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LEAF-LEVEL PHYSIOLOGY, BIOMASS, AND REPRODUCTION OF *PHYTOLACCA AMERICANA* UNDER CONDITIONS OF ELEVATED CO_2 AND ALTERED TEMPERATURE REGIMES

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The effects of increasing air temperature and changing daily temperature regime under conditions of elevated CO_2 on the physiology, biomass, and reproduction of a C_3 plant species were investigated. *Phytolacca americana* L. (Phytolaccaceae) was grown under either ambient (370 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2 at three air temperature regimes (day/night temperatures of 26°/20°C, T_1; 30°/24°C, T_2; and 28°/24°C, T_3). Length of day/night temperature exposure was adjusted so that average daily temperature was 22°C in T_1 and 26°C in T_2 and T_3. Daily temperature regime was different for T_2 and T_3: plants in T_2 experienced a higher maximum daily temperature but for a shorter daily duration than plants in T_3. Elevated CO_2 increased photosynthetic rate, total biomass, and root-to-shoot ratio (RSR) and decreased stomatal conductance and transpiration as well as allocation to reproduction. In contrast, elevated temperatures had no effect on photosynthetic rate, stomatal conductance, or total biomass, but they decreased RSR and increased transpiration, reproductive biomass, and allocation. Both elevated CO_2 and increased temperatures advanced timing of flowering. The plant-level transpiration rate exhibited a unique response to each of the daily temperature regime treatments. These results indicate that elevated CO_2 and increased temperatures elicit different responses at the physiological and whole-plant levels in *P. americana*, with little interaction between the CO_2 and temperature effects. Furthermore, some evidence indicates that a changing daily temperature regime may be an important factor determining plant responses to warming temperatures and should be incorporated into predictions of plant and ecosystem responses to future climate change.

Keywords: elevated CO_2, elevated temperature, *Phytolacca americana*, biomass, root-to-shoot ratio, reproductive allocation, photosynthesis, transpiration.

Introduction

Increasing atmospheric CO_2 concentration and air temperature are both important factors affecting plant physiology, growth, development, and reproduction (Stirling et al. 1998; Morison and Lawlor 1999). The concentration of atmospheric CO_2 has increased during the past 250 yr from ca. 280 to 370 μmol mol^{-1}, and it is predicted to increase to between 540 and 970 μmol mol^{-1} by the year 2100 (Prentice et al. 2001). Average global air temperature increased by ca. 0.6°C in the 20th century (Folland et al. 2001), and it is predicted to increase between 1.7° and 4.9°C by the year 2100 (Cubasch et al. 2001; Wigley and Raper 2001). In addition, most of the rise in global temperature over the past 40 yr can be attributed to a change in daily temperature regime, with nighttime minimum temperature increasing more than daytime maximum temperature (Karl et al. 1993; Horton 1995; Easterling et al. 1997), and this trend is predicted to continue into the 22nd century (Cubasch et al. 2001; Dai et al. 2001).

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The effects of rising atmospheric CO_2 and temperature on plants and ecosystems have been investigated in a number of experiments (Ackerly et al. 1992; Hunt et al. 1996; Stirling et al. 1998; Tjoelker et al. 1998; Wayne et al. 1998; Loiseau and Soussana 2000; Lee et al. 2001; Lewis et al. 2001; Luomala et al. 2003; see reviews by Rawson 1992; Saxe et al. 1998; Morison and Lawlor 1999; Amthor 2001; Norby and Luo 2004; Pendall et al. 2004). However, the warming treatments in most of the elevated CO_2 and temperature studies have not considered how changing aspects of the daily temperature regime may affect plants and ecosystems. For example, if nighttime temperatures increase more than daytime temperatures (Karl et al. 1993; Horton 1995), maintenance respiration in plants could increase (Ryan 1991; Griffin et al. 2002), thus increasing the ratio of dark respiration to photosynthesis and decreasing plant growth. In fact, dark respiration has been found to increase in some crop plants and trees grown under warm nighttime conditions (Manunta and Kirkham 1996; Turnbull et al. 2001, 2004). Furthermore, studies indicate that increasing nighttime temperatures may have a variety of effects on agricultural plants that differ from those of increasing daytime temperatures alone (Seddigh et al. 1989; Mooney et al. 1994; Manunta and Kirkham 1996; Moot et al. 1996).

