



Increased seed consumption by biological control weevil tempers positive CO₂ effect on invasive plant (*Centaurea diffusa*) fitness



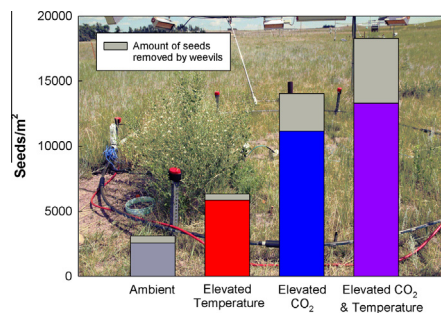
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HIGHLIGHTS

- Elevated CO₂ (but not temperature) increased plant fitness but also agent density.
- Biological control agent thus tempered positive CO₂ effect on plant fitness.
- Both elevated CO₂ and temperature hastened plant phenology.
- Faster plant phenology under elevated CO₂ made plants more attractive to agents.
- Plant and agent phenologies may be better matched with climate change.

GRAPHICAL ABSTRACT



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ABSTRACT

Predicted increases in atmospheric CO₂ and temperature may benefit some invasive plants, increasing the need for effective invasive plant management. Biological control can be an effective means of managing invasive plants, but the anticipated range in responses of plant–insect interactions to climate change make it difficult to predict how effective biological control will be in the future. Field experiments that manipulate climate within biological control systems could improve predictive power, but are challenging to implement and therefore rare to date. Here, we show that free air CO₂ enrichment in the field increased the fitness of *Centaurea diffusa* Lam., a problematic invader in much of the western United States. However, CO₂ enrichment also increased the impact of the biological control agent *Larinus minutus* (Coleoptera: Curculionidae) on *C. diffusa* fitness. *C. diffusa* plants flowered earlier and seed heads developed faster with both elevated CO₂ and increased temperature. Natural dispersal of *L. minutus* into the experimental plots provided a unique opportunity to examine weevil preference for and effects on *C. diffusa* grown under the different climate change treatments. Elevated CO₂ increased both the proportion of seed heads infested by *L. minutus* and, correspondingly, the amount of seed removed by weevils. Warming had no detectable effect on weevil utilization of plants. Higher weevil densities on elevated CO₂ plants reduced, but did not eliminate, the positive effects of CO₂ on *C. diffusa* fitness. Correlations between plant development time and weevil infestation suggest that climate change increased weevil infestation by hastening plant phenology. Phenological mismatches are anticipated with climate change in many plant–insect systems, but in the case of *L. minutus* and *C. diffusa* in mixed-grass prairie, a better phenological match may make the biological control agent more effective as CO₂ levels rise.

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1. Introduction

Increases in atmospheric CO₂ and consequent climate change are expected to dramatically alter plant communities (Thuiller et al., 2005), and may in the process facilitate plant invasion (Dukes and Mooney, 1999). Models, experiments, and observations all provide examples of likely increases in invasion; however, patterns vary widely among studies (Walther et al., 2009; Bradley et al., 2010; Sorte et al., 2013). Furthermore, while many studies have examined the direct effects of climate change on invaders and their competitors (e.g., Smith et al., 2000; Ziska, 2003; Belote et al., 2004; Williams et al., 2007; Dukes et al., 2011), much less is known about the influence of climate change on trophic interactions of invasive plants.

Herbivory and plant disease can either allow for invasion via enemy release or inhibit invasion via biotic resistance (Keane and Crawley, 2002; Mitchell and Power, 2003; Levine et al., 2004; Liu and Stiling, 2006; Parker et al., 2006), and such trophic interactions can depend on their environmental context (Maron and Vila, 2001; Blumenthal et al., 2009). For example, warming-induced range expansion was found to cause enemy release in Europe, where plant species that had moved north were less strongly inhibited by both above-ground herbivores and below-ground pathogens than were congeneric native species (Engelkes et al., 2008). Biological control, which increases biotic resistance through the introduction of specialized enemies, can also depend on environmental context. For example, in a system similar to the one studied here, fertilizer addition increased preference for host plants (*Centaurea diffusa* Lam.) by *Cyphocleonus achates* Fahraeus, while decreasing preference by *Larinus minutus* Gyllenhal (Lejuene et al., 2005).

Climate change is likely to have widespread and varied effects on plant–insect interactions in general (reviewed by Bale et al., 2002; Zvereva and Kozlov, 2006; DeLucia et al., 2012), and biological control in particular (Hellman et al., 2008). Climate is critical to predicting population dynamics and therefore success of biological control agents (Zalucki and van Klinken, 2006), and climate change could either facilitate or inhibit biological control depending on the responses of both the target plant species and their biological control agents (Hellman et al., 2008). For example, climate change, particularly warming, may alter the geographical ranges and success of interactions between biological control agents and their target plants by shifting locations where plants and agents will experience ideal (and non-ideal) temperatures (Lu et al., 2013; Allen et al., 2014). Among the other hypothesized effects of warming on plant–insect interactions is the potential for decreased synchrony of plant and insect phenologies (Bale et al., 2002; DeLucia et al., 2012), which could affect not only herbivory, but also alter pollination dynamics (CaraDonna et al., 2014). Such phenological mismatches may either benefit or harm the plant and insect in question, but for biological control applications, lessened damage to plants via decreased synchrony between the biological control agent and its target plant would be detrimental to control efforts.

