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Effects of Long-term Experimental Warming on Aphid Density in the Field

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ABSTRACT: Global warming is generally predicted to increase the intensity of herbivore pressure on plants. Support for this prediction often comes from short-term studies, or studies conducted in controlled laboratory settings. We examined the effect of long-term experimental warming on an aphid-sagebrush interaction (*Obtusicauda coweni* and *Artemisia tridentata*) in natural field plots in the Rocky Mountains. In no year did we find support for the prediction that warming increased aphid abundance or population growth. In fact, warming decreased aphid density on sagebrush in one year, tended to decrease aphids in a second year, and had no effect in a third year. In enclosures that excluded predators, warming decreased aphid population growth by an amount consistent with observed field density differences. Warming increased sagebrush carbon:nitrogen (C:N) ratio and plant size, but there was no significant correlation between these variables and aphid growth or density. In a separate snow-manipulation experiment in unwarmed plots, the timing of snowmelt did not affect aphid density. In conclusion, warming reduced or did not affect aphid density in each of three years, but this effect could not be explained by differences in plant size, bulk C:N ratio, predation, or snowmelt date. Our results suggest that long-term studies within a natural community context may provide counterexamples to the prediction that warming will increase herbivore pressure on plants.

KEY WORDS: aphid, *Artemisia tridentate*, carbon:nitrogen ratio, drought stress, global warming, herbivory, *Obtusicauda coweni*, plant quality, plant phenology, population growth rate, Rocky Mountains, sagebrush, tritrophic interactions

Global mean surface temperatures have increased by approximately 0.6°C in the last century, the largest 100-year increase for the last 1000 yrs (Houghton *et al.,* 2001). The global mean surface temperature is predicted to increase by another 1.4 to 5.8°C by 2100 (IPCC, 2001). The effects of climate change on species' distribution and abundance could involve not only direct effects on each species in an ecosystem but also effects on species interactions (Jensen *et al.,* 1992; Ayres, 1993; Coley, 1998; Davis *et al.,* 1998a; Davis *et al.,* 1998b). Plant-herbivore interactions are of particular interest because of their agricultural importance, as well as their potential to affect ecosystem nutrient and carbon fluxes (e.g., Frost and Hunter, 2004) and hence feed back to affect atmospheric CO_2. A variety of field, microcosm and laboratory studies generally conclude that insect abundance increases with rising temperatures (reviewed in Cannon, 1998; Bale *et al.,* 2002), and furthermore that temperature is

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the major factor in global climate change that directly affects insect herbivores (Bale *et al.,* 2002). Although insect responses to global climatic change will vary, the effect of global warming on plant-insect interactions has generally been predicted to increase the intensity of herbivore pressure on plants (Cannon, 1998; Coley, 1998; Wilf and Labandeira, 1999; Bale *et al.,* 2002).

Aphids (Hemiptera: Aphididae) are ubiquitous insect herbivores whose growth and abundance could increase, decrease, or be unaffected by global warming. Temperature was tightly linked with the density of aphids or other sucking insects in two modelling studies based on insect survey data (Zhou *et al.,* 1997; Whittaker and Tribe, 1998); both studies found that higher temperatures increased insect density or abundance. However, increased temperature could also decrease growth of some aphid species, depending on their thermal requirements and host specificity (Bale *et al.,* 2002). The outcome of species interactions is often context-dependent (e.g., Thompson and Cunningham, 2002; Gomez, 2003; Bezemer and van Dam, 2005), and temperature could have many indirect impacts on aphid populations via changes in plant phenology or quality (Holopainen and Kainulainen, 2004), increased natural enemy activity (Ayres, 1993) or by non-additive interactions with defensive compounds (Stamp *et al.,* 1994; Yang *et al.,* 1996). Global warming may also lead to changes in soil moisture and therefore plant xylem pressure potential. For example, in montane regions that receive most of their annual precipitation as snow in winter, earlier snowmelt induced by global warming is likely to result in drier summer conditions. Moreover, summer potential evaporation rates are likely to be higher, further decreasing soil moisture levels. To assess the importance of these many possible biotic and abiotic mechanisms for the dynamics of aphids and other insect herbivores, long-term field studies are necessary.