We conducted an experiment investigating how elevated CO_2, increasing air temperature, and changing daily temperature regime may affect the C_3 plant *Phytolacca americana*. Physiological processes are the only mechanisms by which plants can respond directly to rising CO_2 (Drake et al. 1997; Long 1999), and increasing air temperature may modify these responses (Farrar and Williams 1991; Long 1991; Hunt et al. 1996). Thus, we measured the physiological responses of photosynthetic rate, stomatal conductance, and transpiration rate, as well as the whole-plant responses of total biomass accumulation, biomass allocation, phenology, and reproductive allocation.

Material and Methods

Study Organism

Phytolacca americana L. (Phytolaccaceae; pokeweed) is a polycarpic perennial herb common to much of eastern North America, ranging from Quebec and Ontario to northern Mexico (Caulkins and Wyatt 1990). It grows in open disturbed areas, particularly forest edges and canopy gaps (Sauer 1952; Caulkins and Wyatt 1990; Wilson and Shure 1993). It utilizes the C_3 photosynthetic pathway (Basinger 2002) and is a predominantly autogamous species (Armesto et al. 1983). In an earlier study, we found that *P. americana* in its natural habitat grows to an average height of 98 cm, produces an average of 28 g of total biomass, and grows roots to a depth of 15–25 cm, depending on soil conditions (He and Bazzaz 2003). Under natural conditions, this perennial species can grow to reproductive maturity within a single season, making it a desirable species for glasshouse experiments.

Experimental Design and Growth Conditions

The experimental facility consisted of twelve $1.0 \times 1.0 \times 2.0$-m environmentally controlled glass chambers located in a glasshouse facility at Harvard University, Cambridge, Massachusetts. Each chamber received one of six CO_2 and air temperature treatments (two CO_2 treatments $\times$ three temperature treatments $\times$ two chambers per treatment). CO_2 levels in the chambers were maintained at either 370 or 700 μmol mol^{-1}. The three air temperature regimes consisted of day/night temperatures of 26°/20°C (control; T_1), 30°/24°C (4°C increase for both day and night; T_2), or 28°/24°C (2°C increase for day and 4°C increase for night; T_3). In the T_1 and T_2 treatments, the daytime temperature was maintained for 10 h d^{-1}, from 0800 to 1800 hours, and the nighttime temperature for 14 h d^{-1}, from 1800 to 0800 hours Eastern Standard Time (EST). In the T_3 treatment, the daytime temperature was maintained for 14 h d^{-1}, from 0700 to 2100 hours, and the nighttime temperature for 10 h d^{-1}, from 2100 to 0700 hours EST, so that the mean temperature of T_3 was equal to that of T_2. Average daily temperature in T_1 was 22°C, and average daily temperature in T_2 and T_3 was 26°C. Thus, the two elevated-temperature treatments varied in daytime high temperature and daily duration of high temperature; plants in T_2 were exposed to a greater maximum temperature (30°C), but for only 10 h d^{-1}, while plants in T_3 were exposed to a cooler maximum temperature (28°C), but for 14 h d^{-1}.

Temperatures in the control treatment (T_1) were based on the 40-yr mean of summer temperatures (June–August) in eastern Massachusetts, according to data available from the National Climatic Data Center of the National Oceanic and Atmospheric Administration. The T_2 and T_3 treatments were based on predictions of global air temperature change within the next century (Cubasch et al. 2001; Dai et al. 2001; Wigley and Raper 2001).

The glass chambers were exposed to natural sunlight supplemented with light from metal halide lamps to partially compensate for some reduction in natural light by the glasshouse facility. When the photosynthetically active radiation (PAR) was lower than 500 μmol m^{-2} s^{-1} between 0800 and 1800 hours, which occurred on overcast days, the metal halide lamps were automatically turned on. As a result, the minimum PAR level in each chamber was 300 μmol m^{-2} s^{-1}. Daytime relative humidity was ca. 70% in all chambers.

