Ecological Entomology

Ecological Entomology (2020), DOI: 10.1111/een.12878

Advanced phenology of intraguild predators shifts herbivore host plant preference and performance

MARIA MULLINS,[1,2] JAMES DEN UYL,[1,2] EMILY CRUZ,[1,2] SAMANTHA TRAIL,[1,3] BENJAMIN DAVIDSON,[1,4] DIANE CAMPBELL[1,5] and EMILY MOONEY[1,2] [1]Rocky Mountain Biological Laboratory, Crested Butte, Colorado, USA, [2]University of Colorado Colorado Springs, Colorado Springs, Colorado, USA, [3]Florida Atlantic University, Boca Raton, Florida, USA, [4]Vassar College, Poughkeepsie, New York, USA and [5]University of California Irvine, Irvine, California, USA

Abstract. 1. The abundance of insect herbivores is mediated by interactions with higher and lower trophic levels. This research asks (i) how phenological change across trophic levels affects host plant quality and selection for aphids, and (ii) what higher trophic level mechanisms drive aphid abundance.

2. *Ligusticum porteri* is a perennial host for the sap-feeding aphid *Aphis asclepiadis* and intraguild mirid predators (chiefly *Lygus hesperus*) in Colorado. We used long-term observational data to discover that aphids and mirids respond differently to phenological cues. These unique responses can impact aphid abundance through changes to host plant selection and quality.

3. We used behavioural choice assays to assess how advanced mirid phenology influences aphid host plant selection. More alates landed and reproduced on mirid-free control plants relative to host plants with prior mirid feeding. However, this preference did not correlate with aphid performance when we compared aphid relative growth rates between treatments. This suggests that advanced mirid phenology would impact aphid populations more through host plant choice, rather than reductions in host quality. The addition of mirids to experimental aphid colonies also demonstrated reduced aphid colony growth via predation.

4. We measured plant cues involved in host selection and found differences in volatile composition between plants with prior mirid feeding compared to control plants, providing the potential for aphids to detect enemy-free space using volatile cues.

Key words. Aphids, climate change, host selection, intraguild predation, natural enemies.

Introduction

Climate change is predicted to alter herbivore abundance through a variety of mechanisms. Most studies that predict insect herbivore abundance have focused on the direct effects of rising temperatures (Deutsch *et al.*, 2008). However, biotic interactions are key determinants of herbivore abundance in nearly all systems (Karban *et al.*, 2017). Both host plant quality (i.e. bottom-up control) and interactions with predators (i.e. top-down control) can be altered by climate change. For example, higher trophic levels may be more sensitive to elevated temperatures via increased metabolic costs and unique demographic traits, allowing for the release of herbivore populations from top-down control (Nelson *et al.*, 2019; Urban *et al.*, 2017). In addition to changes in the abundance of host plants and predators, climate change affects the *timing* of interactions, e.g. phenological mismatch (Both *et al.*, 2009). In some plants, foliar quality declines as the growing season progresses, resulting in a short window for larval development to synchronise with optimal quality host plants (van Asch *et al.*, 2007). Other experiments have found phenological asynchrony leading to *greater* host plant quality (Schwartzberg *et al.*, 2014), suggesting idiosyncratic responses among systems. Phenological matching with natural enemies may be just as important

Correspondence: Maria Mullins, 1420 Austin Bluffs Pkwy, Colorado Springs, CO 80918. Email: mmullins14@gmail.com

© 2020 The Royal Entomological Society

for determining herbivore abundance. For instance, advanced phenology of a predator relative to an insect herbivore could result in reduced temporal overlap, increasing herbivore populations via decreased predation (Donnelly *et al.*, 2011). Host plant quality and natural enemies will likely both determine herbivore abundance and distribution patterns as warming continues.

Climate change effects on interactions with intraguild predators may have a unique potential to impact herbivore populations. Early arrival on host plants by intraguild predators could both induce plant defences and enhance top-down control (Denno *et al.*, 2000). Through these combined effects, intraguild predators may be so-called 'biotic multipliers' for the effects of climate change on ecological communities (Urban *et al.*, 2017). In this study, we examined phenology-mediated changes of intraguild predator pressure on aphids, a widespread group of sap-feeding insect herbivores. Our study system consists of a perennial host plant (*Ligusticum porteri*) and two hemipteran herbivores that colonise its flowering stalks, aphids (*Aphis asclepiadis*), and their intraguild mirid predators, chiefly *Lygus hesperus*. Year-to-year variation in the flowering phenology of the host plant is primarily determined by snowmelt timing (Iler *et al.*, 2013). Importantly, aphid and mirid abundances have shown differential responses to this phenological cue: in years with late snowmelt, aphids colonise nearly all host plant flowering stalks, while mirid abundance is greatest in years with early snowmelt (Robinson *et al.*, 2017). The degree to which these relationships are related to one another is unknown. However, mirids have the potential to alter both the cues aphids use to select host plants and the ability of a host plant to support aphid colonies (Kersch-Becker *et al.*, 2017; Zhang *et al.*, 2019).

Given their role as both predators and competitors, intraguild mirid predators may affect aphid abundance through several mechanisms. For one, aphid colonisation could be reduced if mirids change the cues aphids use to locate *L. porteri* flowering stalks. Aphids use volatile organic compounds (VOCs) to first find their host plant among a sea of green, and then to perceive the quality of a host plant based on complex volatile bouquets (Bruce *et al.*, 2005). The ability of mirids to elicit natural enemy attracting VOCs has been well studied in other systems (Kessler & Baldwin, 2004). However, the ability of aphids to detect the natural enemy presence, through chemical footprints, or by volatile emissions induced by other competitors is unclear (Dicke, 2000; Hatano *et al.*, 2008). Host plant selection, by apterous alates, is well studied due to its implications for aphid colony establishment and success (Wilson & Leather, 2012). Alates face trade-offs as they choose high quality host plants that will support the growth of a colony, while also avoiding natural enemies (Powell *et al.*, 2006). Seeking out enemy-free space to establish colonies may be as important, if not more, than locating a nutritionally superior host (Wilson & Leather, 2012). As competitors, mirid feeding may also directly reduce host plant quality. Like aphids, mirids feed on host plants using piercing-sucking mouthparts, but mirids create symptomatic damage to stems, leaves, and developing inflorescences (Wheeler, 2001). For Lygus bugs (*Lygus* spp.), this damage is in the form of stippling, i.e. necrotic or chlorotic spots (Wheeler, 2001). Early season damage to plant tissues would likely reduce later quality for sap-feeding aphids (Denno *et al.*, 2000). Mirids may also directly affect aphid colony growth through predation. Lygus bugs show so-called life history omnivory, meaning that adult stages and later instars are more likely to be zoophagous than earlier nymphs (Wheeler, 2001). As such, advanced mirid phenology may allow zoophagous stages to overlap with aphid colony establishment and decrease colony growth rates.

