric water content of soils at each site once in June using a probe (Field Scout, TDR 150 Soil Moisture Meter, Spectrum Technology, Aurora, IL US) at 11.3 cm in depth around three randomly selected host plants. At the end of June, we used three randomly placed pitfall traps to assess ant abundance at each site.

We tested for the effects of snowmelt date on biotic and abiotic responses measured at the population level ($n = 8$) using linear mixed effects models (Qian, 2017; Bates et al., 2015). Response variables included mean soil moisture, flowering phenology, ant abundance in pitfall traps, and aphid and ant abundance on host plants. Flowering phenology was the average phenological score recorded during the final census in June (Ordinal Date: 179), when anthesis generally begins in the host plant (Weber and Wittmann, 2001). We assessed aphid abundance as the total number of host plants colonized per population. We assessed ant abundance on host plants as the cumulative number observed during the June–August census period. All statistical models included the fixed effect of snowmelt date plus the random effect of population. For continuous response variables, we used the 'lmer ()' function from the *lmerTest* package to construct models, and for count

variables, we used the 'glmer ()' (Kuznetsova et al., 2017). We used the 'summary ()' function to obtain test statistics and *P*-values for significance testing, which returned type III analysis of variance tables with Satterthwaite's method (Kuznetsova et al., 2017).

2.3. Water addition experiment

2.3.1. Study design

In 2018, we combined an elevation gradient with experimental water addition to test for multiple effects of snowmelt date on aphid abundance. We randomly selected ten host plants in three replicate populations at high (3109 m.a.s.l.) and six replicate populations at low elevation (2964 m.a.s.l). Using snowmelt dates from the nearest population (see above), loss of snow cover at low elevation sites took place 8 days earlier than at high elevation. In three randomly selected low elevation populations, we added water to individual host plants (3 populations X ten plants = 30). The remaining host plant populations at low elevation remained at ambient soil moisture conditions (3 populations X ten plants = 30). At high elevation sites, we selected ten host plants in each population (n = 30). For plants receiving water addition at low elevation, we added 1.5L of water over a 36 cm radius circle twice weekly beginning on 6-11-18 and ending on 7-9-18.

The motivation for this watering duration was to mimic the input of water from later snowmelts, which would chiefly affect early season soil moisture. Secondly, monsoon rains began in mid-July, which would render the effects of further water addition to be minimal. We monitored changes in both soil moisture and flowering phenology of host plants each week from June through July. We measured soil moisture for 3 randomly selected plants in each population beginning on four dates beginning on June 18 and ending on July 16. We monitored host plant flowering phenology using the index as described above from June 5 through July 23.

2.3.2. Host plant quality

We measured both plant and aphid responses as indicators of host plant quality. On host plants, we measured three responses related to drought stress: photosystem II efficiency (F_v/F_m), relative chlorophyll content (SPAD), and leaf water content. Drought stress can destabilize photosystems, resulting in reduction of photosystem II efficiency and breakdown of chlorophyll in leaves (Heschel et al., 2014; Maxwell and Johnson, 2000; Valladares and Pearcy, 1997). We measured both F_v/F_m and SPAD on two measurement dates (6-23-18 and 7-26-18). We measured F_v/F_m using a chlorophyll fluorometer (miniPPM, EARS, Kanaalweg, Netherlands). For each plant, we recorded three separate measurements taken on leafy bracts; these were then averaged to provide a single measurement for each host plant. We followed the same sampling protocol for relative chlorophyll content (SPAD, Konica-Minolta, Japan). For leaf water content, we sampled the top-most fully expanded leafy bract on each host plant. We kept the bract samples on ice in a cooler until measuring fresh weight (*FW*) and then drying for 144 h at 60 °C to obtain dry weight (*DW*). We then estimated water content as percent of fresh weight ($FW\text{-}DW\cdot FW^{-1}$).

We also used the growth of aphid colonies to measure host plant quality. On 7-10-18, we randomly selected half of the plants (n = 5) in each group (high elevation, low elevation non-watered, and low elevation watered) for experimental aphid colony addition (N = 45). We cleared these plants of all insects using a soft-bristled paintbrush and introduced 5 mature and 5 immature aphid instars to one terminal umbel on each plant. We excluded predators using a sealed fine mesh bag and a guard, which excluded crawling predators with masking tape coated in an insect barrier (Tree Tanglefoot, Contech Enterprises, Marysville, OH). We recorded colony size every three days for thirteen days (DOY 194–203). This approach allows us to test for differences in quality among host plants for aphids (i.e. bottom-up effects) without the influence of site-to-site variation in predation or mutualisms (Mooney et al., 2016).

To test for differences in leaf water content, we used a linear mixed effects model with fixed effect of host plant type (high elevation, low elevation and low elevation watered) and random effects of population. We used a repeated measures approach to determine how soil moisture, flowering phenology, F_v/F_m, and SPAD changed over time in each treatment group (Qian, 2017). Models consisted of the fixed effect of host plant type (high elevation, low elevation and low elevation watered), day of year (D.O.Y.) and the random effect of plant nested within population. For continuous response variable, we constructed models using the 'lmer ()' function from the *lmerTest* package (Kuznetsova et al., 2017). Significance testing proceeded as described above. For significant ($P < 0.05$) effects, we performed *post hoc* contrasts using the *emmeans* package (Lenth, 2019); these included pairwise contrasts of means using the 'emmeans ()' function and tests for heterogeneity of slopes using the 'emtrends ()' function. To test for differences in aphid colony growth, we also used a repeated measures approach with count of aphids as our response variable. Our data fit a Poisson distribution, so we used the 'glmer ()' function to specify the model. We checked for overdispersion using the function 'dispersion_glmer ()' from the *blmeco* package (Korner-Nievergelt et al., 2015). If evidence of overdispersion was found (> 1.4), we added an observation-level random effect (Harrison, 2014). We tested for significance of model terms using likelihood ratio tests performed with the 'drop1 ()' function from the MASS package (Venables and Ripley, 2002).