We studied the effect of increased temperature on the aphid *Obtusicauda coweni* Hunter feeding on big sagebrush, *Artemisia tridentata* Nutt. (Asteraceae), using an ongoing warming experiment initiated in 1991 in the Rocky Mountains of Colorado. Our experimental warming treatment causes the range of microclimate effects expected under real climate warming, including warmer foliage temperatures, earlier snow melt, and warmer and drier soil conditions (Harte *et al.,* 1995), but it does not include changes in precipitation or ambient CO_2. We determined how warming affected aphid density over 3 yrs (1996–1998) and measured impacts on growth rate in one of these years. Additional observations, manipulative experiments and chemical analyses were used to explore some of the mechanisms that could mediate effects of warming on aphid density, including plant size, whole-plant carbon:nitrogen ratio, and phenological differences caused by an earlier snowmelt date.

Materials and Methods

STUDY AREA: Our experiments took place at the Rocky Mountain Biological Laboratory, Colorado, USA (38°53′N, 107°02′W, elevation 2920 m). The warming experiment consisted of ten 3 m × 10 m plots in an ungrazed montane meadow with 2 treatments alternating in space, heated and control ($n = 5$ per treatment). Heated plots have been warmed continuously since January, 1991, with overhead infrared heaters providing a heat flux set to 22 W/m^2 since 1993 (see Harte *et al.,* 1995). The heaters are suspended approximately 1.5 m over treatment plots by towers and cables, have reflective shields designed to provide uniform radiation, and remain on

24 hours per day throughout the year. The heaters shaded less than 2 watts/m^2 from the heated plots and were not placed over control plots. The plots span the slope of a small moraine hill and include a warmer, drier upper zone and a cooler, moister lower zone. This study focused on the dry upper zone because the strongest effects of the warming treatment on soil microclimate, biogeochemistry, and plant growth occur there (Harte and Shaw, 1995; Harte *et al.,* 1995; Saleska *et al.,* 1999).

Soil moisture and temperature data have been collected by monitors every 2 hr all year at 5, 12, and 25 cm depths in 3 locations per plot since establishment. Melt dates are recorded for each plot annually. A detailed analysis of melt dates, soil moisture, and soil temperature over 1991 and 1992 showed that heating advanced snowmelt by about 1 wk, increased soil summer temperatures by up to 3°C, and reduced summer soil moisture levels by about 25% compared to control plots. Responses also varied with season, time of day, weather conditions, and location within each plot (Harte *et al.,* 1995). Other studies have shown that in heated compared to control plots, nitrogen mineralization increases by approximately 0.14 g/m^2-d (Shaw and Harte, 2001), plant phenology of at least 5 species, including *A. tridentata*, advances (Price and Waser, 1998; Dunne, 2000), relative water content and water potential in *A. tridentata* decreases (Loik and Harte, 1997), there is lower above-ground forb biomass and higher above-ground shrub biomass (Harte and Shaw, 1995; De Valpine and Harte, 2001; Perfors *et al.,* 2003), and the carbon budget may shift to create a net carbon loss (Saleska *et al.,* 1999). Further details about the site are described elsewhere (Harte *et al.,* 1995).

To demonstrate that heating influenced plant phenology and soil microclimate during the years of this study (1996–1998), we present a brief analysis of melt date, soil temperature, and soil moisture. Temperature and moisture data were averaged over day, date, depth and location to produce one data point per plot per year. MANCOVA (Scheiner, 1993) was used to analyze the effect of treatment with plot as a gradient (covariate) in each year. In 1998, moisture data were lost from 4 plots due to failed dataloggers. Moisture data are not included in the MANCOVA for this year and are instead analyzed with separate ANCOVA because MANCOVA drops entire replicates with any missing data. Heated plots differed significantly from control plots in 1996 and 1997, and marginally in 1998 (Appendix 1; MANCOVA: 1996: Wilks' $\lambda = 0.21$, $F_{3,5} = 6.33$, $P = 0.037$; 1997: Wilks' $\lambda = 0.14$, $F_{3,5} = 10.44$, $P = 0.014$; 1998: Wilks' $\lambda = 0.38$, $F_{2,6} = 4.92$, $P = 0.054$). Examination of univariate analyses revealed that heating significantly advanced melt date by 9–12 days each year and significantly increased soil temperature by about 1.5°C in 2 of 3 yr. Soil moisture was consistently lower in heated plots, but not significantly so (Appendix 1).

STUDY SYSTEM: *Artemisia tridentata* (hereafter "sagebrush") is a perennial, and the dominant shrub in the dry zone of our plots and over large regions of the western USA (Barbour and Billings, 2000). *Obtusicauda coweni* (hereafter "aphid") is a common and variable species occurring throughout the western United States that feeds on sagebrush, particularly flowering stems (Robinson and Halbert, 1989).