Mature seeds of *P. americana* were collected from a population in Lexington, Massachusetts, in October 1999. On May 31, 2001, seeds were sown into horticultural starter trays. Seeds were stratified for 2 wk at 4°C and then placed in the glass chambers. On June 28, when plants had at least two true leaves, seedlings were transplanted into 5.0-L (17 cm diameter $\times$ 25 cm deep) plastic pots filled with a 1 : 4 mixture of garden soil : Pro-Mix general-purpose growing medium (Premier Horticultural Company, Red Hill, PA). This soil had a background nutrient level of 1.0–2.0 mmol L^{-1} N (NO_3^- and NH_4^+), and no additional fertilizer was added during the experiment. Plants were individually drip-irrigated to maintain soil moisture at 16%–27% by volume. Eight seedlings were randomly assigned to each of the 12 chambers (8 seedlings $\times$ 6 treatments $\times$ 2 replicate chambers), which resulted in 96 total plants in the experiment. The locations of individual plants within each chamber were randomized twice during the growth period to compensate for potential light differences within chambers.

Phenology and Biomass Measurements

As a measure of phenology, we recorded the elapsed time from planting to first flower opening for each individual. Once all plants had reached full flowering stage, 24 plants at the same stage of development were harvested. Total leaf area was measured for each of these plants in order to calculate plant-level transpiration rates. The remaining 72 plants were harvested once seeds were fully mature.

At each harvest, total leaf area per plant was measured with a LI-3100 area meter (Li-Cor, Lincoln, NE). The biomass of each plant was partitioned into leaf, stem, reproductive (fruit), and root components and dried at 65°C to constant weight.

Physiological Measurements

Leaf-level net photosynthesis at saturating light (A_{sat}) and stomatal conductance (g) were measured at the seedling, flowering, and mature seed stages of plant development (65, 91, and 119 d after planting, respectively). Values of A_{sat} and g were measured with an open path gas exchange system

Table 1

Results of Multivariate ANOVA for the Effects of the CO_2 and Temperature Treatments

Source	df (H)	df (E)	Wilks's λ	*P*
CO_2	4	63	0.498	<0.0001
Temperature	8	126	0.420	<0.0001
CO_2 × temperature	8	126	0.817	0.1096

Note. The multiple-response variables included total biomass, reproductive biomass, biomass allocated to reproduction, and root-to-shoot ratio of *Phytolacca americana*. The parameters df (H) and df (E) denote the degrees of freedom for the hypothesis and error sum of squares cross-product matrices, respectively.

(LI-6400 portable photosynthesis system) with a red-blue light source and a CO_2 mixer (Li-Cor). Measurements were conducted on eight plants within each of the six treatments. During both the A_{sat} and g measurements, leaf cuvette temperature was maintained at the level of the daytime temperature treatment in which the plant had been grown, and relative humidity in the leaf cuvette was kept at 50%–65%. The reference CO_2 concentration in the leaf cuvette was maintained at 370 μmol CO_2 mol^{-1} in the ambient-CO_2 treatment and 700 μmol CO_2 mol^{-1} in the elevated-CO_2 treatment. The saturating photosynthetic photon flux density (PPFD) was set at 1500 μmol m^{-2} s^{-1}.

Plant-level transpiration rate (g H_2O m^{-2} d^{-1}) was measured as whole-plant water loss over a 24-hr period. Transpiration was measured in 24 plants at the full flowering stage. Before measurement, the soil of each plant pot was well watered and then sealed with plastic attached both to the edges of the pot and to the base of the plant stems to contain water evaporated from the soil. Mass of the pots was measured before and after the 24-hr period. Immediately after transpiration measurements were completed, the 24 plants were harvested, and total standing leaf area per plant was measured. The daily transpiration rate was calculated for each plant by dividing the 24-hr water loss by total standing leaf area.

Statistical Analyses

Effects of the CO_2 and temperature treatments on total biomass, reproductive biomass, biomass allocated to reproduction (defined as reproductive biomass divided by the sum of the above- and below-ground vegetative biomass), and root-to-shoot ratio (RSR; defined as root biomass divided by aboveground biomass) were analyzed using a two-factor multivariate ANOVA (MANOVA). Wilks's λ was used to test for significance of treatment effects. The response variables listed above, as well as phenology (days to flower following planting) and plant-level transpiration, were also analyzed in a series of subsequent two-factor ANOVA tests to determine which dependent variables exhibited significant responses to the experimental treatments. All ANOVAs were performed using the general linear models procedure, employing type III sums of squares. Significant effects were explored using Scheffé post hoc tests for subsequent multiple comparisons. Two-way repeated-measures ANOVA (Scheiner and Gurevitch 2001) tests were used to determine the main fixed effects of CO_2 and temperature on A_{sat} and g, and their interaction over time. All analyses were conducted in SAS, version 8.01 (SAS Institute 1999). Biomass variables were log-transformed to meet assumptions of normality and homogeneity of variances. Untransformed means are presented in the figures.