We used a long-term observational study and experimental manipulations to determine how advanced phenology of mirids may determine aphid abundance. Our first objective was to assess differential responses to phenological cues across trophic levels using an 8-year observational dataset. We evaluated the response of aphids and mirids to yearly variation in snowmelt date, which is an important phenological cue for host plants and insects in our system (Boggs & Inouye, 2012; Robinson *et al.*, 2017; Mooney *et al.*, 2019). As a part of this objective, we tested how mirid abundance on host plants predicts aphid colonisation and aphid colony growth. We separately evaluated the impacts of adult and nymph mirid abundance on these aphid responses. We hypothesised that if mirid abundance was affecting aphid abundance through their roles as predators, we would see a negative relationship between mirid adult abundance and these aphid responses. If host plant-mediated competition was also a factor, we hypothesised that aphid colonisation and colony growth would likewise relate to mirid nymph abundance. Our second objective was to experimentally test the effects of prior mirid presence on aphid host plant preference, host quality, and volatile emissions. Through behavioural assays, we asked whether winged females would support the enemy-free space hypothesis by choosing to land and initiate colony growth on the plant free of intraguild predators. We then measured experimental aphid colony growth rates to understand competitor-induced changes in plant quality. Growth rates of exclusively sap-feeding insects are commonly used as a measure of host plant quality (Dixon, 1977). Thus, we could test whether alate preference and aphid performance are linked. We then sampled volatile emissions to understand how prior mirid feeding may influence aphid host selection. Lastly, we evaluated how mirids could affect aphid population growth through their roles as predators. We used a field experiment where mirid adults were added to replicate ant-tended aphid colonies. Given that *A. asclepiadis* like many other aphid species is tended by mutualist ants, our objective was to assess mirid impacts on colony growth as they would occur for ant-tended colonies.

Methods

Ligusticum – Aphis – Mirid system

In our study system near Crested Butte, Colorado, the aphid *A. asclepiadis* colonises the umbel inflorescences of *L. porteri* (Apiaceae), a large, perennial herb (Terrell & Fennell, 2009). *A. asclepiadis* is a host-alternating aphid species that overwinters on *Cornus sericea* and migrates to inflorescences in summer (Addicott, 1981; Lagos-Kutz *et al.*, 2016). *A. asclepiadis* has a holocyclic life cycle: females reproduce parthenogenetically in summer months and fall, then they sexually reproduce overwintering eggs that emerge the next spring as the first asexual

generation (Powell & Hardie, 2001). The aphids are polymorphic, producing alate morphs responsible for migration and new colony establishment. Mirid adults and nymphs also exploit host plant umbels. Most of the adults belong to the polyphagous species *L. hesperus* (Hemiptera: Miridae), whose adults feed on the instars of *A. asclepiadis* and other soft-bodied insects (Agusti & Cohen, 2000; Wheeler, 2001; Robinson *et al.*, 2017).

Response of aphids and mirids to snowmelt timing

We used a long-term observational dataset to test for the direct effects of snowmelt timing on the abundance and phenology of aphids and mirids. In brief, it comprises an 8-year (2012–2019) dataset of weekly insect counts from 10 host plants in each of 10 replicate host plant populations near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado ($N = 800$ host plants). Details of the census methods are described by Robinson *et al.* (2017)). We tested for the direct effects of snowmelt timing on two responses for each species: abundance in June (DOY = 173) and maximum abundance achieved in a given study year. Abundance in June signals early season phenology for both species (Robinson *et al.*, 2017). Our response variables were the number of aphid colonies observed per flowering stalk and the number of mirids observed per flowering stalk. Yearly snowmelt date was the date of the first bare ground observed by Billy Barr (see methods in Inouye *et al.*, 2000). We also used this long-term dataset to assess the indirect effects of snowmelt timing on aphid abundance, i.e. how mirid abundance affects the likelihood of aphid colonisation (Y/N) and colony growth. Mirids exhibit life history omnivory such that adults are primarily predators, while earlier instars are phytophagous (Wheeler, 2001). As such, we considered the abundance of adult and instar mirids as separate explanatory variables. Because we began differentiating adult and nymph counts in 2017, these data are host plant observations from three study years ($N = 300$). We separately analysed how adult and nymph abundance in June (DOY = 173) affected the likelihood of colonisation (Y/N). For aphid colony growth, we calculated the maximum relative growth rate (RGR) from counts (X_n) observed across weekly censuses using the formula, $\text{RGR} = \ln(X_2 + 1) - \ln(X_1 + 1))/7$ (Hunt, 1982). For this analysis, we used the abundance of adults and nymphs observed during this growth period as the explanatory variable. We created general linear mixed effects models (glmm) using the *lme4* package with the population as a random effect (Bates *et al.*, 2015). We used the 'lmer()' function for response variables with gaussian-distributed errors and 'glmer()' function for response variables with binomially-distributed errors (Qian, 2017). We used the 'Anova()' function in the *car* package to obtain test statistics and *P*-values for significance ($P < 0.05$) testing (Fox & Weisberg, 2018). This and all the following statistical analyses were performed in R (R Development Core Team, 2018).

Mirid effects on aphid host plant choice and quality

To test the effect of mirids on aphid host plant selection and quality, we experimentally added mirids to host plants. On 6 June 2018, we potted 60 *L. porteri* plants in early phenological stages from five replicate sites near RMBL. Thirty-six plants produced at least one flowering stalk. We protected all flowering stalks with bridal veil mesh bags. We field-collected mirid (*L. hesperus*) adults and placed 10 on the flowering stalks of half ($n = 18$) of the host plants.

When alates appeared on *L. porteri* in the field in mid-July, we began performing pairwise choice trials between mirid-free control plants and plants with prior mirid feeding. One randomly selected plant from each treatment was placed inside an insect chamber (Bugdorm, MegaView Science Education Services Co., Taichung, Taiwan) with a Petri dish containing 10 field-collected alates at plant level (Haahr, 2019). We recorded how many alates landed on each plant after 24–48 h and the number of nymphs produced. We alternated the position of plants within the insect chamber to avoid any positional effects. We performed a total of 19 choice trials. We analysed the behavioural choice assays using the *coin* package in R (Hothorn *et al.*, 2008). Because each group had a zero-inflated Poisson distribution, we used a two-sample permutation test to compare the mean number of alates landing on plants with prior mirid feeding versus mirid-free control plants. We used the same test to measure differences in the number of aphids produced on plants.

To assess the effects of mirids on host plant quality, we experimentally colonised both plant treatments with aphids. On 2 August 2018, we placed 10 mature *A. asclepiadis* aphids on the terminal umbel of 28 host plants ($n = 16$ mirid-free control and $n = 12$ mirid addition). We counted the number of aphids on each umbel every two days for a total of eight days. We analysed differences in colony growth between groups using a repeated measures approach. We used the *lme4* package to create the model with counts as the response variable, plant treatment as the explanatory variable, and plant as a random effect (Bates *et al.*, 2015). 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). Significance testing followed the approach outlined above.