As with warming, elevated CO₂ is likely to have varied effects on plant–insect interactions (Stiling and Cornelissen, 2007; Stiling et al., 2009, 2013; DeLucia et al., 2012; Robinson et al., 2012). One common effect of elevated CO₂ may be a decrease in host plant quality, particularly through increases in C:N ratio (Bezemer and Jones, 1998; Coviella and Trumble, 1999; Hunter, 2001; Zvereva and Kozlov, 2006; Stiling and Cornelissen, 2007; DeLucia et al., 2012; Robinson et al., 2012). Such reductions in plant quality have been shown to increase insect feeding rates to compensate for nutritional deficiencies (e.g., Hughes and Bazzaz, 1997; Stiling

and Cornelissen, 2007; Yin et al., 2010), although insects may also display lower acceptance of and performance on plants grown under elevated CO₂ (Stiling and Cornelissen, 2007; Stiling et al., 2013). While effects of CO₂ on biological control may vary accordingly, the general patterns of decreased plant quality and insect preference with elevated CO₂ may not bode well for biological control applications. Despite considerable evidence that plant–insect interactions may be sensitive to climate change, field experiments testing such effects on biological control remain rare. This lack of research is especially evident when considering combined effects of elevated temperature and CO₂ on plant–insect interactions and biological control (Zvereva and Kozlov, 2006; DeLucia et al., 2012), which can also be highly variable (Johns and Hughes, 2002; Veteli et al., 2002; Johns et al., 2003; Zvereva and Kozlov, 2006; DeLucia et al., 2012; Scherber et al., 2013), leading to cautions against broad interpretations of climate change effects from single-factor studies (Bale et al., 2002; DeLucia et al., 2012; Robinson et al., 2012).

One useful model system for examining the impacts of climate change on biological control is the introduction of the lesser knapweed flower weevil (*L. minutus* Gyllenhal; Coleoptera: Curculionidae) to control diffuse knapweed (*Centaurea diffusa* Lam.). Native to Eurasia, *C. diffusa* is a problematic biennial invasive forb in much of the western United States. It has been shown to decrease native plant biodiversity and livestock forage, reduce land use by wildlife, and can also increase erosion rates (Sheley et al., 1998). As such, there has been much research regarding *C. diffusa* control (reviewed by Muller-Scharer and Schroeder, 1993; Sheley et al., 1998). *L. minutus* is one of several biological control agents for *C. diffusa* (Muller-Scharer and Schroeder, 1993; Lang et al., 2000), and is also used for spotted knapweed (*C. stoebe*) control (Kashefi and Sobhian, 1998; Lang et al., 2000; Bouchier and Crowe, 2011). These weevils have been used in the United States since the early 1990s (Muller-Scharer and Schroeder, 1993; Sheley et al., 1998; Seastedt et al., 2003, 2007). Adult *L. minutus* overwinter in leaf litter or soil near *C. diffusa* and emerge by early May (Kashefi and Sobhian, 1998), prior to *C. diffusa* flowering in July (Sheley et al., 1998). Stem and leaf material are consumed until flowers have developed, at which point flowers are consumed by females for ovary development (Kashefi and Sobhian, 1998). Faster-developing plants are often preferred by *L. minutus* (Seastedt et al., 2003; Lejuene et al., 2005; Bouchier and Crowe, 2011), presumably because adults emerge before flowering and later depend on flowers for food and oviposition. Adult feeding can damage bolting *C. diffusa* stems (Seastedt et al., 2003) and even reduce seed production in some instances (Stephens and Myers, 2013). However, larvae develop inside the flower/seed head of *C. diffusa* and typically consume or damage 100% of the developing seeds (Kashefi and Sobhian, 1998), often having a larger effect than adults on plant control.

Here, we use the Prairie Heating and CO₂ Enrichment (PHACE) experiment to test the effects of free air CO₂-enrichment and infrared warming on interactions between *C. diffusa* and *L. minutus*. Experimental introduction of *C. diffusa* into northern mixed-grass prairie, followed by unplanned colonization by *L. minutus*, provided a unique opportunity to examine how climate change influences the interaction between a problematic invasive plant and a key biological control agent under field conditions. The objectives of this study were to examine how elevated CO₂ and temperature influenced (1) the phenology (defined here as number of early-season flowers and date of seed maturation), growth, and fitness of *C. diffusa*, (2) host selection by *L. minutus*, and (3) the influence of *L. minutus* on *C. diffusa* fitness.