Results

Total Biomass and Biomass Allocation

Results from the MANOVA indicated that both the CO_2 and temperature regimes had significant effects on total biomass and reproductive allocation, with no significant CO_2 × temperature interaction (table 1). Further ANOVA revealed that the total biomass of mature plants was significantly increased by elevated CO_2 (table 2), with enhancement ratios from 1.33 to 1.49 (biomass at elevated CO_2/biomass at ambient CO_2; fig. 1*a*). In contrast, total biomass was not significantly affected by a 4°C increase in mean daily temperature (cf. T_1 with T_2 and T_3). Reproductive output, however, was significantly increased by elevated mean temperature, but it was not significantly affected by elevated CO_2 (table 2; fig. 1*b*). Across temperature treatments, elevated CO_2 significantly increased RSR. In contrast, temperature significantly decreased RSR across the CO_2 treatments (table 2; fig. 1*c*). Total biomass, reproductive output, and RSR all exhibited no significant response to changing daily temperature regime (cf. T_2 with T_3; fig. 1).

Both elevated CO_2 and elevated temperature had significant effects on reproductive allocation (reproductive biomass/sum of above- and below-ground vegetative biomass; table 2). Elevated CO_2 significantly decreased biomass allocation to reproductive organs, while elevated temperature significantly increased it under both ambient and elevated CO_2 (fig. 2). Plants exhibited no significant response in reproductive allocation to changing daily temperature regime.

Phenology

Both elevated CO_2 and increased temperature significantly advanced the timing of flowering (table 2; fig. 3). Plants

Table 2

***F* Values from Two-Factor ANOVAs**

Source	CO_2	Temperature	CO_2 × temperature
Total biomass	17.13^{**}	0.16	0.11
Reproductive biomass	1.21	6.91^{*}	0.53
Repro/vegetative biomass	20.53^{**}	33.58^{**}	1.13
RSR	9.70^{*}	35.90^{**}	0.55
Days to flowering	110.56^{**}	118.79^{**}	0.52
Transpiration rate	38.00^{**}	13.26^{*}	1.04

Note. ANOVAs were conducted separately for total biomass, reproductive biomass, biomass allocated to reproduction, root-to-shoot ratio (RSR), days to flowering, and whole-plant transpiration rate of *Phytolacca americana* grown under two CO_2 concentrations and three temperature regimes. Degrees of freedom in the model were 1 for CO_2 and 2 for temperature and CO_2 × temperature.

* $P < 0.01$.