Mirid effects on host plant volatiles

To test for differences in volatile bouquets, we used dynamic headspace techniques to sample floral volatiles from mirid addition and mirid-free controls (Bischoff *et al.*, 2014). Forty-five scent samples were taken on a total of 35 potted plants. Additionally, we sampled 20 ambient air controls both inside and outside of the field greenhouse. We measured soil moisture using a time-domain reflectometry probe (FieldScout TDR 100, Spectrum Technologies, Aurora, Illinois) as a covariate (Campbell *et al.*, 2019). We sampled mirid addition and mirid-free control plants between 4 July and 17 July, 10 days after mirid addition. Mirids on each plant were removed immediately prior to scent collection. All samples were taken on warm days between 09.00 and 16.00 hours.

We cut nylon bags (Reynolds Oven Bags, Reynolds Consumer Products, Lake Forest, Illinois) into 16 cm × 11 cm × 7 cm

© 2020 The Royal Entomological Society,

bags and placed one over the terminal umbel of each plant. Scents accumulated in the bag for 30 min, then were pumped at 100 ml min^{-1} through scent traps using a micro-air sampler (PAS-500 micro air sampler, Supelco, Bellefonte, Pennsylvania) for 15 min following established scent collection methods (Bischoff *et al.*, 2014). The scent traps consisted of 5 mg Tenax TA® absorbent inside a microcapillary tube and enclosed on both ends with silanised glass wool. The scent traps were cleaned before use by soaking in ethanol and then baking in a clean oven at 180 °C. Vegetative controls consisted of two leaf and stem samples. We sampled ambient air controls ($n = 20$) and vegetative controls using the same method.

Scent samples were analysed using thermal desorption gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS (Shimadzu QP 2020, Shimadzu Corporation, Kyoto, Japan). Thermal desorption occurred on a Markes system with a Unity-xr attached to an ultra-auto loader. Each scent trap was initially desorbed for 5 min at 250 °C. The scent trap was then cold trapped at 40 °C for 2 min, then increased 10 °C every minute until reaching 275 °C where it held for 3 min. We used a DB-5 all-purpose, non-polar GC column (Rtx-5MS) with dimensions (30 m/25 mm/0.25 µm). The GC was coupled with an electron ionization quadrupole mass spectrometer, and helium was the carrier gas. We used Shimadzu GCMS Solutions software to assemble data from the GC-MS. Peaks were initially identified using the NIST14 mass-spectra library with a minimum similarity search of at least 70%. We exported peak areas to Excel and used a filtering script in R (Campbell *et al.*, 2019). The script filtered out contaminant compounds by comparing peak areas of floral samples to ambient air controls, and only plant samples that passed the criterion were used in the final dataset. These compounds were then verified as previously published plant volatiles or insect semiochemicals (El-Sayed, 2019). We calculated the abundance of each compound relative to all other verified compounds in each sample, which produced the final dataset for analysis. We classified all verified compounds into compound classes: monoterpenes, sesquiterpenes, benzenoids, nitrogen compounds, and aliphatics, then compared the relative abundance between host plant treatments. We calculated the relative abundance of each compound per treatment to look for composition differences. We used the 'aov()' function to compare these individual compound abundances across treatments. We used the 'cca()' function and the 'anova()' function within the *vegan* package in R to perform a constrained ordination analysis of the final matrix (Oksanen *et al.*, 2017). To build the model, we used plant treatment as the main constraint and soil moisture and the number of flowers as conditional constraints.

Mirid effects on ant-tended aphid population growth

To assess the impacts of mirids on ant-tended aphid colonies, we experimentally added mirids to replicate aphid colonies on host plants. We randomly selected 10 host plants with flowering stalks in each of six different host plant populations ($N = 60$). On 16 July 2019, we used a soft-bristled paintbrush to add 10 field-collected aphid nymphs to the terminal umbels of each flowering stalk. We enclosed the colony in a fine bridal veil mesh bag and excluded crawling insects using tape covered in an insect barrier (Tanglefoot, Scotts Corporation, Marysville, Ohio). The colonies grew for 3 days, after which we replaced the crawling-insect barrier with a 1 cm section of plastic straw, which allowed ants to access the mesh bag. Eleven experimental aphid colonies either failed to establish, had animals chew their mesh bags, or had their flowering stalk snapped, reducing our sample size ($N = 49$). We counted aphids (alates and instars) on 24th July and randomly assigned half of the plants in each population to a mirid addition treatment. For the mirid addition treatment, five adults, field-collected *Lygus hesperus* were added to each bridal veil mesh bag. We repeated aphid counts three more times, replacing the adult Lygus bugs each time when bags were removed. We compared the growth of colonies, during this time using the repeated measured approach as described above for the host plant quality assessment. We also analysed alate production from colonies using this approach. For these analyses, we added a random factor of population. Correction for overdispersion and significance testing followed the same methods outlined above.

Results

Response of aphids and mirids to snowmelt timing

Aphid abundance responded differently to snowmelt timing than did mirid abundance. While aphid abundance in June does not vary with snowmelt date ($\chi^2 = 1.426$, $P = 0.232$), mirids have increased abundance in June of early snowmelt years (Fig. 1a; $\chi^2 = 21.354$, $P < 0.001$). Relative to the latest snowmelt date (DOY: 156) year, we observed 315% more mirids in the June of the earliest snowmelt (DOY: 114) year. Aphids achieved larger overall abundance in years with later snowmelt dates (Fig. 1b; $\chi^2 = 17.518$, $P < 0.001$). We observed 324% more aphids (colonies per flowering stalk) in the year with the latest snowmelt date than in the year with the earliest snowmelt date. Snowmelt timing does not predict overall mirid abundance on host plant flowering stalks ($\chi^2 = 0.003$, $P = 0.960$). From 2017–2019, we observed 192 aphid colonisation events on 300 monitored host plants. Nymph abundance in June did not affect the likelihood of colonisation ($\chi^2 = 1.560$, $P = 0.212$), but adult abundance at this time significantly reduced the likelihood of aphid colonisation (Fig. 2a: $\chi^2 = 4.905$, $P = 0.0268$). With each additional adult mirid, the likelihood of colonisation was reduced by 41%. We observed similar results for colony growth rates. Nymph abundance during colony growth had no effect on growth rates ($\chi^2 = 1.278$, $P = 0.258$), but greater numbers of adult mirids significantly reduced aphid colony growth (Fig. 2b: $\chi^2 = 4.834$, $P = 0.028$). The model predicts that with each additional adult mirid, aphid RGR would be reduced by 20%.