2. Methods

2.1. Site description

The 2.4 ha study site was located in a northern mixed-grass prairie ecosystem at the USDA-ARS High Plains Grasslands Research Station, about 7 km northwest of Cheyenne, Wyoming, USA (41°11' N, -104°53' W). The primary ecological site is Loamy (Site ID is R067AY122WY). Soils included an Ascalon variant loam (fine-loamy, mixed-mesic) at the north end of the study site, and an Altvan loam (fine-loamy over sandy, mixed-mesic) at the south end. Mean annual precipitation at the study site (136 yr) is 381 (\pm 98 SD) mm, with a typical peak in May. The native plant community consists of about 55% perennial C₃ monocots (particularly *Pascopyrum smthii* [Rydb.] Å. Löve, *Carex duriuscula* C.A. Mey., and *Hesperostipa comata* [Trin. and Rupr.]), 25% perennial C₄ grasses (almost exclusively *Bouteloua gracilis* [H.B.K.] Lag. ex Griffiths), and 20% forbs and small sub-shrubs. While *C. diffusa* was not present at the study site prior to its experimental introduction, it did occur nearby (<2 km) within the same ecosystem. Of the two years for which data are presented, 2012 was warmer and drier than 2011. In 2012, April–September precipitation was 189.5 mm (62% of average) and average temperature over the same period was 16.5 °C (3° above average); for 2011, values were 364.5 mm (119% of average) and 13.5 °C (near average), respectively.

2.2. Experimental design

The PHACE study began in 2006 to examine climate change effects on northern mixed-grass prairie and included factorial combinations of ambient and increased temperature and CO₂. The experiment consisted of four treatments (designated by lowercase and capital letters representing ambient and elevated CO₂ [c,C] and temperature [t,T] respectively) that were replicated five times each: (1) control (ambient conditions; ct), (2) free air CO₂ enrichment (FACE) to 600 ppmv (Ct), (3) infrared heating to increase canopy temperature 1.5 °C during the day and 3 °C at night (cT), and (4) CO₂ enrichment plus heating (CT). The 20 plots were placed randomly within two soil-type blocks (soils described above). Circular 7-m² experimental plots were isolated from surrounding soil using 60 cm-deep plastic barriers. Dummy FACE rings and heaters were used around untreated plots to control for any potential infrastructure effects. Achieved treatment levels were 600.5 ppmv CO₂ \pm 50.4 (SD) (monitored at 1-min intervals over a 40 day period), and +1.6 °C \pm 0.3 (SD) in the day and +3.0 °C \pm 0.3 (SD) at night (monitored at 1-h intervals over a 6 month period). Detailed methods and treatment performance can be found in [Morgan et al. \(2011\)](#).

In October 2007, two 70 cm \times 100 cm subplots were created within each plot to study native and invasive dicots. In each subplot, the soil within two 100 cm \times 20 cm strips (57% of the total area) was disturbed to a depth of 10 cm by turning it over with a Maddox and smoothing it with a rake. This disturbance killed all vegetation within the strips but not adjacent undisturbed vegetation. The two subplots were planted in consecutive years to evaluate interactive effects of the climate change treatments and ambient climate on establishment of planted dicots. Subplots were randomly assigned to years, and planted in November 2007 or November 2008 with five native species (*Artemisia frigida* Willd., *Chenopodium leptophyllum* (Moq.) Nutt. ex S. Watson, *Descurainia pinnata* (Walter) Britton, *Grindelia squarrosa* (Pursh) Dunal, and *Ambrosia tomentosa* Nutt.), and five exotic species (*Cardaria draba* (L.) Desv., *C. diffusa*, *Descurainia sophia* (L.) Webb ex Prantl, *Linaria dalmatICA* (L.) Mill, and *Salsola tragus* L.). *C. diffusa* seed was collected in Boulder, Colorado, USA, in the growing seasons prior to planting. Each subplot received 3.4 g of *C. diffusa* seed

(~4000 seeds/m²), which was applied by hand to both disturbed and undisturbed sections. The soil was then raked, and packed lightly to increase seed-soil contact. In subsequent years, small sections of each sub-plot were harvested for a related study. As a result, subplot areas were reduced to 0.42 m² and 0.35 m² in 2011 and 2012, respectively.

2.3. Data collection

As an unplanned aspect of the PHACE experiment, *L. minutus* independently dispersed into the experimental plots (i.e., weevils were not released as a part of the study). Although flowering *C. diffusa* plants were first recorded in 2009, weevils and damaged seed heads were rarely observed until 2011 (the first year for which data were recorded). Weevils persisted on *C. diffusa* through 2012, the final full year of the experiment. There were no *C. diffusa* plants within the study site prior to this experiment, so immigrating weevils from unassociated prior releases (the nearest of which were unknown) had open choice among the many patches of *C. diffusa* grown in close proximity under different climate change treatments. Other *C. diffusa* biological control agents were not observed, suggesting that if present, they were considerably less abundant than *L. minutus*.