STATISTICAL ANALYSIS—GENERAL POINTS: The unit of replication in this study was the plot. Individual stems within plants were not considered independent units, and neither were plants within the same plot. Data were averaged within plant and then within plot to create one independent data point per plot, and all analyses for warming effects were ANOVA with warming treatment as the main effect and with *n*

= 5 unless otherwise specified. Repeated measures analysis was not considered appropriate here for data taken over multiple years because we did not sample the same plants in plots each year, and different responses were used in 1996 compared to 1997 and 1998 (see *Effect of Warming on Aphid Density*). Therefore, we analyzed data separately for each year (von Ende, 2001). Analysis details for each experiment are provided with the methods below.

Effect of Warming on Aphid Density

In 1996 we surveyed aphids in the warming experiment on 24 July, 2 August and 9 August by counting aphids on every tenth flowering stalk in a 2 m × 2 m square in the dry zone of each plot (12.5 ± 1.09 stalks/plant (mean ± standard error), range 3–28). Relative abundances of aphids among plots were similar on each date in 1996 (correlations of ranked abundances across plots: $n = 10$, $r > 0.91$, $P < 0.0002$ for all comparisons between weeks 1, 2 and 3), so in 1997 and 1998 we surveyed aphids only once (on 16 August and 10 August, respectively), but more thoroughly. We counted the number of flowering stalks with and without aphids on 4 sagebrush bushes in the dry zone (77.1 ± 14.60 stalks/plant, range 4–417). We also counted aphids on up to 5 infested stalks per bush. Because plot was the unit of replication, we averaged data over stems and then plants to produce 1 measure per plot.

For 1997 and 1998, we tested the effect of warming on aphid density per infested stalk, proportion of stalks infested, and overall density (aphids per total stalks). The 1996 data allowed us to estimate only the last of these quantities. All density measures were ln(density+1) transformed for analysis. Proportional data were arcsine-square root transformed. Because there were several plots with 0 aphids in 1996, we used a Wilcoxon 2-sided rank-sum (non-parametric) test for an effect of warming on aphid abundance (Zar, 1996).

Effect of Warming on Population Growth

We conducted an enclosure experiment in 1997 to determine whether warming affected local population growth. On 22 July, we transported alates from non-experimental sagebrush to 3 uninfested flowering stems on each of 4 plants per plot, and confined the alates on the target stalk with a fine mesh organdy bag. Although mesh bags can increase temperature and humidity, a comparison of paper, plastic, pellon, and fabric mesh bags (i.e., bridal veil) showed that mesh bags have the least effects on microclimate compared to unbagged controls (Wyatt *et al.,* 1992). On 1 August, we replaced alates that had died (20 out of 60 in control plots and 24 out of 60 in warmed plots; $\chi^2 = 0.57$, $P = 0.45$) and accounted for the shorter time period for growth in the equation below. Ultimately, 56 out of 60 alates in each treatment survived and were used in analysis.

We counted aphids in each bag on 23 August to estimate population growth rate, r. We used the density-independent population growth equation $\ln(N_t/N_0) = rt$ (Odum and Barrett, 2005), where N_t is the final number of aphids, $N_0 = 1$ is the initial number of aphids, and t is time in days. We calculated an average r for each plot by averaging individual values of r over all stems on which alates survived. We analyzed the effect of treatment on aphid growth using a one-way ANOVA with treatment as the main effect and plot as the unit of replication ($n = 5$) (Zar, 1996).

WARMING EFFECTS MEDIATED BY PLANT SIZE OR CARBON:NITROGEN RATIO: We collected 1 uninfested flowering stalk for chemical analysis from each of the 4 study plants per plot on 9 August 1997. These samples were dried at 60°C for 2 days, ground to powder using a ball and mill, and submitted for nitrogen and carbon measurement using a combustion gas analyzer (UC DANR lab, Pella, 1990). Measuring nitrogen and carbon in phloem would have been ideal since aphids are phloem-feeders, but was beyond the scope of this study. We tested for warming effects on carbon, nitrogen, and carbon:nitrogen (C:N) ratio. We also determined whether aphid density and growth were correlated with C:N ratio within treatment (using plants as the unit of replication) to determine if C:N ratio was a possible mechanism explaining aphid performance.