2.3.3. Ant-aphid mutualism

To determine how the ant-aphid mutualism responded to host plant type (high elevation, low elevation and low elevation watered), we monitored responses by ants and aphid predators to host plants and aphid colonies. We conducted weekly censuses (6-5-18 until 7-23-18) of aphid colonies on host plant flowering stalks. In order to control for interactions between time of day and insect behavior, we altered the order of our surveys daily, so any effects of insect diurnal rhythms were distributed among sites. Weekly censuses also included a count of ants, which we identified to species during these observations. In addition to collecting honeydew from aphids, ants may also be attracted to floral nectaries common in the Apiaceae (Koul et al., 1993). For ants, we considered two responses: abundance on host plants without aphid colonization (n = 90) and recruitment to host plants with aphid colonies (n = 46). Host plants without aphid colonies often had zero ants. Thus, to analyze ant abundance on host plants without aphids, we used a zero-inflated model specified using the 'hurdle ()' function from the *pscl* package (Jackman, 2015). This model included the effects of host plant type and included the number of flowering stalks as a covariate. For ants on host plants with aphid colonies, we used an analysis of covariance approach to test for differences in ant recruitment as a function of aphid colony size (Mooney and Agrawal, 2008). In this analysis, we used mean aphid colony size as the covariate and the cumulative numbers of ants as the response variable. Given the small number (n = 3) of ant-tended aphid colonies observed in two study populations, the mixed effects model including a random effect of population was nearly unidentifiable. Instead, we used a general linear model to analyze recruitment to aphid colonies and specified quasi-poisson-distributed errors to account for overdispersion. To test for differences in predator abundance, we again used a zero-inflated model, which included the effects of host plant type and mean aphid colony size as a covariate. Because aphidophagous predators were overall uncommon, we analyzed the cumulative number of all predators across the census period.

To understand why ant recruitment to aphid colonies might vary among host plants, we analyzed the sugar composition of honeydew from experimental aphid colonies. We placed squares of aluminum foil under aphid colonies to collect honeydew dropped by aphids for 5 h. We were able to sample honeydew from 6 to 8 colonies on each host plant type (N = 20), with most of the populations represented by two

colony samples. After collection, we froze honeydew foils until analysis. We measured honeydew deposition (drops·cm^{-2}) with a stereo dissecting microscope. We then analyzed the samples for its composition of six different sugars: fructose, glucose, sucrose, trehalose, melezitose and raffinose. These sugars are commonly found in honeydew (Pringle et al., 2014). Details of the analytical method can be found in Mooney et al. (2019). We used multiple analysis of variance (MANOVA) via the 'manova ()' function to analyze variation in the six sugars among host plant treatments. We used densities of honeydew droplets as a covariate in these analyses to account for variation in the amount of honeydew collected on each foil. We performed univariate tests for individual sugar responses using the 'summary.aov ()' function. We also tested for differences in honeydew deposition (drops·cm^{-2}) among host plant types using aphid colony size as a covariate; this model was constructed using the 'lm ()' function from the R base package (R Foundation for Statistical Computing, 2020).

3. Results

3.1. Observational study

We observed multiple biotic and abiotic response associated with snowmelt timing in host plant populations (Fig. 1). Later snowmelt dates were associated with increased soil moisture in June ($b = 0.257 \pm 0.053$ S.E.; t-value = 4.858, $P < 0.001$) and also delayed flowering phenology of host plants ($b = -0.090 \pm 0.007$ S.E; t-value = −11.970, $P < 0.001$). As estimated from pitfall collections, ant abundance decreased in sites with later snowmelt dates ($b = -0.028 \pm 0.003$; z-value = −9.771, $P < 0.001$). Greater numbers of host plants were colonized by aphids in populations with later snowmelt dates ($b = 0.015 \pm 0.005$; z-value = 2.589, $P = 0.010$). Ant abundance on flowering stalks showed a similar response, with greater numbers of ants on flowering stalks in populations with later snowmelt dates ($b = 0.016 \pm 0.002$, z-value = 7.920, $P < 0.001$).

3.2. Water addition experiment

3.2.1. Effects on soil moisture and host plant phenology

Across all dates, soil moisture surrounding host plants varied with host plant type (Type: $F_{2,98} = 3.118$, $P = 0.049$: Fig. 2). High elevation host plants had higher levels soil moisture ($\bar{x} = 14.4 \pm 1.3$ S.E.) than low elevation host plants without added water ($\bar{x} = 12.9 \pm 0.8$ S.E.). However, soil moisture surrounding high elevation host plants were similar to low elevation host plants with added water ($\bar{x} = 14.4 \pm 1.2$ S.E.). Soil moisture also declined over the observation period regardless of host plant type (D.O.Y: $F_{1,98} = 35.062$, $P < 0.001$). This seasonal pattern tended to be modified by host plant type (D.O.Y. X Type:

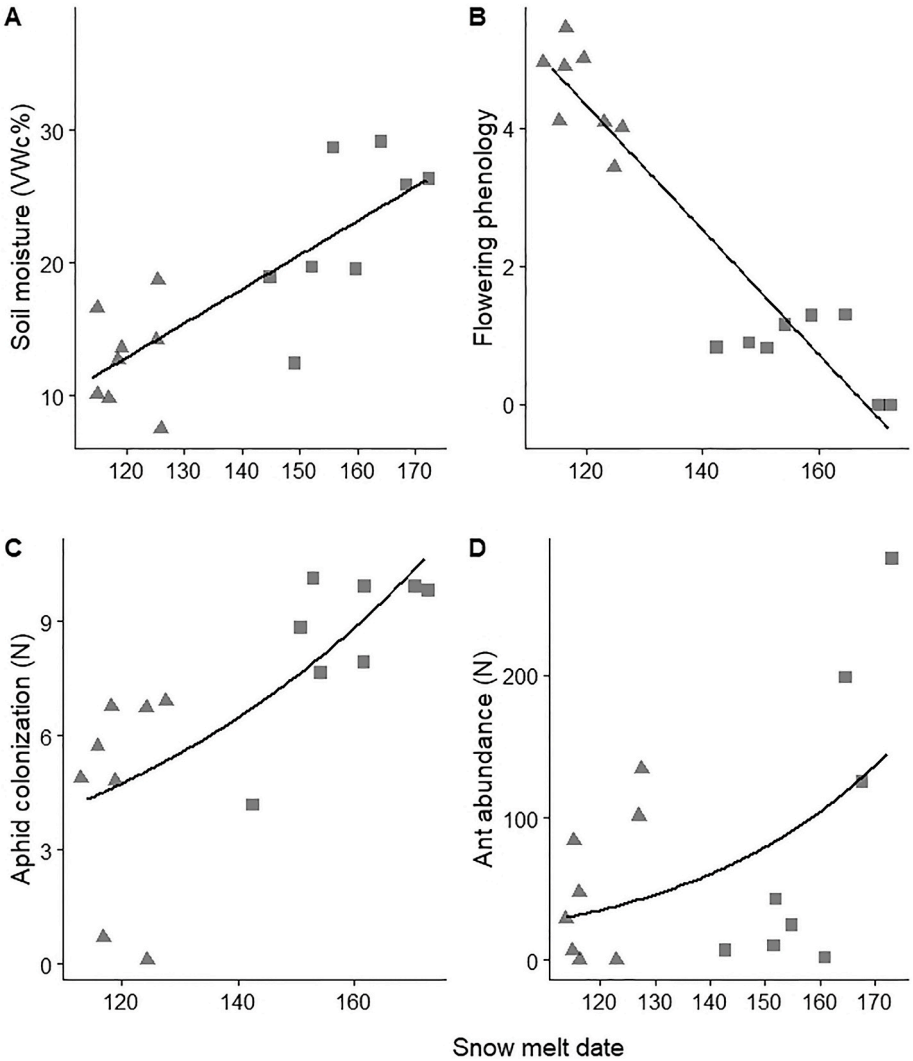

Fig. 1. Associations of snowmelt timing with soil moisture measured in June (A) mean flowering June phenology (B), aphid colonization of *L. porteri* (C) and ant abundance on *L. porteri* (D) observed in eight host plant populations in two years (2018 ▲ and 2019 ■).

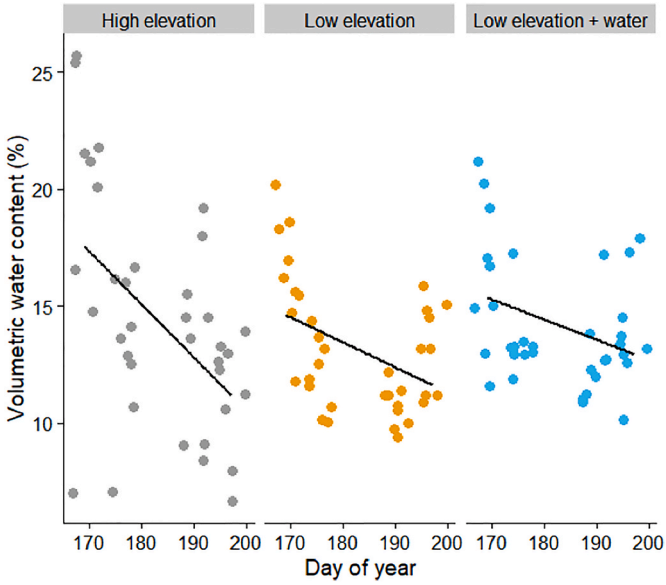

Fig. 2. Volumetric water content (water volume/soil volume = %) surrounding *L. porteri* host plants versus day of year of measurement (ordinal date); points show the mean of three values from three separate host plants, and lines depict trends over time.