Mirid effects on aphid host plant choice and quality

More *A. asclepiadis* alates selected plants without prior mirid colonisation. Grouping all trials, 40 total alates chose mirid-free control plants while 10 total alates chose host plants with prior

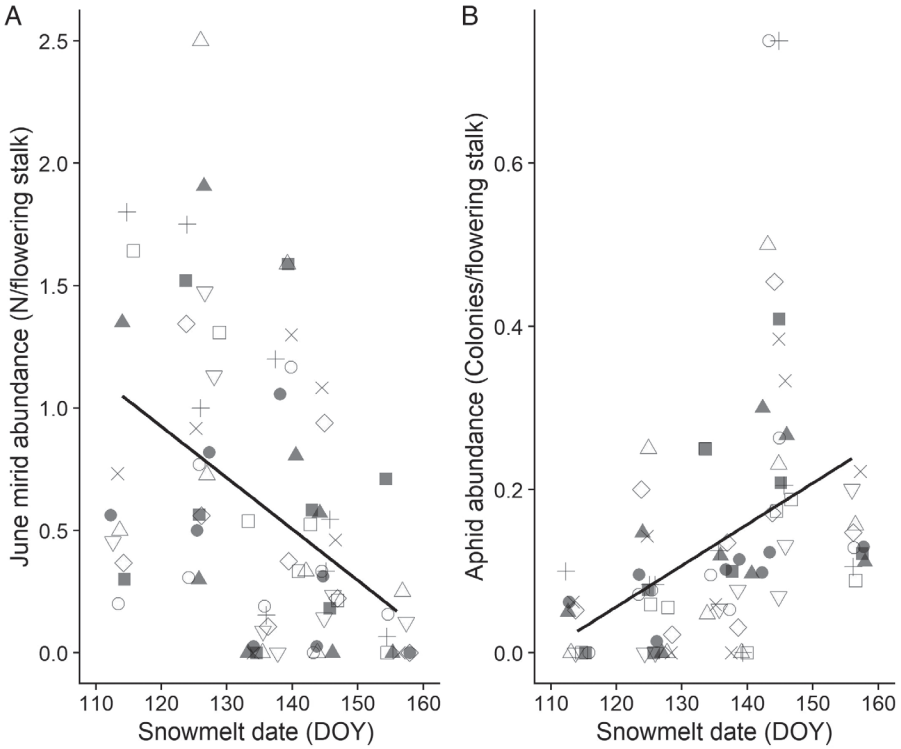


Fig. 1. The response of mirid abundance in June (a) and maximum aphid abundance (b) to snowmelt date; symbols represent 10 different host plant populations; monitoring took place over 8 years (2012–2019). Lines depict predicted values from regression results.

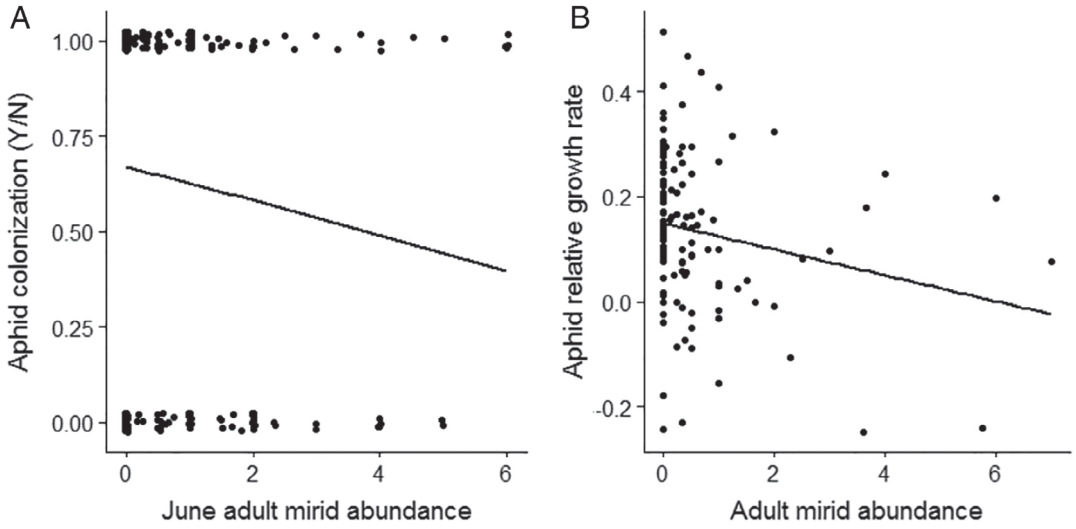


Fig. 2. Relationships between adult mirid abundance in June and the likelihood of aphid colonisation (a) and between adult mirid abundance and relative growth rates of aphid colonies (b); colonisation and relative growth rates observed during three study years (2017–2019). The line for (a) representing predicted values from logistic regression, and the line for (b), represents predicted values from linear regression.

mirid feeding. Averaging across all trials, the mean number of alates choosing mirid-free host plants was (2.12 ± 2.08 SD) and (0.53 ± 0.91 SD) for host plants with prior mirid feeding (Fig. 3a; $z = 2.26$, $P = 0.024$). However, the mean number of aphids produced per alate did not differ between treatments ($z = 1.61$, $P = 0.109$). The overall number of aphids produced after landing was lower for alates on host plants with prior mirid feeding than for mirid-free controls (1.91 ± 0.89 vs. 3.16 ± 5.70, respectively). The alates on mirid-free host plants produced 0.667 aphids *per capita*, while alates on host plants with prior mirid feeding produced 0.588 aphids *per capita*. We did not detect differences in host plant quality through the growth of the

© 2020 The Royal Entomological Society, *Ecological Entomology*, doi: 10.1111/een.12878

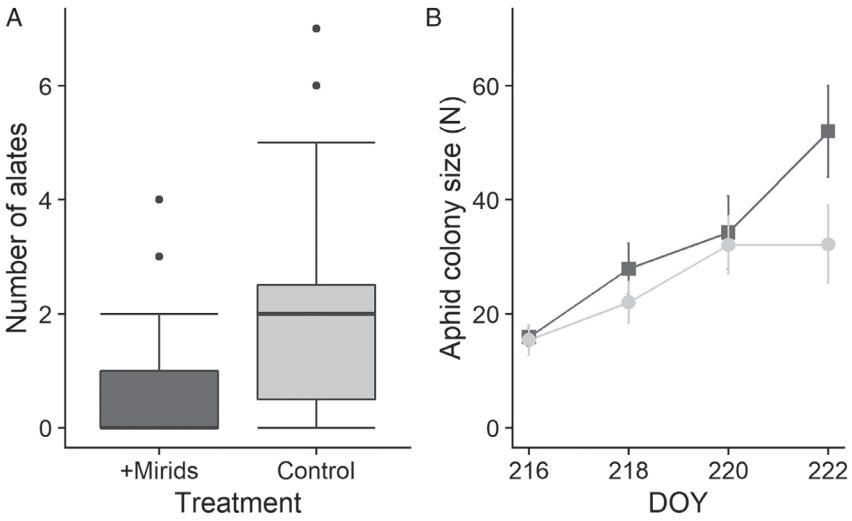


Fig. 3. Comparisons of aphid host plant choice and quality between mirid addition and mirid-free controls. (a) Boxplots showing the preference of *A. asclepiadis* alates to host plant with and without mirids from19 behavioural choice assays. Bold lines show the median of each group, the whiskers indicate maximum and minimum values, and circles show outliers. (bB) Growth of experimental aphid colonies over 1 week.

experimental aphid colonies. The colonies grew over the four censuses (DOY: $\chi^2 = 60.503$, $P < 0.0001$), but this growth did not significantly vary between colonies on mirid-free controls and mirid addition host plants (Fig. 3b: DOY *X* Treatment: $\chi^2 = 1.666$, $P = 0.197$). From the starting point at 10 aphids each, the mean number of aphids per plant at the end of the trial was 30.25 for the mirid addition treatment and 34.06 aphids per plant for the mirid-free control group. Colony size overall did not vary with host plant treatment (Treatment: $\chi^2 = 0.320$, $P = 0.572$).