Because of previous evidence that sagebrush grows faster in warmed plots than in control plots (Harte and Shaw, 1995; Perfors *et al.,* 2003), we tested for a warming effect on plant size (measured as the number of flowering shoots) and correlated plant size with aphid growth and density within treatments, using plants as replicates.

Effect of Snowmelt Date on Aphid Density

We studied the effect of snowmelt date on aphid abundance in 1997 using 3 meadow sites located at elevations of 2770 m, 2940 m, and 3190 m (see Dunne, 2000; Dunne *et al.,* 2003 for full description of sites and manipulation). Each site had 10 4 m × 4 m experimental plots. On 5 plots per site, snow was shoveled in mid spring, advancing snowmelt by about 7 days. Snow removal began when snow pack was about 1 m deep, and ended several weeks later when the tops of *Artemisia* shrubs were exposed. Snow was removed from a 5 × 5 m square in the middle of each plot, and snow depth was reduced by less than one half on any given day. These manipulations provided an earlier snowmelt date without the direct effects on soil temperature and moisture brought about with heaters. However they did cause a slight decrease in summer water availability because of a longer growing season and reduced volume of snowmelt water (Dunne *et al.,* 2003).

We surveyed aphids in a 2 m × 2 m area of each plot on 7 August at the lower site, 25 August at the middle site, and 26 August at the upper site. Because the lower site was sampled at an earlier date than the other two, temporal differences may have been confounded with the 'site' effect. However, our main goal was to determine whether advanced snowmelt would produce the same effects as experimental warming across a range of situations, which could include variation in both site and date. In each sample we counted the proportion of sagebrush flowering stems with aphids and counted aphid abundance on a random sample of up to 10 infested stems. We averaged these data within each plot and tested for an effect of snowmelt advancement on aphid density using ANOVA with site and treatment as main effects (Zar, 1996).

Results

We found that warming significantly decreased aphid density in 1997 but not in 1996 or 1998 (Fig. 1). In 1996, there were on average three times as many aphids per stalk in control compared to warmed plots (Fig. 1c), but several plots had no aphids, severely limiting statistical power (Wilcoxon 2-sided rank-sum test, $P > 0.9$). Aphids were abundant in 1997, and every control plot had more aphids per stalk than every warmed plot (Wilcoxon two-sided rank-sum test, $P = 0.0122$). There were over 3