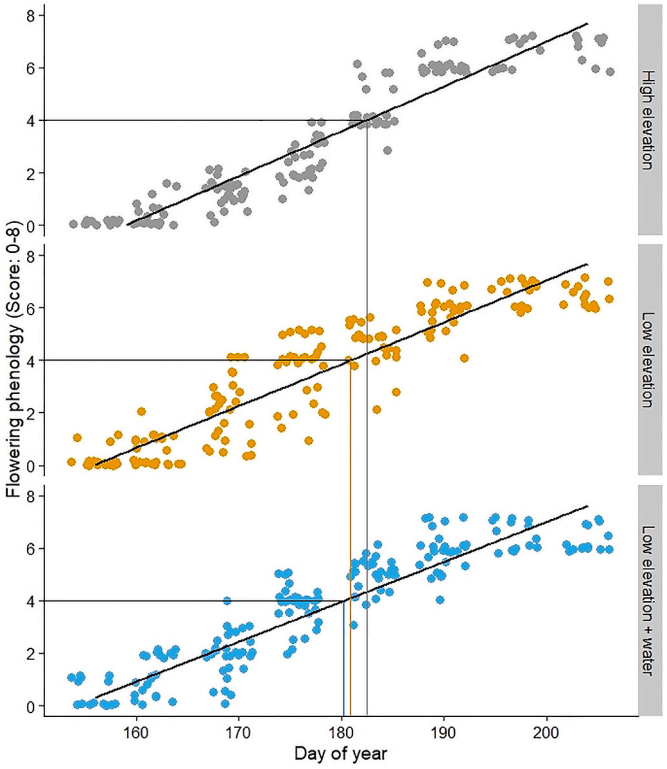

Fig. 3. Flowering phenology of *L. porteri* inflorescences versus day of year of measurement (ordinal date); points show the flowering stage of terminal umbels scored (0–8) using methods described in Robinson et al. (2017). Points were jittered to display individual data points.

$F_{2,98}$ = 3.036, P = 0.053). *Post hoc* tests for heterogeneity of slopes showed a slightly greater rate of soil moisture decline in soils around plants at high elevation (b = −0.221 ± 0.041) than at low elevation where water was added (b = −0.088 ± 0.042), although this difference was not statistically significant (t-ratio = −2.257, P = 0.067). No other *post hoc* comparisons were statistically significant.

Host plant flowering phenology advanced over the measurement period (Fig. 3; D.O.Y.: $F_{1,534}$ = 5551.590, P < 0.001). Overall, host plant flowering phenology at high elevation was delayed relative to host plants at low elevation (Type: $F_{2,338}$ = 8.455, P < 0.001). For example, host plants at low elevation began to flower (model regression score ≥4) by 6-30-18 (ordinal date: 181) whereas host plants at high elevation began to flower the following week 7-2-18 (ordinal date: 183). Changes in flowering phenology over time were also modified by host plant type (Type *X* D.O.Y.: $F_{1,534}$ = 7.336, P < 0.001). *Post hoc* tests for heterogeneity of slopes showed that high elevation plants tended to advance their phenology more quickly (b = 0.173 ± 0.004) than either low elevation watered (b = 0.152 ± 0.004; t-ratio = 3.798, P < 0.001) or unwatered plants (b = 0.159 ± 0.004; t-ratio = 2.450, P = 0.039). Rate of flowering phenology change was similar between low elevation watered and non-watered plants (t-ratio = 1.389, P = 0.347).

3.2.2. Effects on host plant quality

Host plant physiological responses and aphid colony growth reflected differences in quality with host plant type. Both photosystem II efficiency (F_v/F_m) and chlorophyll content (SPAD) declined between measurement dates, but this pattern varied with host plant type (Fig. 4). Across all plant types, F_v/F_m declined from the first to the second measurement date (Day of Year: $F_{1,169}$ = 205.766, P < 0.001). This seasonal decline tended to vary among host plant types (Type *X* D.O.Y.: $F_{2,169}$ = 2.750, P = 0.067). *Post hoc* tests for heterogeneity of slopes showed that this trend was driven by a tendency for declines in low elevation, non-watered plants (b = −1.016 ± 0.100) to be greater than for host plants at high elevation (b = −0.699 ± 0.100; t-ratio = −2.232, P = 0.072). Declines for low elevation watered host plants were similar to those at high elevation (b = −0.771 ± 0.100; t-ratio = 0.505, P = 0.869). We did not observe a significant main effect of host plant type on F_v/F_m (Host plant type: $F_{1,167}$ = 1.764, P = 0.174). Similar patterns emerged for chlorophyll content (SPAD) of leafy bracts. Regardless of plant type, SPAD declined across the two measurement dates (D.O.Y: $F_{1,167}$ = 120.232, P < 0.001). This seasonal decline differed among host plant types (Type *X* D.O.Y.: $F_{2,167}$ = 4.406, P = 0.014). As with F_v/F_m, *post hoc* tests for heterogeneity of slopes showed that this differential response was driven by differences in the rate of SPAD decline between host plants at high elevation (b = −0.241 ± 0.056) and the rate of decline in low elevation without added water (b = −0.476 ± 0.056; t-ratio = 2.964, P = 0.011). No other *post hoc* contrasts indicated significant slope differences (P > 0.05). Overall, SPAD of leafy bracts tended to be higher for host plants at high elevation ($\bar{x}$ = 25.1 ± 1.3) than those on low elevation host plants with ($\bar{x}$ = 21.5 ± 1.3) or without added water ($\bar{x}$ = 23.1 ± 1.3). However, *post hoc* contrasts of mean SPAD among these plant types were not significant (P > 0.05). Leafy bract water content did not vary with host plant type ($F_{2,6}$ = 2.769, P = 0.141). Although not significantly different, mean leaf water contents tended to be higher for host plants at high elevation ($\bar{x}$ = 75.4% ± 1%) than host plants at low elevation with ($\bar{x}$ = 74.0% ± 1%) or without ($\bar{x}$ = 72.8% ± 1%) added water.