** $P < 0.001$.

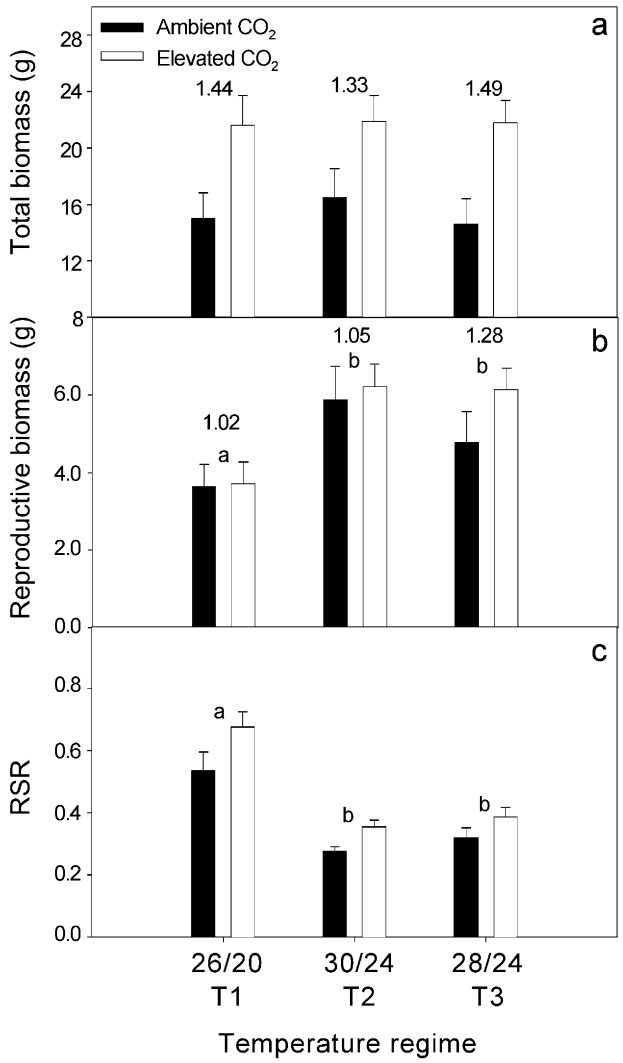

Fig. 1 Total biomass (*a*), reproductive biomass (*b*), and root-to-shoot ratio (*c*) of *Phytolacca americana* grown at three temperature regimes (day/night temperature 26°/20°C, 30°/24°C, or 28°/24°C) under either ambient (370 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2. CO_2 enhancement ratios (biomass at elevated CO_2/biomass at ambient CO_2) for total biomass and reproductive biomass are shown above each bar pair. Different letters above each bar pair in *b* and *c* represent a significant difference ($P < 0.05$) in means across CO_2 treatment, based on Scheffé post hoc comparisons. Mean + 1 SE is shown for each treatment ($n = 12$).

flowered an average of 3 d earlier under elevated CO_2 and an average of 6 d earlier under elevated temperature (cf. T_1 with T_2 and T_3). Changing daily temperature regime, however, did not affect time to flowering (cf. T_2 with T_3).

Leaf-Level Physiology

Leaf-level photosynthetic capacity at saturating light (A_{sat}) decreased with plant development (fig. 4*a*). At the time of flowering (91 d after planting), A_{sat} increased under elevated CO_2 but was not significantly affected by elevated temperature or changing daily temperature regime (table 3; fig. 4*a*). The CO_2 effect became less pronounced by the time seeds were mature (119 d after planting), thus contributing to a significant $CO_2 \times$ time interaction. Similarly, stomatal conductance (*g*) decreased with plant development (fig. 4*b*). Elevated CO_2 led to a significant reduction in *g* at both the flowering and mature seed stages, while elevated temperature and changing daily temperature regime did not significantly affect *g*, resulting in a significant temperature $\times$ CO_2 interaction (table 3; fig. 4*b*).

Transpiration

Plant-level transpiration rate significantly decreased under elevated CO_2 but increased under elevated temperature (table 2; fig. 5). In addition, changing daily temperature regime affected transpiration; transpiration rate was highest under conditions of the higher daily temperature and shorter daily duration of high temperature, compared with the treatment with lower high temperature and longer daily duration of high temperature (cf. T_2 with T_3; fig. 5).

Discussion

Physiological Impacts of Elevated CO_2 and Temperature

Short-term photosynthetic rate is predicted to increase with rising atmospheric CO_2 concentrations, while stomatal conductance and transpiration are predicted to decrease (reviewed in Long 1999). All three physiological processes of *Phytolacca americana* in this study responded to elevated CO_2 as predicted. Rising temperatures, in contrast, are predicted to cause an increase in transpiration rate and stomatal conductance under well-watered conditions (Leuning 1995; Monteith 1995; Grace 1997). In our study, transpiration exhibited the predicted response, but stomatal conductance exhibited no response to increasing temperature. Furthermore,

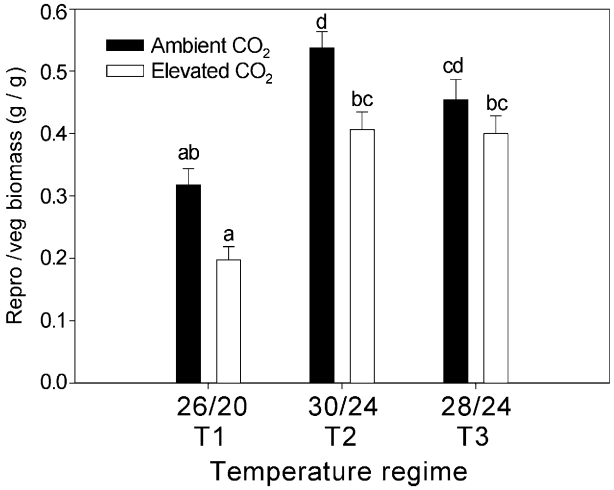

Fig. 2 Reproductive allocation of *Phytolacca americana*. Different letters above each bar represent a significant difference ($P < 0.05$) in mean ratios, based on Scheffé post hoc comparisons. Mean + 1 SE is shown for each treatment ($n = 12$).