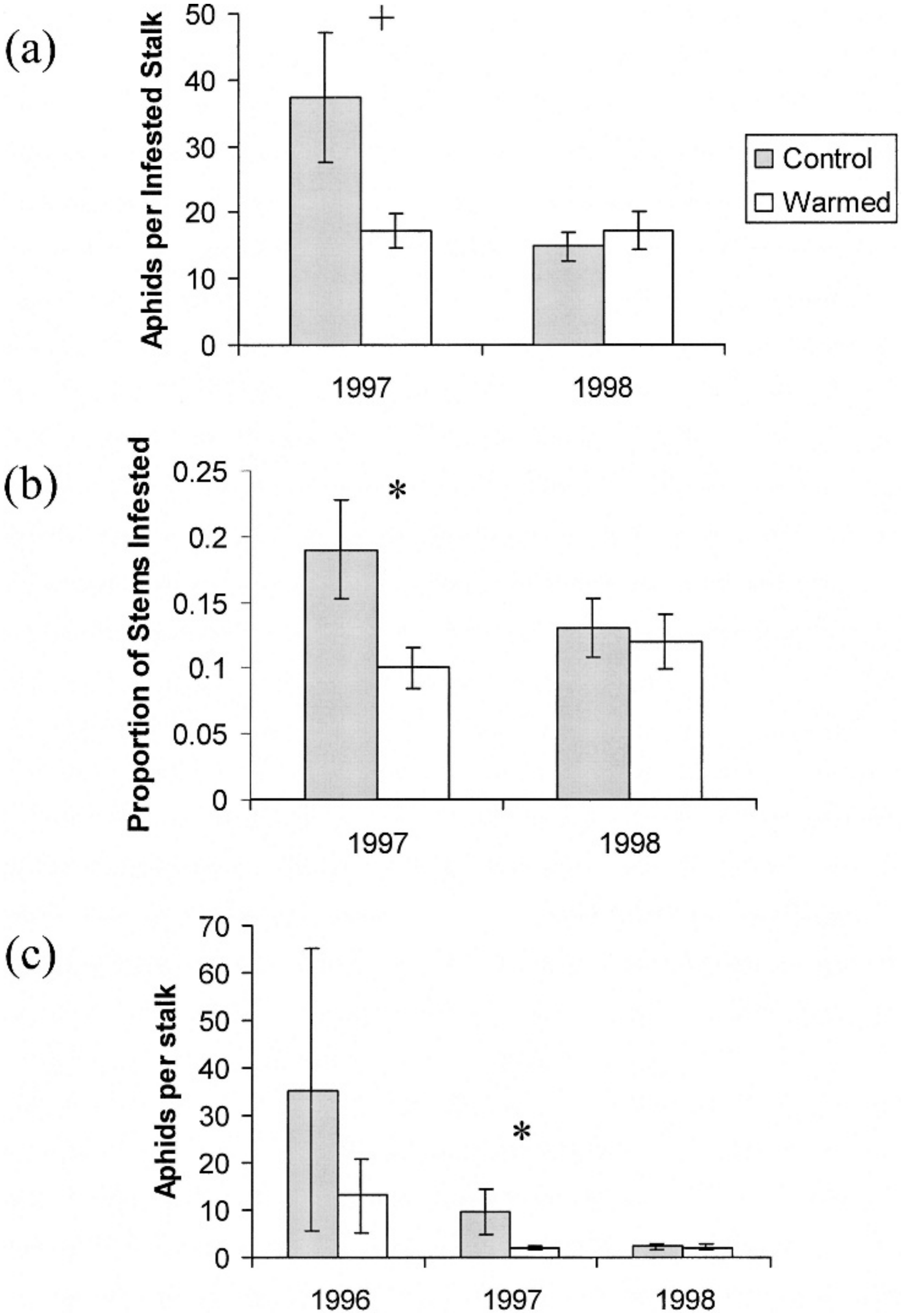

Fig. 1. Effect of experimental warming on (a) density per infested stalk, (b) proportion of stalks infested, and (c) density per stalk. Data from 1996 include zero counts from several plots. Error bars represent one standard error; + $P < 0.10$, * $P < 0.05$.

times as many aphids per stalk in control compared to warmed plots ($F_{1,8} = 8.05$, $P = 0.022$), the proportion of stems infested was almost twice as high ($F_{1,8} = 5.54$, $P = 0.046$), and there was a marginally higher abundance per infested stalk ($F_{1,8} = 4.91$, $P = 0.058$). However, in 1998 none of these measures was significantly different between treatments ($F_{1,8} < 0.4$, $P > 0.5$ for all).

In the 1997 enclosure experiment, aphid population growth in enclosures was marginally reduced in warmed plots compared to control plots ($F_{1,8} = 4.88$, $P = 0.06$, Fig. 2a), an effect consistent with the effect of warming outside enclosures.

Sagebrush in warmed plots had lower nitrogen content, higher C:N ratio, and equivalent carbon content compared to control plots (Fig. 2b; nitrogen: $F_{1,8} = 6.36$, $P = 0.04$; C:N ratio: $F_{1,8} = 6.89$, $P = 0.03$; carbon: $F_{1,8} = 0.01$, $P = 0.55$). However, there was no significant relationship between aphid growth and C:N ratio in either control or warmed plots ($F_{1,15} = 2.7$, $R^2 = 0.15$, $P = 0.12$, and $F_{1,16} = 1.9$, $R^2 = 0.11$, $P = 0.19$, respectively) or between aphid densities and C:N ratio ($P > 0.5$ for all density measures). Sagebrush plants were marginally larger on warmed plots than control plots ($F_{1,8} = 4.96$, $P = 0.06$; Fig. 2c), but there was no relationship between plant size and aphid growth for control plots, warmed plots, or both together ($R^2 < 0.03$, $P > 0.4$ for all analyses).

In the snowmelt manipulation experiment, aphid abundance was highest at the middle elevation, moderate at the low elevation and lowest at the high elevation ($F_{2,24} = 17.48$, $P = 0.0001$ for proportion stems infested; $F_{2,24} = 9.12$, $P = 0.0011$ for aphids per infested stem). However, there were no significant effects of snowmelt manipulation or site*manipulation interactions for any measure of aphid abundance ($F < 1.1$, $P > 0.35$ for all), suggesting that changes in snowmelt date alone were not responsible for the effects on aphid growth or density.