To quantify all weevil and plant metrics examined below, every *C. diffusa* inflorescence within each plot was harvested each year. From late-August to mid-October in both years, *C. diffusa* stems were harvested weekly as seeds matured. Inflorescences were harvested when >50% of seed heads contained darkened, hardened seeds. After the total number of seed heads from each treatment was counted, the proportion of seed heads infested by *L. minutus* was determined from random samples of 60 seed heads from each subplot within each treatment for each year. Similarly, for each subplot in each year, a subset of 30 seed heads was used to quantify the mean number and mass/m² of seeds per uninfested seed head. We observed 100% damage or removal of seeds by weevils in the infested seed heads in the subsamples (i.e., there were no intact seeds to count in any infested seed heads). Weevil infestation data were calculated at the end of each growing season using total plant harvest from each subplot (i.e., weevil data were quantified for the season as a whole rather than at each plant harvest date). A plot-level weighted average for proportion of infested seed heads was calculated using total number of seed heads in each subplot as the weighting factor to account for differences in *C. diffusa* size between subplots.

To estimate the number of seed heads infested by *L. minutus*, the proportion of infested seed heads was multiplied by the total number of seed heads in a subplot. Next, seed number and mass/m² removed by weevils was estimated by multiplying the mean seed number and mass/m² from uninfested seed heads by the estimated number of seed heads infested by weevils. Finally, to estimate the total potential seed number and mass/m² that would have been produced if *L. minutus* had been absent, the values for total seed number and mass/m² in uninfested seed heads and estimated seed number and mass/m² removed by weevils were summed.

To evaluate potential mechanistic explanations for weevil plant preference, both *C. diffusa* size and phenology metrics by plot were quantified. Because female *L. minutus* utilize seed heads for food and oviposition, number of seed heads/m² was used as a proxy for plant size. For phenology, both early-season (flowering) and late-season (seed head maturation) measurements were used, as different seasonal plant phenological measurements can be affected independently by climate change ([CaraDonna et al., 2014](#)), and because weevils are active on plants for the full growing season. For the early-season measurement, the number of open flowers/m² in July in each subplot was counted from overhead photographs taken once every ~21 days. The selected photograph date for each

year represented the first instance among photograph dates where detectable flowering (i.e., >1%) was present, at which point differences between plots/treatments were already emerging. For 2011, the photograph date was 27 July; for 2012, the photograph date was 13 July. For late-season phenology, a mean date (day of year) of seed head maturity was calculated as the average day of year when plants within a given subplot were ready to be harvested. Weighted plot-level averages of mean maturation day of year were then calculated using total stem number in each subplot as the weighting factor.

2.4. Statistical analysis

Effects of elevated CO₂ and temperature treatments on the weevil and plant variables described above were tested using two-way, repeated measures mixed models in JMP 10.0.0 (REML method; SAS Institute Inc., Cary, NC, 2012). These models included CO₂, temperature, CO₂ × temperature interaction, year, and year × treatment interactions as fixed effects, with block and ring(CO₂, temperature) as random effects. Data for all models except proportion of *L. minutus*-infested seed heads and mean seed head maturation date were square root transformed to alleviate residual heteroscedasticity.

Anticipating that *L. minutus* preferred larger plants and/or more phenologically advanced plants, we tested whether treatment effects on proportion of infested seed heads were robust to the inclusion of open flowers/m² in July, mean seed head maturation day of year, or seed heads/m² as covariates in the model. If inclusion of these explanatory variables made originally significant effects non-significant, it was concluded that that explanatory (mechanistic) variable could account for the treatment effect. To present these relationships visually, linear regressions were performed across all plots (treatment replicates) on these same three variables vs. proportion of seed heads infested by *L. minutus*. Because of the general lack of year × treatment interactions from the mixed models (Table 1), these regressions were performed on data which were averaged across 2011 and 2012 for each plot. Similarly, all figures depict mean values of response variables across 2011 and 2012.

3. Results

Elevated CO₂ increased both the proportion of seed heads infested by *L. minutus* (Fig. 1 and Table 1) and the number of seeds removed by *L. minutus* (Fig. 2 and Table 1). No temperature or CO₂ × temperature interaction effects existed for proportion of infested seed heads or number of seeds removed by weevils (Table 1). Along with effects on weevils, elevated CO₂ also substantially increased *C. diffusa* fitness (seeds/m²), both for actual seed number (seeds in uninfested seed heads) and potential seed number (actual seed number + estimated seed number removed by *L. minutus*; Fig. 2 and Table 1). This positive CO₂ effect on seeds/m² corresponds with a positive CO₂ effect on total number of seed heads/m² produced (Table 1; Fig. 3c). Note that seed mass (g/m²) results were very similar to seed number results, so only seed number results are presented. Both elevated CO₂ and temperature increased the number of open flowers/m² in July (Table 1 and Fig. 3a). Although trends were similar across years, the temperature effect was only significant in 2011 ($F = 12.86_{(1,16)}$; $P = 0.0025$), leading to the significant year × temperature interaction for open flowers/m² in July (Table 1). Both elevated CO₂ and temperature also reduced mean seed head maturation day of year (Table 1 and Fig. 3b).