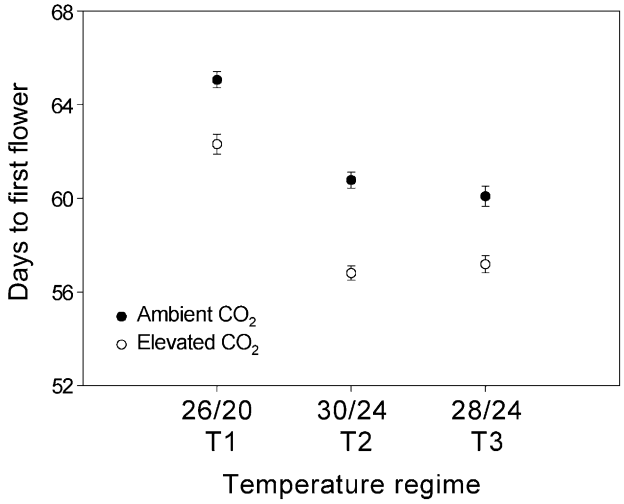

Fig. 3 Flowering phenology of *Phytolacca americana*. Mean ± 1 SE is shown for each treatment ($n = 12$).

it has been predicted that high temperatures may increase the magnitude of the positive photosynthetic response to elevated CO_2 (Farrar and Williams 1991; Long 1991; Kirschbaum 1994; Drake et al. 1997) because of potential increases in maintenance respiration and photorespiration in C_3 plants (Farrar and Williams 1991; Long 1991; Ryan 1991; Griffin et al. 2002) causing increased sink demand for carbohydrates. While some evidence supports this prediction (Sage et al. 1995; Ziska and Bunce 1997; Turnbull et al. 2002), the results of the present study do not. The value of A_{sat} increased under elevated CO_2 but exhibited no further increase with warm temperatures. Thus, although we did not directly measure respiration, the photosynthesis results indicate that respiration did not increase, or it may not have increased enough under warm temperatures to increase carbohydrate sink demand detectably. Recent studies with Douglas fir (*Psuedotsuga menziesii*) seedlings also found that the effect of elevated CO_2 on net photosynthetic rate was largely independent of temperature (Lewis et al. 2001). This leads us to conclude that the CO_2 × temperature effect on photosynthetic rate found in other studies may be species specific or may occur primarily at temperatures higher than those applied in this study (cf. the 4°C temperature increase in this study and Lewis et al. 2001 and the 10°–11°C increase in Sage et al. 1995; Ziska and Bunce 1997; Turnbull et al. 2002).

Of all the variables measured in this experiment, transpiration rate was the only one to exhibit a response to change in daily temperature regime. Transpiration rate was highest in the elevated-temperature treatment with the higher daily maximum temperature and shorter duration of high temperature. Thus, it appears that transpiration rate in a 24-hr period may be more strongly related to maximum temperature than to the duration of high temperatures throughout the day. Interestingly, plants exhibited a similar transpiration response to the daily temperature regime treatments at both ambient and elevated CO_2.

Biomass Accumulation and Allocation under Elevated CO_2 and Temperature

Many plants generally exhibit long-term, slightly elevated photosynthetic rates under elevated CO_2 (Drake et al. 1997; Curtis and Wang 1998; Morison and Lawlor 1999; Körner 2000; Sholtis et al. 2004), resulting in increased biomass accumulation (Jackson et al. 1995; Farnsworth et al. 1996; Jablonski 1997; Curtis and Wang 1998; Lilley et al. 2001). As predicted, *P. americana* responded to elevated CO_2 with an increase in total plant biomass, which likely could be attributed at least partially to the observed increase in A_{sat}.

In contrast, elevated temperatures could have a negative effect on plant biomass, in part because growth rate can increase (Ackerly et al. 1992), leaving determinate plants with less time for biomass accumulation before flowering (Rawson 1992; Morison and Lawlor 1999). However, some studies have reported that elevated CO_2 can at least partially compensate for the negative effect of high temperatures on plant biomass (Morison and Lawlor 1999; Lilley et al. 2001) or that the positive effects of elevated CO_2 on biomass are even greater at high temperatures (Farrar and Williams 1991; Hunt et al. 1996; Tjoelker et al. 1998). Despite these predictions, however, we found that *P. americana* exhibited no total biomass response to increased temperature, nor was there a significant CO_2 × temperature interaction.