Discussion

Global warming is predicted to increase herbivore pressure on plants (Cannon, 1998; Coley 1998; Wilf and Labandeira, 1999; Bale *et al.,* 2002). However, we examined the effect of experimentally manipulated warming over the long term in a field setting and found no evidence that warming increased aphid growth or abundance. In fact, warming decreased aphid growth and abundance in at least 1 of 3 yrs. By contrast, short-term field and microcosm studies (under 1 yr duration) have generally supported the prediction that experimental warming increased growth rates (Strathdee *et al.,* 1993; Hill and Hodkinson, 1995) and/or abundance (Strathdee *et al.,* 1993; Bezemer *et al.,* 1998). Additionally, a long-term observational study found that temperature explained 75% of the variation in abundance of a sucking insect at a high-altitude site, and a 1-year manipulation increased insect abundance by 157% with a 1°C change in mean temperature (Whittaker and Tribe, 1998). Other examples of long-term manipulative studies are rare. Two studies have experimentally manipulated warming over 2 to 3 yrs, and found contrasting responses in the abundance of sucking insects in response to experimental warming. Whereas the psyllid *Strophingia ericae* increased markedly within months of the heating manipulation (Miles *et al.,* 1997), the Auchenorrhyncha only increased in response to winter warming when it was combined with supplemented summer rainfall (Masters *et al.,* 1998). Furthermore, one of these studies found that temperature increased psyllid abundance and advanced psyllid phenology in the first but not the