Beyond the CO₂ and temperature effects, there were multiple significant year effects (Table 1). For instance, seed production

Table 1
Model results for effects of elevated CO₂ and warming on *C. diffusa* and *L. minutus* impact on *C. diffusa*. Bolded values highlight significant effects ($P < 0.05$).

Variable	Proportion of <i>L. minutus</i> -infested seed heads		Potential seed No./m ²		Actual seed No./m ²		Seed No./m ² consumed		Open flowers/m ² in July		Mean day of year of seed maturation		No. of seed heads/m ²	
	$F_{(df)}$	P	$F_{(df)}$	P	$F_{(df)}$	P	$F_{(df)}$	P	$F_{(df)}$	P	$F_{(df)}$	P	$F_{(df)}$	P
CO ₂	6.47 _(1,14.7)	0.0227	17.27 _(1,15)	0.0008	14.30 _(1,15)	0.0018	25.37 _(1,15)	0.0001	21.24 _(1,16)	0.0003	11.90 _(1,15.1)	0.0035	16.56 _(1,15)	0.0010
Temperature	1.72 _(1,14.7)	0.2100	0.21 _(1,15)	0.6515	0.10 _(1,15)	0.7552	0.55 _(1,15)	0.4686	6.51 _(1,16)	0.0213	5.14 _(1,15.2)	0.0384	0.02 _(1,15)	0.8812
CO ₂ × Temperature	0.86 _(1,14.7)	0.3676	0.00 _(1,15)	0.9952	0.06 _(1,15)	0.8147	0.79 _(1,15)	0.3869	1.00 _(1,16)	0.3330	0.03 _(1,15.2)	0.8634	0.00 _(1,15)	0.9456
Year	5.82 _(1,13.8)	0.0304	12.89 _(1,16)	0.0025	13.17 _(1,16)	0.0023	5.74 _(1,16)	0.0291	3.25 _(1,16)	0.0902	76.43 _(1,14.2)	<0.001	7.55 _(1,16)	0.0143
Year × CO ₂	1.27 _(1,13.8)	0.2796	1.79 _(1,16)	0.1998	1.37 _(1,16)	0.2597	4.23 _(1,16)	0.0563	3.80 _(1,16)	0.0690	1.22 _(1,14.2)	0.2885	0.27 _(1,16)	0.6136
Year × Temperature	0.07 _(1,13.8)	0.7958	3.60 _(1,16)	0.0759	3.20 _(1,16)	0.0927	3.00 _(1,16)	0.1023	11.46 _(1,16)	0.0038	0.48 _(1,14.2)	0.4997	0.95 _(1,16)	0.3437
Year × CO ₂ × Temperature	0.02 _(1,13.8)	0.9008	0.20 _(1,16)	0.6643	0.24 _(1,16)	0.6319	0.00 _(1,16)	0.9767	0.01 _(1,16)	0.9246	0.95 _(1,14.2)	0.3464	1.61 _(1,16)	0.2224

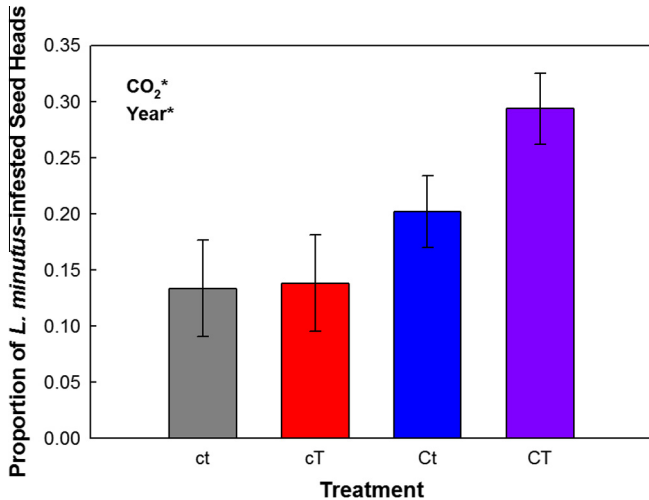


Fig. 1. Mean (\pm SE) proportion of *C. diffusa* seed heads infested by *L. minutus* across CO₂ (c = ambient; C = 600 ppmv enrichment) and warming (t = ambient; T = 1.5/3 °C day/night warming) treatments. Treatment means and errors were calculated using plot values which were averaged across 2011 and 2012. Significant treatment effects are listed in the figure: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

