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LEAF-LEVEL PHYSIOLOGY, BIOMASS, AND REPRODUCTION OF *PHYTOLACCA AMERICANA* UNDER CONDITIONS OF ELEVATED CARBON DIOXIDE AND INCREASED NOCTURNAL TEMPERATURE

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Rising atmospheric CO_2 and increasing air temperatures are predicted to increase future plant growth, but plant responses to increasing temperatures could be complicated by the fact that nocturnal temperatures may increase more than diurnal temperatures. The C_3 forb *Phytolacca americana* L. (Phytolaccacea) was grown under either ambient (370 μmol mol^{-1}) or elevated (740 μmol mol^{-1}) CO_2 in either of two nocturnal temperature treatments (26°/20°C or 26°/24°C day/night). We predicted that elevated CO_2 would increase photosynthetic rate and enhance plant biomass, while elevated nocturnal temperature would increase dark respiration rate and decrease biomass. Thus, increased nocturnal temperature was predicted to diminish the generally positive effects of elevated CO_2 on plant growth. Plants grown under elevated CO_2 responded as expected, with 69% greater photosynthetic rate and 35% larger whole-plant biomass for the first part of the growing season. Contrary to the predictions, however, increased nocturnal temperature had no negative effect on respiration rate or biomass. In fact, plants grown at higher nocturnal temperatures flowered 1.5 d earlier and exhibited a 32% increase in biomass allocation to reproduction. Thus, higher nocturnal temperatures did not diminish the generally positive effects of elevated CO_2 on *P. americana* growth. Instead, these results indicate that elevated CO_2 and increasing nocturnal temperatures of the future could have a neutral or even positive effect on *P. americana* population growth.

Keywords: C_3 forb, CO_2, nighttime temperature, phenology, photosynthesis, respiration.

Introduction

Atmospheric CO_2 concentration and air temperature are both increasing in the global environment (Easterling et al. 1997; Crowley 2000), and both are key variables affecting plant physiology, growth, development, and reproduction (Saxe et al. 2001). The consequences of rising atmospheric CO_2 and temperature could be profound for plants and ecosystems. Plants generally exhibit elevated photosynthetic rates (Drake et al. 1997; Curtis and Wang 1998; Norby et al. 1999; Körner 2000) and enhanced growth and biomass accumulation (e.g., Jackson et al. 1995; Jablonski 1997; Curtis and Wang 1998; Lilley et al. 2001; He et al. 2005) when grown under conditions of elevated CO_2. Under conditions of increased temperature, plant photosynthetic rate may increase (Saxe et al. 2001; Turnbull et al. 2002), but maintenance respiration rate may also increase (Ryan 1991; Bolstad et al. 1999; Tjoelker et al. 2001*b*), potentially leading to smaller plant size. Growth rates have been found to increase under elevated temperatures (Ackerly et al. 1992), but some studies on plants with determinate growth report reduced plant size because of a reduction in the time for biomass accumulation before flowering (Rawson 1992; Morison and Lawlor 1999).

When plants are exposed to both elevated CO_2 and increased temperatures, it has been shown that elevated CO_2 can at least partially compensate for the negative effects of high temperatures on plant biomass (Morison and Lawlor 1999; Lilley et al. 2001). In contrast, other studies report that the positive effects of elevated CO_2 on photosynthetic rate and biomass may be enhanced at high temperatures because of a respiratory-driven reduction in leaf carbohydrate sink size and increase in photosynthetic rate (Farrar and Williams 1991; Hunt et al. 1996; Tjoelker et al. 1998; Llorens et al. 2003). The interactive effects of increasing CO_2 and temperature have been investigated in a large number of experiments (e.g., Ackerly et al. 1992; Hunt et al. 1996; Stirling et al. 1998; Tjoelker et al. 1998; Wayne et al. 1998; Leverenz et al. 1999; Loiseau and Soussana 2000; Lee et al. 2001; Lewis et al. 2001; He et al. 2005; see reviews by Rawson 1992; Saxe et al. 1998; Morison and Lawlor 1999; Amthor 2001; Fuhrer 2003; Norby and Luo 2004; Pendall et al. 2004). More research is needed, however, because the temperature treatments in most of these studies have consisted of an increase in both diurnal and nocturnal temperatures by a constant increment. This is despite the fact that nocturnal temperatures are predicted to increase more than diurnal temperatures (Cubasch et al. 2001; Dai et al. 2001) and that increasing nocturnal temperatures may have different consequences for

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plant growth compared with constant-increment changes in both day and night temperatures. In particular, greater warming at night can increase dark respiration (Manunta and Kirkham 1996; Turnbull et al. 2002, 2004; Fu and Huang 2003), which could increase the ratio of dark respiration to photosynthesis and contribute to a decrease in plant growth (Manunta and Kirkham 1996; Fu and Huang 2003).