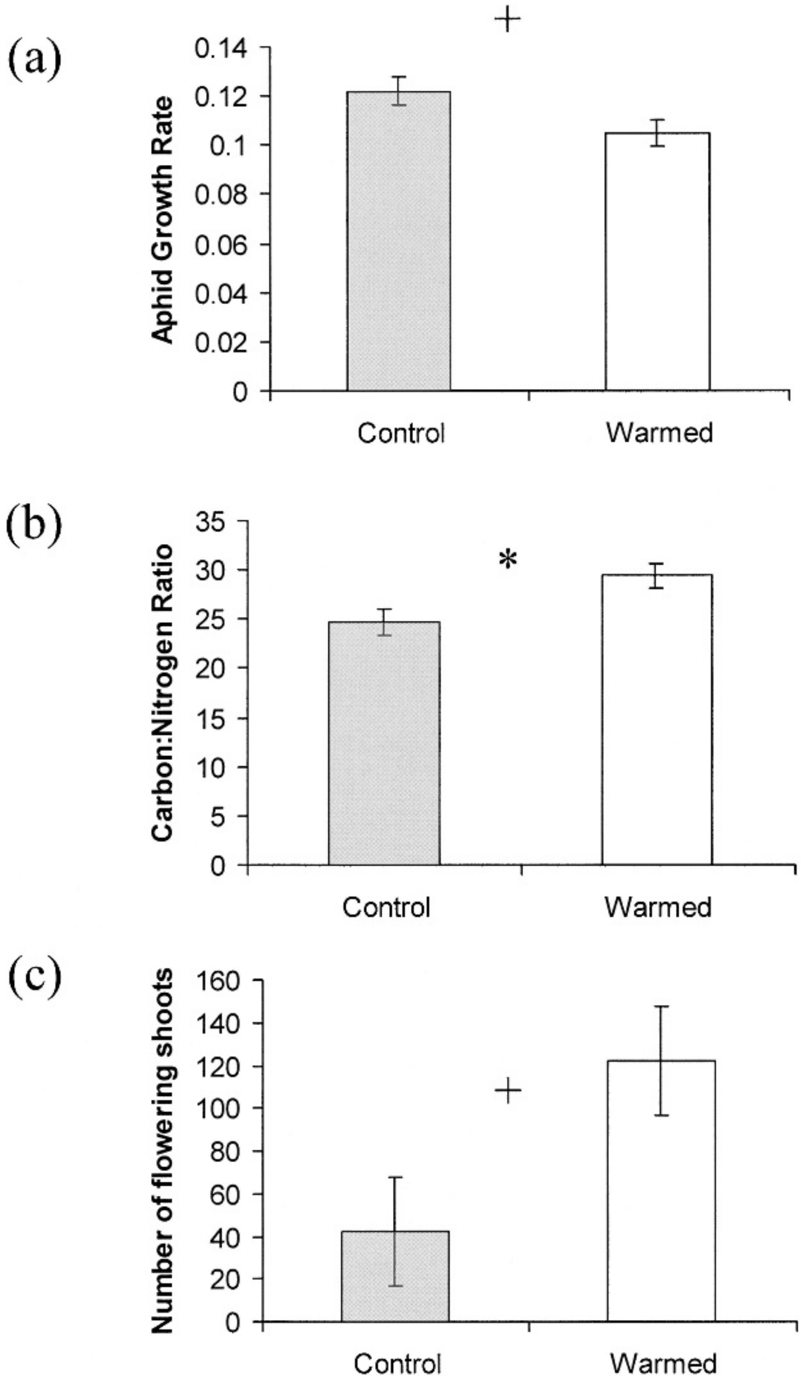

Fig. 2. Effect of experimental warming in 1997 on (a) local aphid population growth rate, *r*, (b) sagebrush carbon:nitrogen ratio, and (c) average number of flowering shoots per sagebrush. Error bars represent one standard error; + $P < 0.10$, * $P < 0.05$.

second year of the study (Miles *et al.,* 1997), suggesting that warming effects can change annually or as a study progresses. The results of our current study are consistent with recent results measuring herbivore damage and pathogen attachment to six common plant species in the same plots in 2002 (Roy *et al.,* 2004). Although herbivory and pathogens generally increased in heated plots, the number of stems with aphid damage was highest in control plots. Thus, even in a high-altitude site where insect population dynamics are more likely to be limited by temperature (as in Whittaker and Tribe, 1998), the long-term impacts of global warming in a community context may vary between species and be different from those predicted by short-term or laboratory studies.

The scarcity of long-term field studies that experimentally manipulate warming is undoubtedly due to the logistical difficulty and expense of such a manipulation, even at a replication of $n = 5$ as in our study. Although this study has low replication, it has unusual power to test for long-term community-wide impacts of small changes in temperature. Nonetheless, one concern about our results may be that we lacked the power to detect effects. In spite of the low replication, we found significant effects of warming on aphid growth in the *opposite* direction of that predicted in 1 of 3 yrs. In the other 2 yrs, we found no significant effect of warming. Examination of our graphs demonstrates that in 1998, means between treatments were very similar. A follow-up power analysis using our 1998 data shows that we would have needed 106, 246, and 6167 replicates to get a significant difference between treatments for the 3 aphid responses at alpha = 0.05. Thus, our failure to get a significant result in 1998 is not due to poor replication, but rather to a lack of effect in that year. In 1996, we found a trend similar to that of 1997 (Fig. 1c) that would likely be significant with greater replication. But this would only reinforce our main assertion that warming did not increase aphid abundance, contrary to predictions and other research. Thus, we feel confident in our assertion that warming *did not* have the predicted effect of increasing aphid growth and abundance.

In 1997, our enclosure experiment showed that warming marginally reduced aphid growth at a level consistent with field densities. Because aphids were enclosed in bags, these reduced growth rates occurred in the absence of natural enemies or other species, such as mutualist ants, that can vary between heated and control plots (Roy *et al.,* 2004). While enclosures may have influenced results by increasing microclimate temperature or humidity, our enclosures did not cause results that were qualitatively different from other field measures. Thus, while natural enemies or other species may play a role in regulating aphid dynamics in this system, the reduced aphid growth in response to warming occurred even in the absence of such interactions. Furthermore, in contrast to the results of Roy *et al.* (2004), we found no evidence in a separate experiment that an earlier snowmelt date was responsible for the treatment effects. The results of our enclosure and snowmelt study suggest that either increased temperature directly reduced aphid growth, or that temperature reduced aphid growth via effects on plant traits such as water content, nutritional quality or plant defenses.

It is possible that temperature directly reduced aphid growth. Direct effects of temperature on the intrinsic rates of increase of aphid populations are generally inverted-*U*-shaped between 5 and 30°C, with the optimal temperature varying between species (Wellings and Dixon, 1987). Although many aphid species may be limited by low temperature, others can have upper thresholds as low as 25°C

(Harrington *et al.,* 1995). However, at high-latitude or high-elevation sites, it is more common to find aphids limited by low rather than high temperature thresholds (e.g., Hill and Hodkinson, 1995; Strathdee *et al.,* 1995). Growth chamber studies of *O. cowenii* growth rates in response to temperature change are necessary to determine whether such direct effects exist. Disentangling the direct and plant-mediated impacts of temperature on herbivores may help provide more precise predictions of the effects of global warming in the Rocky Mountains.