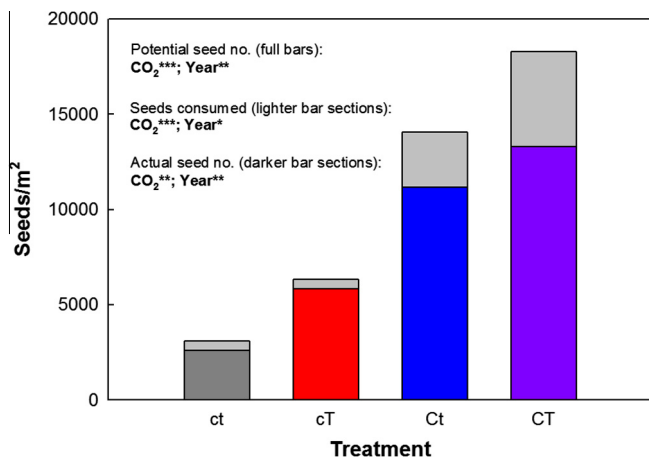


Fig. 2. Effects of *L. minutus* infestation on *C. diffusa* seed numbers/m² for CO₂ and warming treatments (see text for abbreviations). Darker (lower) bar sections represent actual seed production from seed heads uninfested by *L. minutus*. Lighter (upper) bar sections represent estimated seed number removed by *L. minutus*. Total stacked bar height represents estimated total seeds production if no *L. minutus* were present. Treatment means were calculated using plot values which were averaged across 2011 and 2012. Significant treatment effects are listed in the figure: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

was over 260% higher in 2011 across treatments, with 171% more seed heads produced. Weevils removed 181% more seeds in 2011, even though 7.4% more seed heads were infested in 2012 across treatments. Finally, seed head maturation occurred 17 days earlier across treatments in 2012. These year effects did not impact primary CO₂ or temperature effects on either *L. minutus* or *C. diffusa* (no meaningful year \times treatment interactions existed in the models), and can be attributed to 2011 being relatively cooler and wetter than 2012.

Regression results showed that *L. minutus* infestation rates were higher on both earlier-flowering (Fig. 4a) and faster-developing (Fig. 4b) *C. diffusa* plants, whereas the relationship between *L. minutus* preference and seed heads/m² was not significant (Fig. 4c). Including mean seed head maturation day of year as a covariate in the above model for infestation rate removed the significant CO₂ effect on weevil infestation ($P = 0.1255$; $F_{(1,28)} = 2.49$),

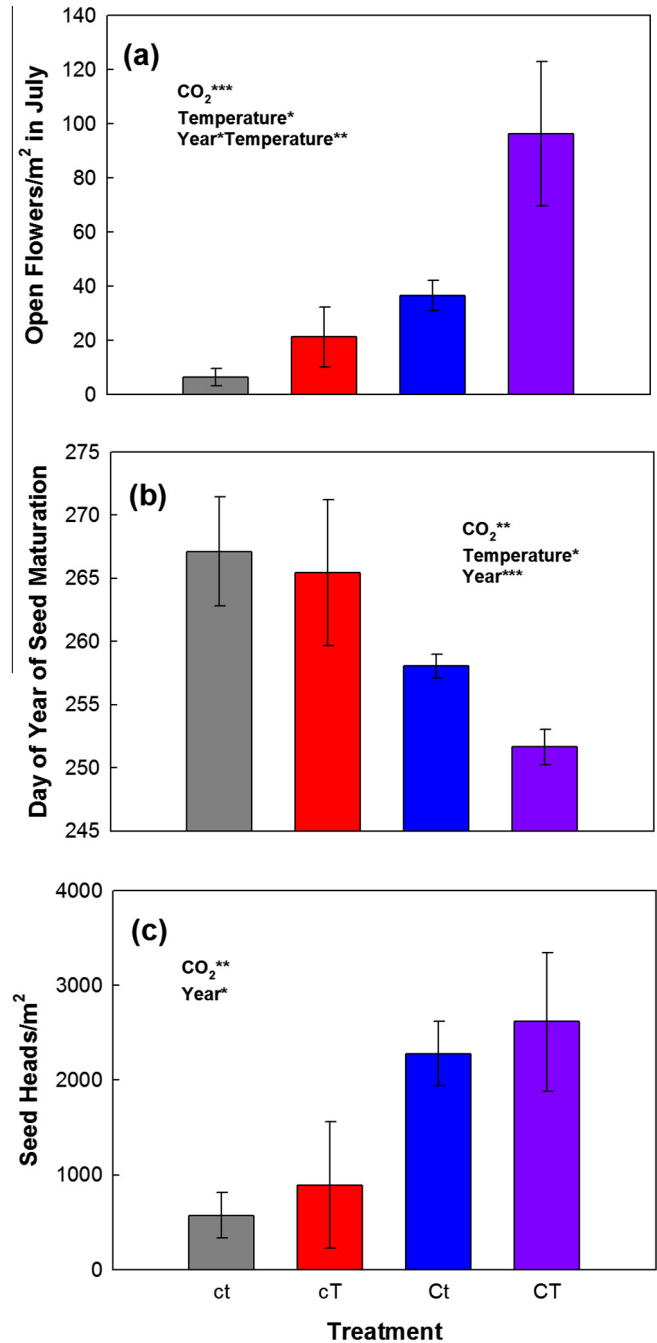


Fig. 3. Effects of elevated CO₂ (c = ambient; C = 600 ppmv enrichment) and warming (t = ambient; T = 1.5/3 °C day/night warming) on number of open *C. diffusa* flowers/m² in July (a), mean day of year of seed head maturation (b), and number of seed heads/m² (c). Bars represent mean (\pm SE) calculated from plot values which were averaged across 2011 and 2012. Significant treatment effects are listed in each panel: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

indicating that earlier maturation could account for CO₂ effects on infestation. In contrast, CO₂ effects were less significant but still present when open flowers/m² in July ($P = 0.0423$; $F_{(1,28)} = 4.53$), or seed heads/m² (proxy for plant size; $P = 0.0148$; $F_{(1,28)} = 6.75$) were included as covariates.