Growth of experimental aphid colonies varied with host plant type, also indicating differences in quality (Fig. 5). Regardless of host plant type, the experimental aphid colonies tended to increase in size over time (Day of year: L.R. χ^2 = 2.850, df = 1, P = 0.091). Rate of growth over time varied with host plant type (Type *X* D.O.Y.: L.R. χ^2 = 13.094, df = 2, P = 0.001). *Post hoc* tests for heterogeneity of slopes showed the greatest rates of colony size increase over time on high elevation host plants (b = 0.417 ± 0.109) relative to colonies on low elevation host plants without added water (b = −0.185 ± 0.123; z-ratio = 3.676, P < 0.001). No other *post hoc* slope contrasts indicated significant differences in aphid colony growth with host plant type. Significant differences in overall aphid colony size (Type: L.R.

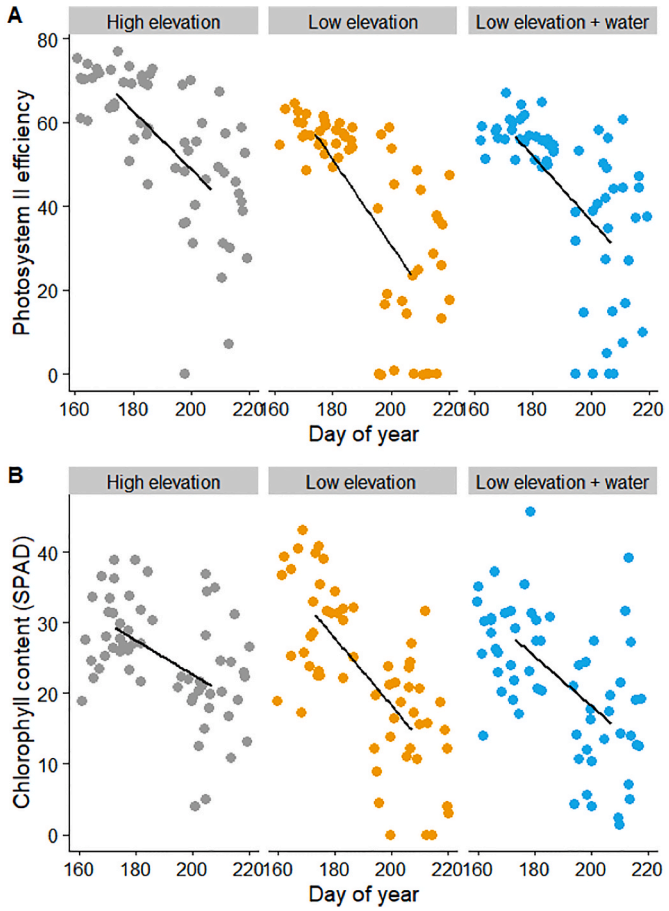

Fig. 4. Declines in photosystem II efficiencies (A) and chlorophyll contents (B) of each host plant type across the experimental period. Measurements were taken on two dates (DOY 174 and 207) and jittered on the plot to show individual points. Individual points represent measurement taken on the leaf bracts of host plants.

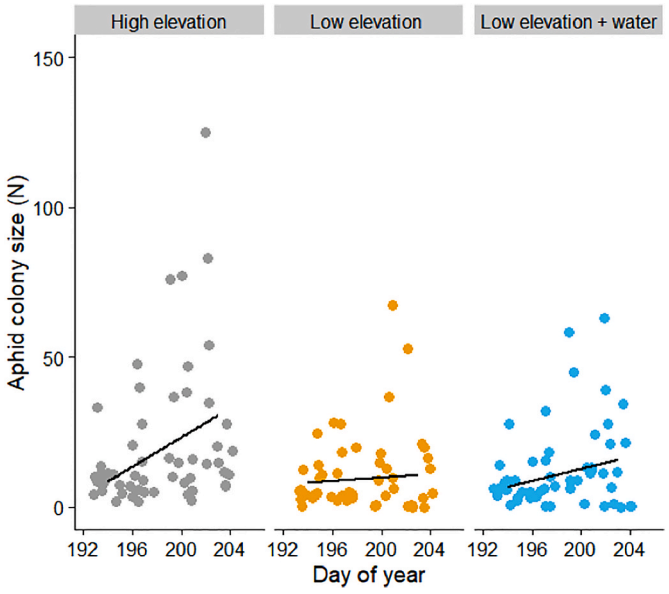

Fig. 5. Growth of experimental aphid colonies reared on each host plant type. Points indicate counts of aphid colonies size across census dates.

$\chi^2 = 6.406$, $df = 2$, $P = 0.041$) reflected that mean aphid colony sizes were generally greater on high elevation host plants than on low elevation host plants with (z-ratio $= 2.184$, $P = 0.074$) or without (z-ratio $= 2.662$, $P = 0.021$) added water.

3.2.3. *Effects on the ant-aphid mutualism*

We counted ants on many host plants, with 30 of 90 plants having ants when no aphid colonies were present (Fig. 6). Notably, most (85%) ants on flowering stalks at high elevation were *Formica rufa*, and most (81%) of ants on flowering stalks at low elevation were *Tapinoma sessile*. Low elevation plants without added water tended to have a greater likelihood of never hosting ants than high elevation plants (z-value $= -2.187$, $P = 0.029$), but no other significant effects were revealed by the zero hurdle model coefficients. When ants did occur, host plants at high elevation tended to have more ants than those at low elevation with added water (z-value $= -1.692$, $P = 0.091$). Among the few plants in low elevation populations with ants ($n = 9$), counts were higher on those without added water (z-value $= 2.104$, $P = 0.035$). No other count model coefficients were significant.