The interactive effects of elevated CO_2 and increased nocturnal temperatures on plants and ecosystems have not been well studied. In fact, to our knowledge, only two such studies have been conducted (Turnbull et al. 2004; Volder et al. 2004). Volder et al. (2004) found that *Phalaris aquatica* exhibited enhanced biomass production under elevated CO_2 but no biomass response to increased nocturnal temperature. In contrast, Turnbull et al. (2004) found that elevated CO_2 enhanced photosynthesis of *Populus tremuloides* saplings, while increased nocturnal temperature enhanced both dark respiration and photosynthesis. The authors speculated that elevated CO_2 might enhance the potentially positive effect of elevated nocturnal temperatures on whole-plant growth, but no whole-plant growth measurements were reported.

We report here the results of an experiment in which we measured both the leaf-level physiology and whole-plant growth responses of a C_3 plant, *Phytolacca americana* L. (Phytolaccaceae; common pokeweed), grown under conditions of elevated CO_2 and increased nocturnal temperature. In an earlier, separate experiment (He et al. 2005), we measured the leaf-level physiology and whole-plant growth responses of this species when grown under conditions of elevated CO_2 and increased diurnal and nocturnal temperatures. In that experiment, *P. americana* exhibited increased photosynthetic rate and biomass accumulation in response to elevated CO_2, and it exhibited no change in either, but an increase in biomass allocation to reproduction, in response to increases in both diurnal and nocturnal temperature together. In the experiment described here, we exposed *P. americana* to elevated CO_2 and increased nocturnal temperature to explicitly test how future increasing nocturnal temperatures could affect plant responses to future elevated CO_2. Furthermore, we explicitly measured nocturnal respiration in this experiment, which was not included by He et al. (2005), as a mechanistic explanation for any whole-plant biomass response to increased nocturnal temperature. We predicted a similar response to elevated CO_2 as that measured by He et al. (2005), with plants expected to exhibit greater photosynthetic rate, increased biomass accumulation, greater ratio of belowground biomass to aboveground biomass (root to shoot ratio [RSR]), increased reproductive biomass, and increased biomass allocation to reproduction (see also Amthor 1995; Saxe et al. 1998; Poorter and Navas 2003). When plants were grown under both elevated CO_2 and higher nocturnal temperature, however, we predicted that increased nocturnal temperature would increase dark respiration rate and decrease the ratio of gross photosynthesis to dark respiration, in accordance with the findings of Manunta and Kirkham (1996) and Fu and Huang (2003). Thus, elevated nocturnal temperatures were predicted to generally diminish the positive effects of elevated CO_2 on whole-plant growth, as measured by total biomass and RSR.

Material and Methods

Study Organism

Phytolacca americana is a polycarpic perennial herb common to much of eastern North America, ranging from Quebec and Ontario to northern Mexico (Caulkins and Wyatt 1990). Seeds used in this experiment were collected from a population in Lexington, Massachusetts, in October 1999. *Phytolacca americana* is predominantly an autogamous species (Armesto et al. 1983) and utilizes the C_3 photosynthetic pathway (Basinger 2002). In its natural habitat, adult plants can reach a height of more than 1 m and produce a taproot 30 cm in length (Uva et al. 1997). In the aforementioned earlier experiment (He et al. 2005), we found that *P. americana* exhibited a tendency toward decreased total biomass and reproductive allocation under conditions of narrowed diurnal temperature range (difference in daytime maximum and nighttime minimum temperature), indicating that this species may be particularly sensitive to changes in temperature regime.

Experimental Design and Growth Conditions

Plants were grown in 12 $1.0 \times 1.0 \times 2.0$-m environmentally controlled glass chambers at Harvard University, Cambridge, Massachusetts. The 12 chambers were arranged in a row within a greenhouse facility and divided into three blocks that minimized potential light differences among chambers within blocks. Each chamber within a block received one of four CO_2 and air temperature treatments (two CO_2 treatments $\times$ two temperature treatments). Atmospheric CO_2 concentration in the chambers was maintained at 370 (ambient treatment) or 740 μmol mol^{-1} (elevated treatment) for 24 h d^{-1}. Air temperature was maintained at diurnal/nocturnal temperatures of 26°/20°C (control treatment) or 26°/24°C (+4°C elevated nocturnal temperature treatment). Diurnal temperature and light conditions were maintained for 14 h d^{-1} from 0500 to 1900 hours EST; nocturnal temperatures and dark conditions were maintained for the other 10 h d^{-1}.