4. Discussion

Elevated CO₂ more than doubled *C. diffusa* fitness, even in the presence of *L. minutus*. Increases in invasive species success with

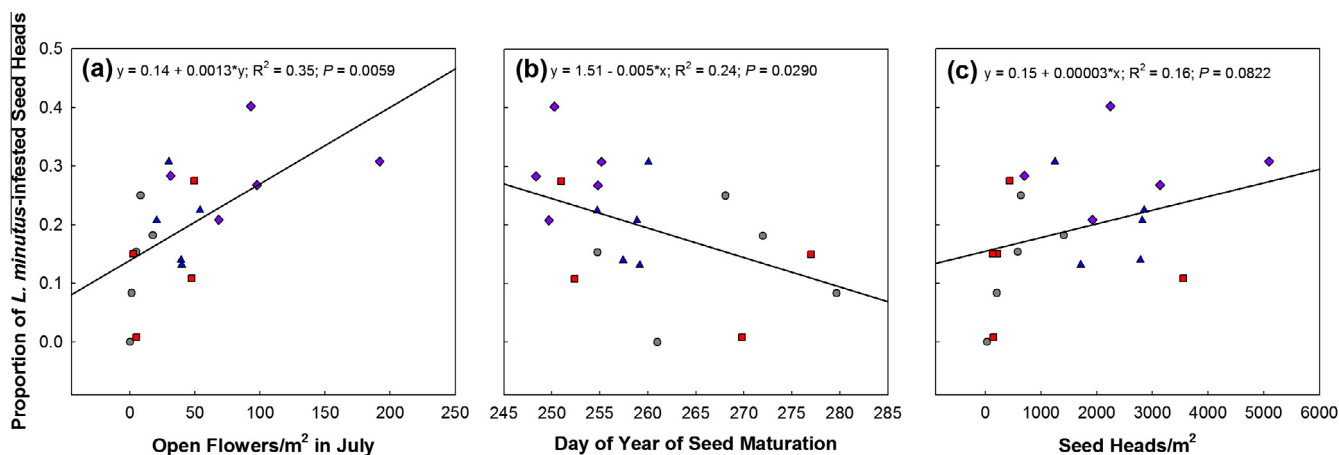


Fig. 4. Relationship between open *C. diffusa* flowers/m² in July (a), mean day of year of seed head maturation (b), and number of seed heads/m² (c) on proportion of *L. minutus*-infested seed heads. Data points represent averages across 2011 and 2012 for individual plots. For treatments: circles = ct; squares = cT; triangles = Ct; diamonds = CT (see text for abbreviations).

elevated CO₂ have been observed in a number of other ecosystems (Smith et al., 2000; Ziska, 2003; Belote et al., 2004; Dukes et al., 2011), but it remains unclear how general this pattern is (Williams et al., 2007; Sorte et al., 2013). Previous work within the PHACE experiment showed that elevated CO₂ strongly facilitated the invasive perennial forb, *Linaria dalmatica*, likely due to increases in the availability of both soil water and carbon, while temperature had little effect (Blumenthal et al., 2013). Similarly, *C. diffusa* responded much more strongly to elevated CO₂ than to warming, despite its very different biennial life history.

As elevated CO₂ increased *C. diffusa* fitness, it also increased the amount of seed consumed by *L. minutus*, limiting but not eliminating the net effect of CO₂ on *C. diffusa*. Across years and warming treatments, *L. minutus* reduced *C. diffusa* fitness by 10.7% with ambient CO₂ and 24.3% with elevated CO₂. However, the percentages of seed heads infested in this study were lower than previously reported infestation levels of ~40–60% (Smith and Mayer, 2005; Seastedt et al., 2007; Myers et al., 2009; Stephens and Myers, 2013). Thus, it is possible that purposeful *L. minutus* population augmentation, or even additional years of infestation in this study, could have further increased weevil numbers and more strongly mitigated the increased success of *C. diffusa* under elevated CO₂. It should also be noted that we were unable to account for adult *L. minutus* damage to *C. diffusa* (e.g., Stephens and Myers, 2013) prior to larval seed consumption, so our estimates of total lost seed production due to *L. minutus* may be conservative.