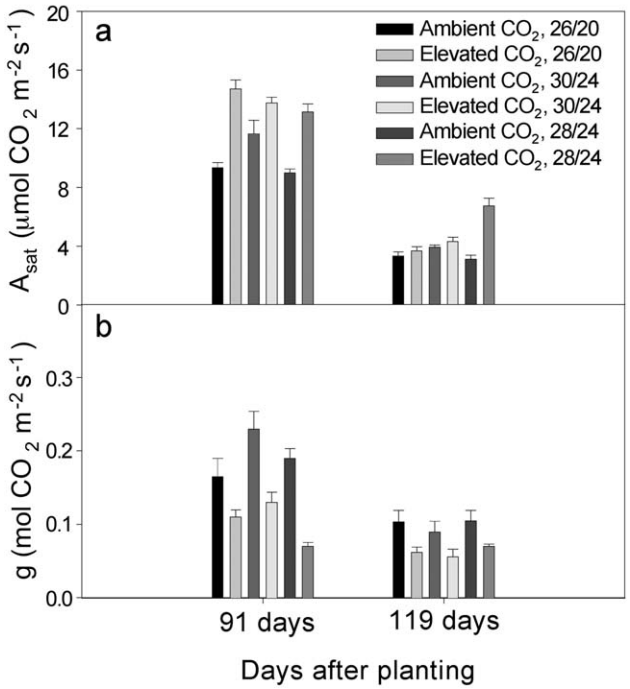

Fig. 4 Leaf-level net photosynthesis at saturating light (A_{sat}) and stomatal conductance (g) of *Phytolacca americana*. Measurements were taken at the following three stages of development: seedling (August 4, 65 d after planting), flowering (August 30, 91 d), and mature seed (September 27, 119 d). Results from the flowering and mature seed stages are shown because they are most relevant for comparisons with the biomass data presented in figs. 1 and 2 and the transpiration data presented in fig. 5. Mean + 1 SE is shown for each treatment ($n = 8$).

Table 3

Results of Repeated-Measures ANOVA for Leaf-Level Net Photosynthesis at Saturating Light (A_{sat}) and Stomatal Conductance (g)

Source	df (H)	df (E)	A_{sat} (μmol CO_2 m^{-2} s^{-1})	g (mol H_2O m^{-2} s^{-1})
CO_2	1	123	196.74^{***}	7.19^{**}
Temperature	2	123	0.91	0.67
Temperature × CO_2	2	123	3.38^{*}	23.17^{***}
Time	2	123	908.01^{***}	288.79^{***}
CO_2 × time	2	123	34.75^{***}	1.39
Temperature × time	4	123	3.56^{**}	0.45

Note. In the analysis, CO_2 and temperature treatments were fixed factors and time was the repeat factor. Parameters A_{sat} and g were measured three times: at the seedling, flowering, and mature seed stages of development. The parameters df (H) and df (E) denote the degrees of freedom for the hypothesis and error sum of squares cross-product matrices, respectively.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

If plants grown under elevated CO_2 produce excess carbohydrates, roots are predicted to act as carbohydrate sinks, and the RSR increases (Farrar and Williams 1991; Farnsworth et al. 1996; Gregory et al. 1997). In contrast, high temperatures have been shown to decrease RSR (Farrar and Williams 1991; Mitchell et al. 1993; Gregory et al. 1997). As predicted, the *P. americana* RSR increased with elevated CO_2 and decreased with elevated temperature, with no interaction between CO_2 and temperature responses.

Phenology and Reproductive Allocation Responses to Elevated CO_2 and Temperature

Some studies have shown that elevated CO_2 advances plant phenology (Garbutt and Bazzaz 1984; LaDeau and Clark 2001), but the response in reproductive allocation seems to vary among species and community types (Jackson et al. 1995; Farnsworth et al. 1996; Schäppi 1996; Jablonski 1997; Leishman et al. 1999; Amthor 2001). A free-air CO_2 enrichment (FACE) experiment in a 19-yr-old loblolly pine (*Pinus taeda*) plantation showed increased fecundity of forest trees because of both earlier reproductive maturation and higher proportional allocation to reproduction (LaDeau and Clark 2001). We found, however, that while elevated CO_2 advanced *P. americana* phenology, it decreased allocation to reproduction.