Mirid effects on host plant volatiles

The floral volatile profile of *L. porteri* was characterised by high relative emissions of monoterpenes. β-myrcene, γ-terpinene, D-limonene, M-cymene, and isoterpinolene made up over 80% of the relative emissions in both plant treatments (Table 1; Fig. 4). The permutation test for the constrained correspondence analysis showed that plant treatment, the main constraint, significantly explained 5.7% of the total variation seen in emissions ($F_{2,30} = 2.383$, $P = 0.009$; Fig. 5). The number of flowers and soil moisture, the environmental conditions, made up an additional 8.7% of the total variation. Each compound class made up a similar proportion of volatiles from both mirid addition and mirid-free control plants (Table 2), and we recorded differences in relative abundances of individual compounds between treatments (Table 1). The compounds with the greatest differences in relative abundances (over 2% between treatments) were oxoisophorone, γ-terpinene, isoterpinolene, β-myrcene, M-cymene, and linalool, which together explained over 70% of the variation seen between treatments (Fig. 5). Only one compound, oxoisophorone, was significantly higher in the mirid-free control group than mirid addition after Benjamini–Hochberg false discovery rates were applied ($P = 0.007$). The simper() function outputs the following cumulative contributions of the most influential compounds to overall dissimilarities between treatment groups: isoterpinolene 17.5%, γ-terpinene 34.8%, β-myrcene 49.0%, D-limonene 59.5%, M-cymene 68.7%, and linalool 73.3%.

Mirid effects on ant-tended aphid population growth

The ant-tended aphid colonies grew over the census period (DOY: $\chi^2 = 77.498$, $P < 0.0001$), but the addition of adult mirids significantly reduced this growth (Fig. 6a; DOY *X* Treatment: $\chi^2 = 5.077$, $P = 0.024$). At the end of the census period, aphid colonies on host plants without mirids were 108% larger in mean size than those with added mirids. There was a similar trend for mirid addition to reduce the number of alates observed across the census period (Fig. 6b; DOY *X* treatment: $\chi^2 = 2.757$, $P = 0.097$). We did not find an overall effect of mirid addition on colony size (treatment: $\chi^2 = 2.313$, $P = 0.128$), although mirid addition significantly reduced the number of alates these colonies produced (treatment: $\chi^2 = 6.866$, $P = 0.009$). Colonies without mirids produced 137% more alates than colonies with mirids. There was a trend for more alates to be produced by colonies over the census period (DOY: $\chi^2 = 3.220$, $P = 0.073$).

Discussion

Our long-term observational data suggest that advanced phenology of intraguild predators has negative effects on both host plant colonisation and subsequent colony growth. Importantly, we see these effects when we examine the abundances of adult mirids, although nymph abundance has no apparent effect on aphid colonisation or growth. This suggests mirids are impacting aphid abundance through their roles as predators, given that adult mirids are more likely to be zoophagous (Agusti &

Table 1. Results table from the volatile analysis.

Compound	Biosynthetic class	CAS number	Mirid addition	Mirid-free control
γ-Terpinene	Monoterpene	99-85-4	30.76	26.19
β-Myrcene	Monoterpene	123-35-3	20.86	16.80
D-Limonene	Monoterpene	5989-27-5	13.32	13.73
M-Cymene	Benzenoid	535-77-3	11.42	8.44
Isoterpinolene	Monoterpene	586-63-0	9.37	15.50
α-Thujene	Monoterpene	2867-05-02	3.18	2.59
M-Cymen-8-ol	Monoterpene	5208-37-7	2.68	2.17
3-Carene	Monoterpene	13466-78-9	2.03	0.55
p-Mentha-1,5,8-triene	Monoterpene	21195-59-5	1.47	1.26
β-Bisabolene	Sesquiterpene	495-61-4	1.01	0.32
trans-β-Ocimene	Monoterpene	3779-61-1	0.77	1.59
Calamanene	Sesquiterpene	483-77-2	0.74	0.95
Isoelemicin	Benzenoid	0487-12-7	0.73	0.52
Linalool	Monoterpene	78-70-6	0.44	3.68
α-Terpinyl acetate	Monoterpene	80-26-2	0.41	0.37
β-Ocimene	Monoterpene	13877-91-3	0.29	0.54
Oxoisophorone	Irregular terpene	1125-21-9	0.13	3.50
Alloocimene	Monoterpene	7216-56-0	0.12	0.17
Amyl acetate	Aliphatic	628-63-7	0.09	0.34
Lilac aldehyde	Monoterpene	53447-45-3	0.07	0.40
Carveol	Monoterpene	99-48-9	0.05	0.06
β-Isophorone	Irregular terpene	0471-01-02	0.02	0.04
β-Copaene	Sesquiterpene	18252-44-3	0.02	0.02
Anisole	Benzenoid	100-66-3	0.01	0.01
Pentadecanone	Aliphatic	2345-28-0	0.01	0.06
β-Caryophyllene	Sesquiterpene	87-44-5	0.01	0.03
Ethylbenzene	Benzenoid	100-41-4	0.00	0.03
Methyl salicylate	Benzenoid	119-36-8	0.00	0.16

Verified compounds are identified by its Chemical Abstract Service (CAS) number, the biosynthetic class which it belongs to, and its relative percent abundance among both plant treatments.

Cohen, 2000; Wheeler, 2001). Aphid population decreases in early snowmelt years may be a straightforward result of higher predation. Experimental results also suggest that the pattern from the long-term study is largely due to the role of mirids as predators, rather than through effects on host plant quality. We detected impacts on aphid colony growth and alate production when mirids were added to colonies. However, when we allowed mirids to feed on host plants prior to aphids, we did not detect a similar effect on colony growth.

We see greater abundances of mirids on host plant flowering stalks in June during the early snowmelt years. Aphids do not show a similar response in June. Mechanisms behind these unique responses could relate to diverging life histories. For example, the mirid *L. hesperus* overwinter as adults in diapause among leaf and plant litter, while A. *asclepiadis* overwinter as eggs on woody secondary host plants (Beards & Strong, 1966; Addicott, 1978). Upon snowmelt in spring, adult mirids would be able to reproduce and rapidly increase population sizes. We may also be seeing the influence of advanced host plant flowering phenology. Mirids are avid feeders on floral resources in umbels (Wheeler, 2001), attracting them to flowering hosts in large numbers (Pan *et al.*, 2013). The flowering of host plants in June brought on by early snowmelt would attract mirids at this time. Early increases in mirids are associated with decreases in the later establishment and growth of aphid colonies. Mirids attracted by floral resources may