Greater infestation by *L. minutus* appeared to be caused by earlier *C. diffusa* phenology with elevated CO₂. Earlier phenology has previously been associated with greater *L. minutus* infestation of *C. diffusa* (Seastedt et al., 2003; Lejuene et al., 2005; Bouchier and Crowe, 2011). While both elevated CO₂ and warming hastened *C. diffusa* phenology, elevated CO₂ did so more strongly, both early and late in the season, which may explain why only CO₂ significantly increased *L. minutus* infestation. Elevated CO₂ probably accelerated phenology by increasing plant growth. In biennial plants, rosettes often need to attain a minimum size to flower, so larger individuals often flower earlier (e.g., Gross, 1981). In contrast, for perennial species, CO₂ has been shown to lengthen the growing season, but have little effect on reproductive phenology in the PHACE study (Reyes-Fox et al., 2014). Thus, it may be that biennial species respond differently to elevated CO₂ because their flowering time is particularly sensitive to plant size.

Effective use of biological control in the future will require predicting how agents will perform in changing climates (Zalucki and

van Klinken, 2006; Hellman et al., 2008). Previous work has suggested that elevated temperatures can increase feeding activity of biological control agents (Forno and Bourne, 1986), but can also alter control success by shifting the geographic ranges in which plants and insects experience suitable thermal minima and maxima (Lu et al., 2013; Allen et al., 2014). Although effects of CO₂ on biological control have received less attention, elevated CO₂ can increase endophagous larval success of some biological agents by counteracting warming-induced leaf loss (Johns et al., 2003). The current study, which uniquely tests how CO₂ and temperature influence biological control in the field, demonstrates that biological control efficacy can be sensitive to elevated CO₂. Furthermore, the likely role of phenological matching suggests that future biological control success will require understanding not only the climate sensitivities of the target plant and the agent, but also the climate sensitivity of their interaction.

For phytophagous insects, elevated CO₂ is expected to reduce host plant quality through a variety of changes to physical and chemical leaf qualities, particularly increases in C:N ratios (Bezemer and Jones, 1998; Coviella and Trumble, 1999; Hunter, 2001; Zvereva and Kozlov, 2006; Stiling and Cornelissen, 2007; DeLucia et al., 2012). These reductions in plant quality can lead to increased plant consumption to compensate for nutritional deficiencies (e.g., Hughes and Bazzaz, 1997; Stiling and Cornelissen, 2007; Yin et al., 2010), but also lower preference for and performance on plants (Stiling and Cornelissen, 2007; Stiling et al., 2013). Under elevated temperatures, varied effects have been reported across insect and plant species (Lemoine et al., 2014), again including reductions in host plant quality (Bauerfeind and Fischer, 2013). Warming may also alter the phenologies of plants and insects, leading to potential mismatches (Bale et al., 2002; DeLucia et al., 2012). Although CO₂ could have reduced host plant quality in this study (as observed for other species in the PHACE experiment; Dijkstra et al., 2010), the potentially reduced quality clearly did not deter weevils from utilizing the elevated CO₂ plants. Thus, it seems likely that CO₂ increased *L. minutus* infestation primarily by hastening *C. diffusa* phenology, (as in Lejuene et al., 2005). In this case, it appears that change in plant phenology resulted in phenological matching, rather than mismatching, between the plant and the insect. Given the lack of similar studies, however, it is difficult to assess how common such a CO₂ response might be for other plant–insect interactions.

Although the preference for accelerated plant phenology by *L. minutus* seems clear (Seastedt et al., 2003; Lejuene et al., 2005;

Bourchier and Crowe, 2011), it is unclear from this study how *L. minutus* phenology will change with increased temperature and CO₂. Weevil measurements were limited here to their infestation rates, and did not include observations of adult weevils. There is little reason to expect CO₂ to directly influence *L. minutus* behavior or phenology, and while temperature might have been more likely to directly influence weevils (i.e., quicken development and spring emergence; Bale et al., 2002; DeLucia et al., 2012), such effects would not explain the observed CO₂ results here. The fact that *L. minutus* could have moved among plots means that weevils that emerged or developed earlier with higher temperature could have colonized any of the treatments within the study. Such movement could have diluted any influence of *L. minutus* phenology on overall treatment effects. However, in a future with elevated CO₂ and temperature in all locations, earlier *L. minutus* development (or at least earlier emergence from overwintering) might be expected to maintain, if not accentuate, the preference for earlier-developing *C. diffusa*. This seemingly provides a counter example to the many expected phenological mismatches between plants and insects with climate change (Bale et al., 2002; DeLucia et al., 2012).

Maintaining effective invasive species management will require adaptation to climate change (Dukes and Mooney, 1999; Hellman et al., 2008). With respect to biological control, such adaptation requires predicting the climate response of complex and species-specific interactions. For the system studied here, *L. minutus* is already considered to be one of the most effective biological control agents for *C. diffusa* (Seastedt et al., 2003; Myers et al., 2009), and it would seem given our results that this will remain true as CO₂ and temperature change. Selecting such biological control agents and beginning to utilize them more frequently, even ahead of anticipated increases in CO₂ and temperature, may be an effective strategy for mitigating the anticipated benefits to invasive plants from climate change.

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