The length of diurnal/nocturnal temperature and light exposure was chosen to mimic the typical length of daylight hours in eastern Massachusetts during the month of August. Temperatures in the control treatment mimicked the 40-yr mean of summer temperatures (June–August) in eastern Massachusetts, as reported by the National Climatic Data Center of the National Oceanic and Atmospheric Administration. The elevated nocturnal temperature regime was set in accordance with predictions of global average air temperature change within the next century (Cubasch et al. 2001; Dai et al. 2001; Wigley and Raper 2001).

During the day, plants were exposed to natural sunlight and supplemental light from metal halide lamps positioned over each chamber. The supplemental lamps were used to partially compensate for reduction in natural sunlight caused by light filtration through the greenhouse facility and growth chambers. Maximum average photosynthetically active radiation (PAR) in the chambers on a clear, sunny day was 400 μmol m^{-2} s^{-1}; minimum PAR on overcast days was 120 μmol m^{-2} s^{-1}. Relative humidity was ca. 30%–40% in all chambers.

Eight plants were grown in each of the 12 chambers for a total of 96 plants in the experiment. Plants were grown in 6.2-L containers (15 cm diameter × 41 cm deep) filled with Metro-Mix 200 general purpose growing medium (Scotts, Marysville, OH). Each plant was fertilized with 19 g of Osmocote slow-release fertilizer (14% : 14% : 14% N : P : K by volume; Scotts) to maintain conditions of saturating nutrient supply. Plants were individually drip irrigated to maintain adequate soil moisture at 25%–35% by volume. The locations of individual plants within each chamber were randomized every 3–4 wk during the experiment to compensate for potential light differences within chambers.

Seeds were sown into horticultural starter trays on July 25, 2003, and stratified at 4°C for 2 wk. On August 9, seeds were moved to greenhouse chambers set at a constant temperature of 23°C. Half the seeds were placed in a chamber with an atmospheric CO_2 concentration of 370 μmol mol^{-1}, and half the seeds were placed in a chamber at 740 μmol mol^{-1} CO_2. Seedlings used in the experiment all germinated on August 18 and had at least four true leaves at the time of transplanting. On September 6, 19 d after germination, seedlings were transplanted into the 6.2-L containers and placed in chambers according to the CO_2 conditions under which they germinated. The diurnal/nocturnal temperature treatments were started at this time.

Leaf-Level Physiological Measurements

Leaf-level net photosynthesis at saturating light (A_{sat}), stomatal conductance (g), and nocturnal respiration were measured at three stages of plant development: preflowering (October 3, 27 d after germination), flowering (October 20, 44 d after germination), and fruiting or reproductive maturity (December 4, 108 d after germination). Measurements were taken on two plants per chamber at the preflowering and fruiting stages and on four plants per chamber at the flowering stage. The A_{sat} and g were measured with an open-path gas exchange system (LI-6400 portable photosynthesis system) with a red-blue light source and a CO_2 mixer (Li-Cor, Lincoln, NE). Measurements were taken during the day between 1000 and 1300 hours EST. During each measurement, leaf cuvette temperature was maintained at 26°C, and relative humidity in the leaf cuvette ranged from 25% to 40%. The reference CO_2 in the leaf cuvette was maintained at 370 μmol mol^{-1} in the ambient CO_2 treatment and 740 μmol mol^{-1} in the elevated CO_2 treatment. Flow rate was set at 500 μmol s^{-1}. Saturating PPFD was held constant at 1500 μmol m^{-2} s^{-1}; this PPFD level was determined in accordance with protocols established in earlier studies (He and Bazzaz 2003; He et al. 2005). The A_{sat} and g are reported here on a leaf area basis (μmol CO_2 m^{-2} s^{-1} and mol H_2O m^{-2} s^{-1}, respectively).

Nocturnal leaf-level respiration rate was measured on the same dates and on the same leaves as A_{sat} and g. Respiration measurements were taken between 2030 and 2330 hours EST; all plants had been in the dark and exposed to the nocturnal temperature treatments for at least 1.5 h by the time respiration measurements were started. We measured the plants in random order to compensate for potential changes in respiration rate throughout the night. During each measurement, leaf cuvette temperature was maintained at 20°C in the control temperature treatment and 24°C in the elevated temperature treatment. Relative humidity in the leaf cuvette ranged from 10% to 20%. The reference CO_2 in the leaf cuvette was maintained at 370 μmol mol^{-1} in the ambient CO_2 treatment and 740 μmol mol^{-1} in the elevated CO_2 treatment. Flow rate was set at 200 μmol s^{-1}. Plants were exposed to essentially no light; PAR levels measured below 0.5 μmol m^{-2} s^{-1} in the leaf cuvette during all measurements. Leaf-level respiration measurements are reported here on a leaf area basis (μmol CO_2 m^{-2} s^{-1}).