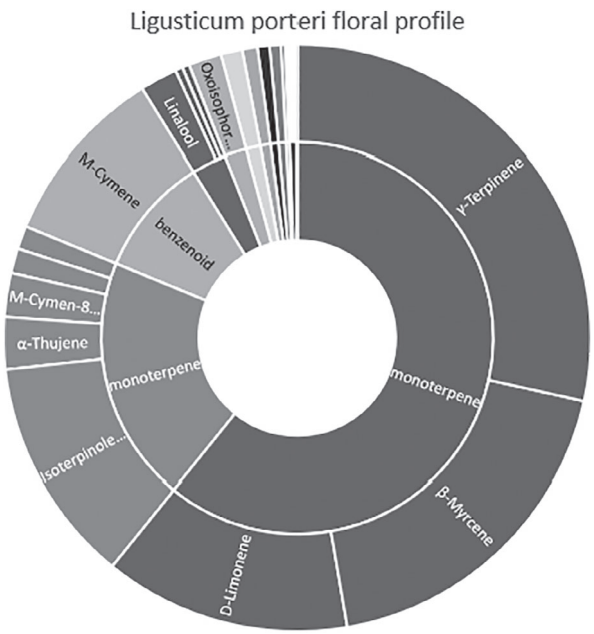


Fig. 4. Pie chart displaying the relative abundance of chemical compounds found in *L. porteri*. The outer ring shows individual compounds, and the inner ring classifies each compound according to its biosynthetic class.

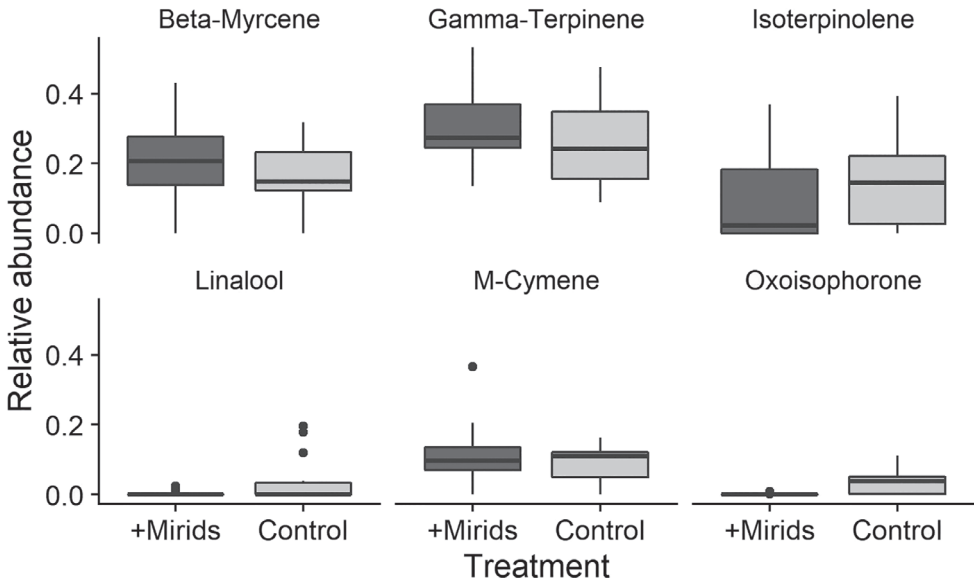


Fig. 5. Boxplots displaying the six chemical compounds that had the highest differences in relative composition among plant treatments.

then subsequently prey on alates. Similar attractive effects of flowering plants is a key component of biological control by mirids in agricultural systems (e.g. Zhu *et al.*, 2014).

Our results also suggest that *A. asclepiadis* support the enemy-free space hypothesis during host selection. Their ability to detect and avoid *L. hesperus* when possible would be advantageous in finding an enemy-free host to start a colony, especially in years with high mirid abundance. However, other studies have demonstrated that this detection ability creates a trade-off and the potential for herbivores to end up on hosts with lower nutritional quality, which can result in slower colony growth (Wilson & Leather, 2012). Still, dealing with competitor-induced effects, such as low nitrogen content in phloem sap, maybe less fraught than choosing a plant dense with natural enemies as "it is better to compete for food than to have no food at all" (Dicke, 2000). Choosing a host plant with fewer mirid predators may be especially impactful for colony reproduction, i.e. alate production. Our experimental results show that – even when tended by ants – aphid colonies with mirids present produce fewer alates. Alate production is often a density-dependent response, so larger colony sizes in mirid-free colonies likely result in more alates (Applebaum & Heifetz, 1999). With predator pressure, alates can increase the prevalence of aphid colonies as they seek out enemy-free space in a host plant population if enemy-free plants are available (Ríos Martínez & Costamagna, 2017). Enemy-free space may be unavailable in years with early snowmelt and high mirid abundance. This would effectively reduce the number of aphid colonies within a host plant population. Having fewer colonies makes local extinction at this scale more likely (Ríos Martínez & Costamagna, 2017), which is the pattern we observe in our long-term data.

Our analysis of floral volatiles suggests the signals aphids could be using to locate enemy-free host plants. To the best of our knowledge, this study is the first characterisation of the floral volatile profile for *L. porteri*, despite several studies of chemotypic variation of roots (e.g. Smith *et al.*, 2018). The floral volatile profile of *L. porteri* is similar to other *Apiaceae* species, where large amounts of monoterpenes (up to 90%) are characteristic across genera (Borg-Karlson *et al.*, 1993). Specifically, large relative amounts of limonene, γ-terpinene, 3-carene, and myrcene are found in multiple *Apiaceae* species similar to *L. porteri* (Borg-Karlson *et al.*, 1993). We also found the impacts of mirid herbivory on the volatile profile of host plant flowering stalks. After correcting for multiple comparisons, only one compound – oxoisophorone – was significantly greater in mirid-free controls than on plants with mirids. This suggests mirid feeding *inhibits* the release of this volatile, which is a constituent of floral scent in a few other plant species (Raguso *et al.*, 2003; Chess *et al.*, 2008) and is a potent attractor of butterfly and moth pollinators (Andersson *et al.*, 2002). Most studies document how herbivore damage *increases* emissions of volatile compounds (Vet & Dicke, 1992; Dicke & van Loon, 2000). Oxoisophorone is a norisoprenoid derived from β-carotene (Winterhalter & Rouseff, 2001). Mirid feeding causes damage to inflorescences and leaves characterised by chlorosis and stippling, i.e. brown or white spots (Wheeler, 2001). Mirid damage could be impacting either the pigment precursor or the synthesis

Table 2. Shows the relative abundance of each chemical compound class for both plant treatments, grouping all verified compounds.