Obtusicauda coweni could be negatively affected by changes in plant quality due to warming, such as water stress, nutritional quality or plant defenses. In our sites, warming decreased growing-season gravimetric soil moisture by approximately 10 to 20% each year (Harte *et al.,* 1995) and relative water content and water potential in *A. tridentata* (Loik and Harte, 1997). White (1984) has argued that aphids and other herbivores perform better on water-stressed plants, while we found that aphid density was lower in warmed plots where plants were more likely to be stressed. Thus, water stress either does not play an important role in this system, or it affects aphid growth differently than predicted. We found an increase in *A. tridentata* C:N ratio in warmed plots that may reflect a change in nutritional status. We were unable in this study to measure nitrogen content of phloem specifically, which would provide more conclusive evidence for a change in nutritional quality. Other studies have found that temperature affects sugar production and export, the ratio of xylem to phloem, phloem amino acid content and phloem loading (Salonen and Suhonen, 1995; Jiao and Grodzinski, 1996; Equiza and Tognetti, 2002; Minchin *et al.,* 2002), all of which could impact aphid growth. While we did not explicitly address the role of plant defenses in this study, the reduced aphid growth in warmed plots would be consistent with an increase in plant defenses. Surprisingly little is known about the effect of temperature on the production of plant defenses overall (Ayres, 1993; Veteli *et al.,* 2002). Higher temperatures diluted phenolics in willows (Veteli *et al.,* 2002), but did not affect total phenolics of Scots pine (Holopainen and Kainulainen, 2004). Furthermore, temperature and plant defenses can have non-additive effects on herbivore performance (Stamp *et al.,* 1994; Yang *et al.,* 1996), so that it may be difficult to predict the impacts of changes in defense at varying temperature ranges.

In conclusion, we found that long-term experimental warming in a field setting did not increase aphid density in any year of a 3-year study. The effects of warming varied from reducing aphid density and growth to having no effect over the 3 yrs. Our results contradict the general finding from short-term, microcosm studies that aphids will increase in response to global warming, and also demonstrate that warming impacts can be temporally variable. This research demonstrates the importance of long-term manipulative field studies to determine the impact of global warming on herbivore population dynamics within natural community contexts.

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Appendix 1. Summary of melt date (day of year), soil temperature (°C) and soil moisture (% dry mass) data in heated and control plots for the years 1996-1998. Means, standard errors, and ANCOVA *F* values for treatment (1 d.f.) and plot as covariate (1 d.f.), and error sums of squares (7 d.f. except moisture in 1998, which is 3 d.f.) are reported. MANCOVA values for combined microhabitat response each year are reported in the text. * $P < 0.05$; ** $P < 0.01$: **** $P < 0.0001$.

	1996			1997			1998		
	Melt date	Temp.	Moisture	Melt date	Temp.	Moisture	Melt date	Temp.	Moisture
Control Plots									
1	139	11.27	25.4	142	14.59	19.9	141	13.96	20.6
3	138	10.54	24.4	150	14.29	23.3	138	13.68	21.6
5	138	10.56	27.0	140	13.35	25.6	133	13.53	22.6
7	130	11.91	20.8	137	15.09	19.4	118	15.73	.
9	128	11.65	25.7	132	14.19	20.3	115	14.59	.
Mean	134.6	11.19	24.66	140.2	14.30	21.70	129.0	14.30	21.6
SE	2.32	0.29	1.05	2.97	0.28	1.19	5.28	0.40	0.58
Heated Plots									
2	135	12.55	23.4	134	14.97	20.4	139	15.65	17.6
4	134	12.79	24.3	128	16.17	24.1	128	16.36	22.2
6	124	14.10	24.1	116	16.05	22.1	117	16.22	20.2
8	116	12.39	20.4	103	14.12	18.7	108	15.49	.
10	119	13.44	22.6	110	14.65	21.3	108	15.6	.
Mean	125.6	13.05	22.96	118.2	15.19	21.32	120.0	15.86	20.0
SE	3.85	0.32	0.71	5.70	0.40	0.90	6.01	0.18	1.33
Treatment	9.85*	18.25**	1.32	24.09**	3.50	0.02	4.78[a]	10.78*	2.65
Covariate	26.54**	1.46	0.89	14.91**	0.57	0.37	88.12****	0.54	2.16
Error (SS)	84.40	2.95	28.43	263.80	4.44	42.06	94.2	3.58	7.35

[a] $P = 0.065$