Elevated temperatures also have been shown to advance phenology (Seddigh et al. 1989; Rawson 1992; Moot et al. 1996; Morison and Lawlor 1999), but in some studies the advanced phenology left less time available for accumulating resources before fruit formation and reduced reproductive allocation (Mitchell et al. 1993; Moot et al. 1996). In some experiments, elevated CO_2 offset the negative effects of elevated temperature on reproductive allocation (Rawson 1995; Amthor 2001). In the present study, we found that phenology advanced and reproductive allocation increased under elevated temperatures, with no interaction between elevated CO_2 and temperature. This could be because *P. americana* is a perennial that exhibits indeterminate growth, whereas most studies reporting decreased reproductive allocation under elevated temperatures have been conducted on annual species exhibiting determinate growth (Mitchell et al. 1993; Rawson 1995; Moot et al. 1996; Amthor 2001). We speculate that in *P. americana*, the shorter time to reproduction allowed less time for resource accumulation before fruiting. However, whereas this contributes to a reduction in reproductive allocation in many determinate plants, in *P. americana* it may have contributed more strongly to reduced allocation to root biomass (as could be seen by the reduced RSR under high temperatures) and allowed more allocation to reproductive biomass. In fact, it has been shown that flowering can mark a transition when considerably less allocation to roots occurs (Gregory et al. 1997).

Conclusions

Predictions of leaf-level responses to elevated CO_2 and air temperature are founded on physiological theory and are often used as the basis for estimating net primary productivity responses to climate change in global carbon cycle models (Long 1991; Geider et al. 2001). However, whole-plant responses to elevated CO_2 and air temperature often do not follow the clear trends predicted from leaf-level responses. As predicted, we found that *P. americana*'s photosynthetic rate increased with elevated CO_2, while stomatal conductance and transpiration decreased. In addition, total biomass and RSR increased, but reproductive allocation decreased. In contrast, elevated temperature had no effect on photosynthetic rate, stomatal conductance, or total biomass, but it decreased the RSR and increased transpiration, reproductive biomass, and reproductive allocation. Both elevated CO_2 and warm temperatures advanced phenology. Although leaf-level

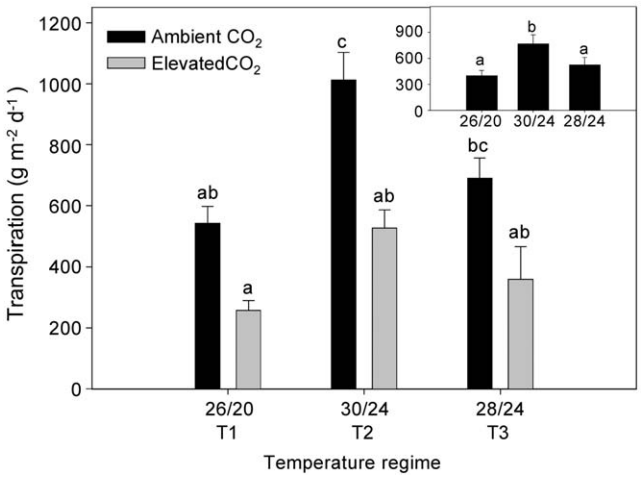

Fig. 5 Whole-plant transpiration rates (g H_2O m^{-2} d^{-1}) for *Phytolacca americana*. Mean + 1 SE is shown for each treatment ($n = 4$). The inset shows mean transpiration rate of each temperature regime across CO_2 treatments (values are mean + 1 SE, $n = 8$). Different letters above each bar represent a significant difference ($P < 0.05$) among the means, based on Scheffé post hoc comparisons.

photosynthetic models predict that biomass and reproductive responses should exhibit $CO_2 \times$ temperature interactions, *P. americana* exhibited no such interactions in this study. Thus, because such uncertainty exists in predicting whole-plant responses to changing CO_2 and temperature based on leaf-level responses, it is important to incorporate experimental measurements of whole-plant responses into predictive climate change carbon cycle models.

Furthermore, we found in this study that *P. americana*'s transpiration rate exhibited a unique response to changes in daily temperature regime, with transpiration increasing significantly when maximum daily temperature rose and exhibiting less response to changes in duration of high temperatures. Thus, we also suggest that careful consideration of changing daily temperature regime may be important for developing predictive understanding of plant and ecosystem responses to changing atmospheric CO_2 and air temperature.

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