Patterns of ant recruitment to aphid colonies also showed the influence of host plant type. As expected, larger aphid colonies recruited more ants (Aphids: L.R. $\chi^2 = 58.599$, $P < 0.001$). However, this recruitment pattern differed among host plant types (Fig. 7; Aphids X Type: L.R. $\chi^2 = 15.644$, $P < 0.001$). This interaction was driven by differences in recruitment rate between colonies on high elevation host pants relative to those on low elevation host plants with added water (z-ratio $= -3.821$, $P < 0.001$). Specifically, fewer tending ants were found on high elevation host plants as mean colony size increased ($b = 0.005 \pm 0.001$) than were observed on low elevation host plants with added water ($b = 0.016 \pm 0.003$). Host plant type had no overall effect on the number of ants tending colonies (Host plant type: L.R. $\chi^2 = 2.146$, $P = 0.342$).

Host plant type also influenced the benefits exchanged in the ant-aphid mutualism. Sugar composition of aphid honeydew tended to differ with host plant type (Supplemental Table 1). This was primarily due to increased sucrose concentration in honeydew of aphids feeding on either high elevation or low elevation watered host plants relative to aphids on low elevation plants without added water (Fig. 8). Honeydew deposition (drops·cm^{-2}) increased with aphid colony size (Aphids:

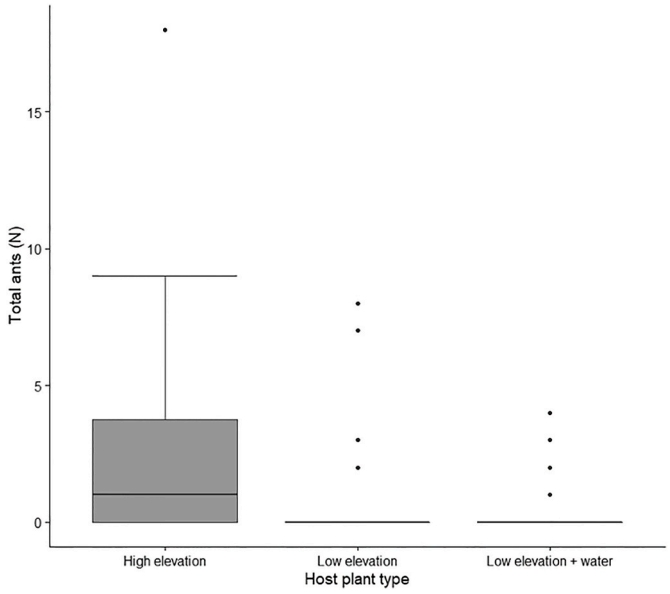

Fig. 6. Cumulative abundance of ants on host plants without aphid colonies in each host plant type.

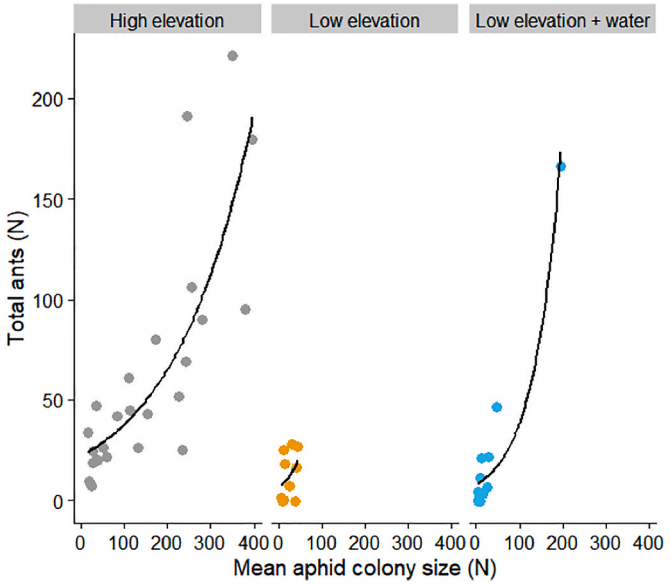

Fig. 7. Cumulative number of ants observed tending aphid colonies on each host plant type. Lines depict general linear models.

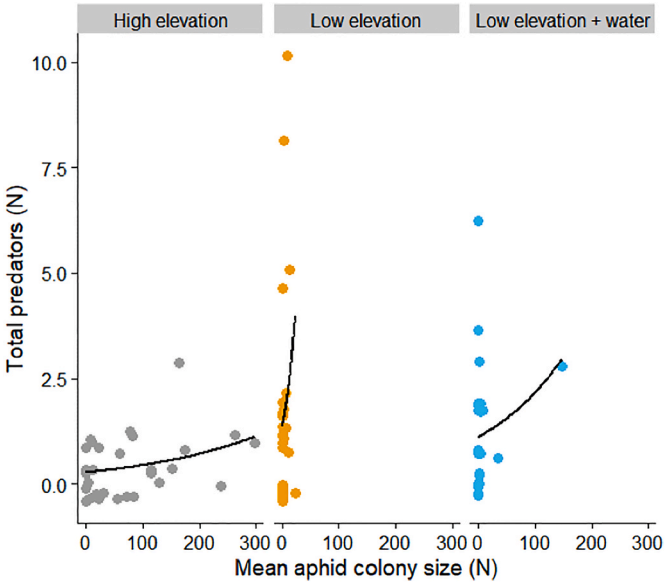

Fig. 9. Cumulative number of predators observed on each host plant type as a function of aphid colony size. Lines depict general linear models.