	Mirid addition	Mirid-free control
Relative percent of biosynthetic class in each treatment		
Monoterpenes	85.82	85.59
Benzenoids	12.16	9.15
Sesquiterpenes	1.77	1.32
Irregular terpenes	0.16	3.54
Aliphatics	0.09	0.40

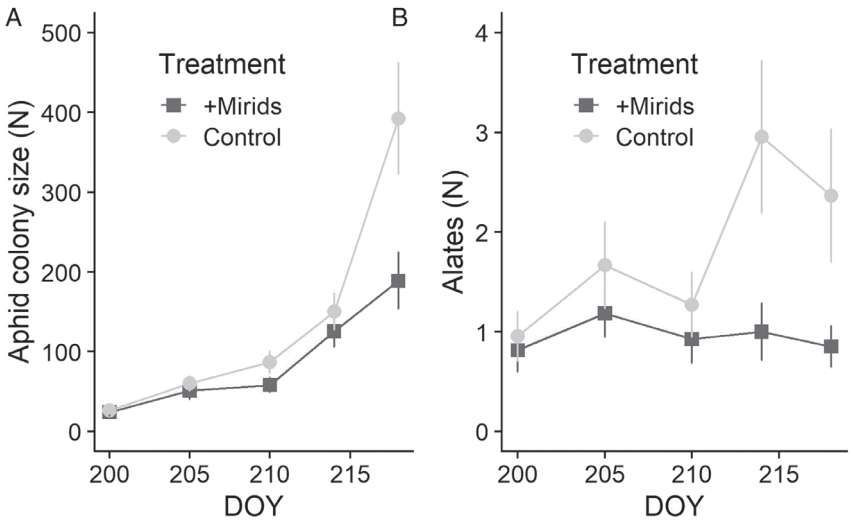


Fig. 6. Response of experimental aphid colony growth to mirid addition across the eight-day census period. (a) Aphid colony size (*n*) of colonies with mirid addition compared to controls. (b) Alate production from aphid colonies with and without mirid addition.

pathway, which relies on intact cell membranes (Cheng *et al.*, 2007). Mirid damage reduces nectar in damaged inflorescences, also demonstrating the impacts on floral resources (Samocha & Sternberg, 2010). Other compounds – β-myrcene, γ-terpinene, M-cymene, D-limonene, and linalool – showed responses to mirid herbivory, although these changes were not significant ($P > \alpha$) after adjustment for multiple comparisons. Despite the statistical similarity of volatile bouquets between host plants with and without mirid herbivory, aphids demonstrated remarkable specificity for mirid-free plants. This is especially relevant when it comes to intraguild predators, in which case aphids may avoid plants based on cues from competitors and potentially decreased plant quality, or avoid the direct threat of an enemy-dense space (Dicke, 2000). Future work could use Y-tube choice trials to determine which specific volatile cues would replicate this pattern.

Conclusions

Aphids, which importantly make up the bottom of many terrestrial food webs, will experience the effects of climate change both directly through changes in temperature, and additionally through changes in the timing and overlap with interacting species as our study exemplifies. Unique responses across taxa to phenological cues such as earlier snowmelt can have cascading effects on important processes such as host plant selection. Our results support other research in host plant-herbivore-predator systems that top-down effects from higher trophic levels mediate the relationship between herbivores and their hosts directly through predation and indirectly through changes in VOCs. This work supports the notion in ecology that a multi-trophic approach is needed when examining the effects of global warming, phenological change, and cascading effects on herbivore populations.

Acknowledgements

This research was funded by the National Science Foundation with a grant awarded to Emily Mooney (NSF Award ID #: 1655914). Graduate student work was also partially funded by the Colorado Native Plant Society Marr Fund, the University of Colorado, Colorado Springs Graduate Research Stipend, and the Rocky Mountain Biological Laboratory Graduate Research Fellowship. The research was completed at the Rocky Mountain Biological Laboratory with help from the RMBL Research Committee and Jennie Reithel. We also thank Robert Raguso for co-leading an NSF-funded workshop at RMBL on volatile analysis [DBI: 1624073]. Emily Cruz, Charlotte DiBiase, Alexander Mitchell, and Alex Shapiro provided field research help for the multiyear observational study. The authors declare no conflict of interest.

References

Addicott, J. (1981) Synonymy of *Aphis heraclella* Davis 1919 with *Aphis helianthi* Monell 1879 (Homoptera: Aphididae). *The Canadian Entomologist*, **113**, 167–169.

Addicott, J.F. (1978) Niche relationships among species of aphids feeding on fireweed. *Canadian Journal of Zoology*, **56**, 1837–1841.

Agusti, N. & Cohen, A. (2000) *Lygus hesperus* and *L. lineolaris* (Hemiptera: Miridae), phytophages, zoophages, or omnivores: evidence of feeding adaptations suggested by the salivary and midgut digestive enzymes. *Journal of Entomological Science*, **35**, 176–186.

Andersson, S., Nilsson, L.A.A., Groth, I. & Bergström, G. (2002) Floral scents in butterfly-pollinated plants: possible convergence in chemical composition. *Botanical Journal of the Linnean Society*, **140**, 129–153.

Applebaum, S.W. & Heifetz, Y. (1999) Density-dependent physiological phase in insects. *Annual Review of Entomology*, **44**, 317–341.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Beards, G.W. & Strong, F.E. (1966) Photoperiod in relation to diapause in *Lygus hesperus* knight. *Hilgardia*, **37**, 345–362.

Bischoff, M., Jürgens, A. & Campbell, D.R. (2014) Floral scent in natural hybrids of Ipomopsis (Polemoniaceae) and their parental species. *Annals of Botany*, **113**, 533–544.

Boggs, C.L. & Inouye, D.W. (2012) A single climate driver has direct and indirect effects on insect population dynamics. *Ecology Letters*, **15**, 502–508.

Borg-Karlson, A.K., Valterová, I. & Nilsson, L.A. (1993) Volatile compounds from flowers of six species in the family Apiaceae: bouquets for different pollinators? *Phytochemistry*, **35**, 111–119.

Both, C., van Asch, M., Bijlsma, R.G., van den Burg, A.B. & Visser, M.E. (2009) Climate change and unequal phenological changes across four trophic levels: constraints or adaptations? *The Journal of Animal Ecology*, **78**, 73–83.

Bruce, T.J., Wadhams, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science*, **10**, 269–274.

Campbell, D.R., Sosenski, P. & Raguso, R.A. (2019) Phenotypic plasticity of floral volatiles in response to increasing drought stress. *Annals of Botany*, **123**, 601–610.

Cheng, A.-X., Lou, Y.-G., Mao, Y.-B., Lu, S., Wang, L.-J. & Chen, X.-Y. (2007) Plant Terpenoids: biosynthesis and ecological functions. *Journal of Integrative Plant Biology*, **49**, 179–186.

Chess, S.K.R., Raguso, R.A. & Lebuhn, G. (2008) Geographic divergence in floral morphology and scent in *Linanthus dichotomus* (Polemoniaceae). *American Journal of Botany*, **95**, 1652–1659.

Denno, R.F., Peterson, M.A., Gratton, C., Cheng, J., Langellotto, G.A., Huberty, A.F. *et al.* (2000) Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology*, **81**, 1814–1827.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. *et al.* (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, **105**, 6668–6672.

Dicke, M. (2000) Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology*, **28**, 601–617.

Dicke, M. & van Loon, J.J. (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, **97**, 237–249.

Dixon, A. F. G. (1977) Aphid ecology: life cycles, polymorphism, and population regulation. *Annual Review of Ecology and Systematics*, **8**, 329–353.

Donnelly, A., Caffarra, A. & O'Neill, B.F. (2011) A review of climate-driven mismatches between interdependent phenophases in terrestrial and aquatic ecosystems. *International Journal of Biometeorology*, **55**, 805–817.