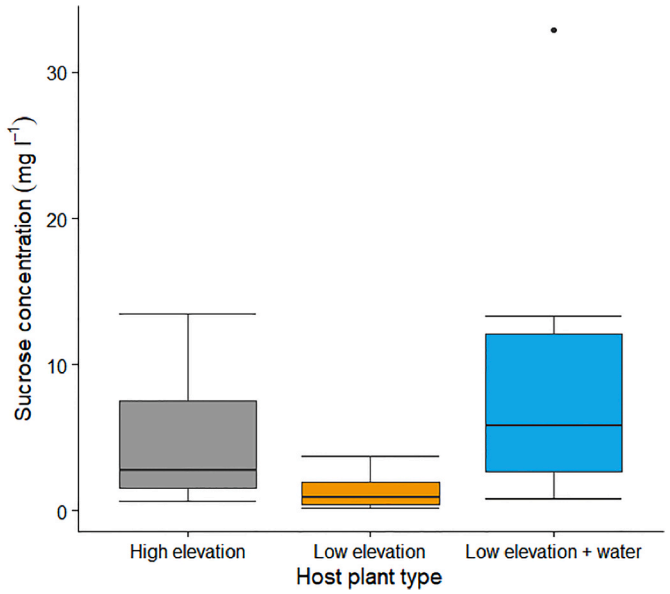

Fig. 8. Concentrations of sucrose from aphid honeydew collected from colonies on each host plant type.

$F_{1,14}$ = 8.743, P = 0.010), but this did not vary with the type of host plants the colonies were feeding upon (Aphids X Type: $F_{2,14}$ = 0.323, P = 0.729). Likewise, there was no overall effect of host plant type on honeydew deposition (Host plant type: $F_{2,14}$ = 1.725, P = 0.214).

We counted aphid predators on half (n = 45) of the host plants we monitored, and host plant type influenced the likelihood of observing aphid predators (Fig. 9). We were more likely to observe zero predators on high elevation host plants relative to host plants at low elevation without added water (z-value = 2.031, P = 0.042). However, the likelihood of observing predators was similar between high elevation host plants and low elevation host plants with added water (z-value = 0.979, P = 0.328). No other significant effects were indicated by the zero hurdle model coefficients. For the subset of plants where predators occurred, we observed significant effects of host plant type on predator abundance. As aphid colony size increased, more predators were observed on low elevation host plants without added water than on high elevation host plants (z-value = 2.140, P = 0.0324) or low elevation host plants with added water (z-value = 2.238, P = 0.025). No other significant effects were indicated by the count model coefficients.

4. Discussion

Our multiyear observational study showed that later loss of snow cover at high elevations was associated with increased early season soil moisture, delayed host plant phenology and increased ant abundance on host plants. To evaluate the influence of early season host plant drought stress on aphid abundance, we combined an elevation gradient with experimental water addition. We matched soil moisture surrounding low elevation host plants to that of high elevation sites during the early summer period, when snow has disappeared and before monsoonal precipitation. Physiological signals demonstrated that adding water early in the season reduced host plant drought stress. Specifically, watered plants at low elevation retained similar levels of photosystem II efficiency and chlorophyll content as at high elevation; these responses are particularly sensitive to protracted periods of drought, as has been shown in other experimental field studies (Resco et al., 2008).

Despite reduced physiological signals of drought stress, aphid colonies on low elevation watered host plants still achieved significantly smaller sizes than at high elevation, suggesting it was not a major mechanism determining the effect snowmelt date on aphid abundances. Instead, our results indicate other mechanisms, specifically host plant phenology, hold more influence over aphid populations. Low elevation watered and unwatered plants initiated flowering and senescence earlier than high elevation plants. Earlier snowmelt date is likely responsible for this effect: snowmelt timing is a key cue in year-to-year variation in flowering phenology for *L. porteri* (Iler et al., 2013). *A. asclepiadis* is likely sensitive to flowering phenology: *A. asclepiadis* colonization sequentially tracks inflorescences of *L. porteri* and two other hosts in our system (Addicott, 1981, 1978). While some aphid species can be so-called 'senescence feeders' (Tariq et al., 2012; White, 2015), this type of tracking corresponds to 'flush feeding', suggesting the importance of matching colonization to high quality host plant stages. Such trophic mismatching is a key effect of climate change for other plant-herbivore systems (Renner and Zohner, 2018) and could explain our results. Relatively few studies have documented such a strong effect

of mismatch between aphid herbivores and their host plants (Bell et al., 2015). Alternatively, this pattern could reflect differences in host plant defenses between high and low elevation. However, such patterns have typically been found over much larger elevational ranges, e.g. 1,000m (Rasmann et al., 2014).

Another mechanism our results suggest may hold significant control over aphid abundances is ant attendance of aphids. Higher ant attendance correlated with higher aphid colony sizes as aphids receive significant predator protection from ants. However, our pitfall traps show ambient ant abundances decreased with increasing elevation, yet high elevation host plants had far more ants on leafy bracts than their low elevation counterparts. This suggests a difference in ant behavior according to our host plant types. Honeydew composition is an important factor in attracting and determining the intensity of ant attendance (Katayama et al., 2013) (Blüthgen and Fiedler, 2004). While honeydew composition did differ between aphids from high elevation and low elevation non-watered plants, it does not account for increased ant attendance at high elevations in comparison to low elevation watered sites with similar honeydew compositions. Honeydew quality appears to only have a mild effect on aphid abundances through mediating ant attendance in this ecosystem. Differential ant species composition between elevations may partially explain differences in recruitment rates.