El-Sayed AM (2019) *The Pherobase: Database of Pheromones and Semiochemicals* [WWW Document]. URL https://www.pherobase.com [accessed on 2019].

Fox, J. & Weisberg, S. (2018) *An R Companion to Applied Regression*. Sage Publications, Thousand Oaks, California.

Haahr, M. (2019) Random.org [WWW document]. URL https://www.random.org/ [accessed on 2019].

Harrison, X.A. (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, **2**, e616.

Hatano, E., Kunert, G., Michaud, J.P. & Weisser, W.W. (2008) Chemical cues mediating aphid location by natural enemies. *European Journal of Entomology*, **105**, 797–806.

Hothorn, T., Hornik, K., Van De Wiel, M. A., & Zeileis, A. (2008) Implementing a Class of Permutation Pests: the Coin Package. *Journal of Statistical Software*, **28**, 1–23.

Hunt, R. (1982) *Plant Growth Curves. The Functional Approach to Plant Growth Analysis*. Edward Arnold Ltd, London, U.K.

Iler, A., Inouye, D., Hoye, T.T., Miller-Rushing, A.T., Burkle, L.A. & Johnston, E.B. (2013) Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate. *Global Change Biology*, **19**, 2348–2359.

Inouye, D.W., Barr, B., Armitage, K.B. & Inouye, B.D. (2000) Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences*, **97**, 1630–1633.

Karban, R., Grof-Tisza, P. & Holyoak, M. (2017) Wet years have more caterpillars: interacting roles of plant litter and predation by ants. *Ecology*, **98**, 2370–2378.

Kersch-Becker, M.F., Kessler, A. & Thaler, J.S. (2017) Plant defences limit herbivore population growth by changing predator-prey interactions. *Proceedings. Biological Sciences*, **284**, 20171120.

Kessler, A. & Baldwin, I.T. (2004) Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*. *Plant Journal*, **38**, 639–649.

Korner-Nievergelt, F., Roth, T., Felten, S. von, Guélat, J., Almasi, B. & Korner-Nievergelt, P. (2015) *Bayesian Data Analysis in Ecology Using Linear Models with R, BUGS, and Stan*. Academic Press, Cambridge, Massachusetts.

Lagos-Kutz, D., Favret, C., Giordano, R. & Voegtlin, D.J. (2016) The status of the members of the *Aphis asclepiadis* species group (Hemiptera: Aphididae) in The United States of America. *Annals of the Entomological Society of America*, **109**, 585–594.

Mooney, E., Davidson, B., Den Uyl, J., Mullins, M., Medina, E., Nguyen, P. *et al.* (2019) Elevated temperature alters an ant-aphid mutualism. *Entomologia Experimentalis et Applicata*, **167**, 891–905.

Nelson, A.S., Pratt, R.T., Pratt, J.D., Smith, R.A., Symanski, C.T., Prenot, C. *et al.* (2019) Progressive sensitivity of trophic levels to warming underlies an elevational gradient in ant–aphid mutualism strength. *Oikos*, **128**, 540–550.

Oksanen, A.J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., *et al.* (2017).

Pan, H., Lu, Y., Wyckhuys, K.A.G. & Wu, K. (2013) Preference of a polyphagous Mirid bug, *Apolygus lucorum* (Meyer-Dür) for flowering host plants. *PLoS One*, **8**, e68980.

Powell, G. & Hardie, J. (2001) The chemical ecology of aphid host alternation: how do return migrants find the primary host plant? *Applied Entomology and Zoology*, **36**, 259–267.

Powell, G., Tosh, C.R. & Hardie, J. (2006) Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, **51**, 309–330.

Qian, S.S. (2017) *Environmental and Ecological Statistics in R*, 2nd edn. CRC Press, Taylor & Francis Group, Boca Raton, Florida.

R Development Core Team. (2018) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Viena, Austria.

Raguso, R.A., Levin, R.A., Foose, S.E., Holmberg, M.W. & McDade, L.A. (2003) Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in Nicotiana. *Phytochemistry*, **63**, 265–284.

Ríos Martínez, A.F. & Costamagna, A.C. (2017) Dispersal to predator-free space counterweighs fecundity costs in alate aphid morphs. *Ecological Entomology*, **42**, 645–656.

Robinson, A., Inouye, D.W., Ogilvie, J.E. & Mooney, E.H. (2017) Multitrophic interactions mediate the effects of climate change on herbivore abundance. *Oecologia*, **185**, 181–190.

Samocha, Y. & Sternberg, M. (2010) Herbivory by sucking mirid bugs can reduce nectar production in *Asphodelus aestivus* Brot. *Arthropod-Plant Interactions*, **4**, 153–158.

Schwartzberg, E.G., Jamieson, M.A., Raffa, K.F., Reich, P.B., Montgomery, R.A. & Lindroth, R.L. (2014) Simulated climate warming alters phenological synchrony between an outbreak insect herbivore and host trees. *Oecologia*, **175**, 1041–1049.

Smith, B., Lowe, L., Owens, J. & Mooney, E. (2018) Chemotypic variation in oshá (*Ligusticum porteri*) in Colorado, USA. *Journal of Applied Research on Medicinal and Aromatic Plants*, **10**, 34–40.

Terrell, B. & Fennell, A. (2009) Oshá (bear root) *Ligusticum porteri* JM Coult. & Rose var. porteri. *Native plants journal*, **10**, 110–118.

Urban, M.C., Zarnetske, P.L. & Skelly, D.K. (2017) Searching for biotic multipliers of climate change. *Integrative and Comparative Biology*, **57**, 134–147.

van Asch, M., van Tienderen, P.H., Holleman, L.J.M. & Visser, M.E. (2007) Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biology*, **13**, 1596–1604.

Vet, L.E.M. & Dicke, M. (1992) Ecology of Infochemical by natural enemies in a Tritrophic context use chemical information in Tritrophic interactions. *Annual Review of Entomology*, **37**, 141–172.

Wheeler, A.G. (2001) *Biology of the Plant Bugs (Hemiptera: Miridae): Pests, Predators, Opprotunists*. Cornell Univeristy Press, Ithaca, New York.

Wilson, M.R. & Leather, S.R. (2012) The effect of past natural enemy activity on host-plant preference of two aphid species. *Entomologia Experimentalis et Applicata*, **144**, 216–222.

Winterhalter, P. & Rouseff, R. (2001) *Carotenoid-Derived Aroma Compounds: an Introduction*, pp. 1–17. ACS Publications, Washington, DC.

Zhang, N.X., van Wieringen, D., Messelink, G.J. & Janssen, A. (2019) Herbivores avoid host plants previously exposed to their omnivorous predator *Macrolophus pygmaeus*. *Journal of Pest Science*, **92**, 737–745.

Zhu, P., Lu, Z., Heong, K., Chen, G., Zheng, X., Xu, H. *et al.* (2014) Selection of nectar plants for use in ecological engineering to promote biological control of Rice pests by the predatory bug, *Cyrtorhinus lividipennis*, (Heteroptera: Miridae). *PLoS One*, **9**, e108669.

Accepted 7 April 2020

Associate Editor: Christopher Hassall

© 2020 The Royal Entomological Society,