Ant species composition was significantly different between elevations, likely as a result of higher daytime temperatures at low elevation. *T. sessile,* the most prevalent ant species at low elevation sites, exhibits a large thermal tolerance exceeding 30 °C without any effect on activity levels (Toennisson et al., 2012) Conversely, ants in the Formica group, more prevalent at high elevation sites, reduce activity precipitously after 20 °C (Wiebe and Gow, 2013). This may explain why we see a distribution of ant species across our elevational gradient and may play a role in the effect of host plant type on predator abundance. However, research by Nelson et al. (2019) in this same geographic area shows a reduced effect of ants on aphid abundances with increasing elevation. This, along with our data showing larger aphid colony sizes at high elevations even when ants were excluded, suggests our observations of strong associations of ants with aphid colonies at high elevations may have not had a strong effect on aphid abundances. Regardless, the strength and influence of ant-aphid mutualisms in this ecosystem have been shown to be highly species specific (Addicott, 1979) and justifies further research to clarify the effect of species specificity and elevation on this mutualism.

5. Conclusions

Host plant phenology emerges as the most likely major mechanism by which snowmelt timing exerts control over aphid abundances by inducing a phenological mismatch. Few ecological studies have examined the importance of host plant phenological matching for aphids in the context of climate change (Bell et al., 2015; Blanchard et al., 2019; Tariq et al., 2012). Aphids are notably advancing arrival in the spring (Bell et al., 2015), but our results suggest later season processes mediated by host plant senescence may be a similarly important response to climate change. Effects of host plant drought stress emerge for plant quality and honeydew composition, but are likely only minor mechanisms by which snowmelt affects aphid abundances. The effect of predation on aphids is modified by the behavior and species assemblage of ant mutualists which showed significant variability with elevation. We recommend further study on the influence of snowmelt date on this ant mutualism. Overall, this study highlights the importance of considering multi-trophic interactions when describing the impacts of snowmelt date on insect abundance.

Author contribution

James Den Uyl: Conceptualization, Investigation, Data curation, Formal Analysis, Writing- Original draft preparation, Writing-Reviewing and Editing. Maria Mullins: Writing - Review & Editing, Investigation. Shane Heschel: Conceptualization, Methodology, Resources, Writing-Reviewing and Editing. Emily Mooney: Funding Acquisition, Conceptualization, Formal Analysis, Visualization, Writing-Reviewing and Editing.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This research was supported by a grant from the National Science Foundation Division of Environmental Biology to E.Mooney (NSF DEB: 1655914). This funding source had no role in the design, implementation, writing, or any other aspect of this project. Dr. Emily Mooney is an assistant professor at the Department of Biology at the University of Colorado Colorado Springs. Maria Mullins and James Den Uyl were graduate students at the University of Colorado Colorado Springs for the duration of the study. Dr. Shane Heschel is an associate professor at Colorado College in Colorado Springs. The Rocky Mountain Biological Station also provided a graduate student fellowship to James Den Uyl and had a role in reviewing the design of the project.

Supplemental Table 1

Response	Effect	Df	Pillai	Approx F	Df (n,d)	*P*-value
All sugars	Density	1	0.9364	22.086	6,9	6.63E-05
	Host plant type	2	0.99119	1.6375	12,20	0.1591
	Density *X* Host plant type	2	1.12751	2.1538	12,20	0.06235
	Residuals	14				
Response	Effect	Df	SS	MS	F-ratio	P-value
Fructose	Density	1	967.45	967.45	65.1682	1.23E-06
	Host plant type	2	70.71	35.36	2.3816	0.1288
0	Density *X* Host plant type	2	44.84	22.42	1.5103	0.2547
	Residuals	14	207.84	14.85		
Glucose	Density	1	44.937	44.937	12.9548	0.002903
	Host plant type	2	1.328	0.664	0.1914	0.827905
	Density *X* Host plant type	2	3.708	1.854	0.5344	0.597492
	Residuals	14	48.563	3.469		
Sucrose	Density	1	696.95	696.95	125.8081	2.21E-08
	Host plant type	2	100.86	50.43	9.1032	0.002933
	Density *X* Host plant type	2	244.01	122	22.0232	4.75E-05
	Residuals	14	77.56	5.54		
Trehalose	Density	1	0.08948	0.089476	0.7718	0.3945
	Host plant type	2	0.11656	0.058281	0.5027	0.6154
	Density *X* Host plant type	2	0.14971	0.074856	0.6457	0.5392
	Residuals	14	1.62311	0.115937		
Melezitose	Density	1	9835.4	9835.4	14.8596	0.001751
	Host plant type	2	269.6	134.8	0.2037	0.818113
	Density *X* Host plant type	2	524.4	262.2	0.3962	0.6802
	Residuals	14	9266.4	661.9		
Raffinose	Density	1	1.8354	1.8354	52.9912	4.04E-06
	Host plant type	2	0.15189	0.07594	2.1926	0.1485
	Density *X* Host plant type	2	0.06627	0.03314	0.9567	0.4079
	Residuals	14	0.4849	0.03464		

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