Whole-Plant Biomass and Phenology Measurements

An intermediate harvest was conducted during the experiment when all plants had reached the full flowering stage. This harvest was conducted on October 28–30, 71–73 d after germination. At that time, four plants from each chamber were harvested. Once the remaining plants had all produced mature fruits, we conducted the final harvest of the last four plants per chamber. This harvest was conducted December 9–17, 113–121 d after germination. At each harvest, total leaf area per plant was measured with a LI-3100 area meter (Li-Cor), and aboveground biomass of each plant was partitioned into leaf, stem, and reproductive components. In addition, at each harvest, root biomass of two plants in each chamber was excavated. Biomass was dried at 65°C to constant weight. As a measure of phenology, we recorded the elapsed time between germination and first flower opening for each plant.

Statistical Analysis

Effects of the CO_2 and temperature treatments on all plant responses were analyzed in a two-factor ANOVA framework for randomized block designs (Quinn and Keough 2002). Leaf-level physiological plant responses were analyzed separately for the preflowering, flowering, and fruiting stages. The physiological responses included net photosynthesis at saturating light (A_{sat}; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g; mol H_2O m^{-2} s^{-1}), and respiration (μmol CO_2 m^{-2} s^{-1}). Whole-plant responses were analyzed separately for the intermediate and final harvests. For the intermediate harvest, the whole-plant responses included total plant biomass, RSR (calculated as root biomass divided by aboveground biomass), total leaf area, and leaf mass per area (LMA; calculated as total leaf mass divided by total leaf area per plant). At the final harvest, the whole-plant responses included the four listed above as well as total reproductive biomass and biomass allocated to reproduction (calculated as reproductive biomass divided by the sum of the above- and belowground vegetative biomass). Phenology was measured as days elapsed between seedling emergence and appearance of the first flower and was analyzed separately in the same ANOVA framework.

For each response variable, we calculated the average value for each chamber and used these averages in the ANOVAs. Thus, each chamber was considered an experimental unit, and the experiment contained a total of 12 experimental units divided into three blocks (replicates). All ANOVAs were conducted in JMP version 4.0.2 (SAS Institute 2000), with the block term entered as a random effect, the CO_2 and temperature terms entered as fixed effects, and the expected mean square approach utilized. The Bonferroni procedure

was used to adjust significance levels when repeated measures were taken over the course of the experiment (Quinn and Keough 2002). Thus, results were deemed statistically significant for the physiological measurements when $P < 0.017$ ($\alpha = 0.05/3$) and for the whole-plant biomass responses when $P < 0.025$ ($\alpha = 0.05/2$). Reproductive measurements were conducted once during the experiments, so results were deemed statistically significant when $P < 0.05$.

Results

Leaf-Level Physiology

Net photosynthesis at saturating light (A_{sat}) was significantly greater under elevated CO_2 at the preflowering stage of plant development (table 1), with A_{sat} increased 69% (fig. 1). No other physiological measures at any stage of development exhibited a significant response to the CO_2 treatment. In addition, the elevated nocturnal temperature treatment did not elicit a significant response in any of the leaf physiological measures, including nocturnal respiration, at any stage of development (table 1).

Whole-Plant Biomass, Biomass Allocation, and Phenology

Total plant biomass was significantly greater under elevated CO_2 at the intermediate harvest (table 2), with an enhancement ratio of 1.35 (elevated CO_2 divided by ambient CO_2; fig. 2), although by the end of the experiment, this biomass enhancement was no longer apparent (table 2; fig. 2). In contrast, total plant biomass exhibited no significant response to the elevated nocturnal temperature treatment either during the growing season or at the end (table 2), and RSR exhibited no significant response to either the CO_2 or temperature treatment at any point in the growing season (table 2). During the growing season, total leaf area was unaffected by elevated CO_2 and was significantly greater under elevated nocturnal temperatures (table 2), while total leaf mass was significantly greater under elevated CO_2 and was unaffected by nocturnal temperature (results not shown). Consequently, LMA during the growing season was enhanced under elevated CO_2 but was unaffected by elevated nocturnal temperature (table 2; fig. 2).

Phytolacca americana reproduction exhibited a significant positive response to elevated nocturnal temperature and no significant response to elevated CO_2 (table 2). Both reproductive biomass and allocation of biomass to reproduction (reproductive biomass divided by vegetative biomass) were greater in plants grown under high nocturnal temperatures (fig. 3). In fact, allocation to reproductive biomass was enhanced by 32% when nocturnal temperature was increased from 20° to 24°C. Furthermore, elevated nocturnal temperature significantly advanced phenological development (table 2), with plants flowering an average of 1.5 d earlier under the elevated nocturnal temperature treatment (fig. 3).

Table 1

Two-Factor ANOVA Results for Leaf-Level Physiological Measurements of *Phytolacca americana*

Dependent variable	CO_2	Temperature	CO_2 × temperature
Preflowering stage:			
Photosynthesis (A_{sat})	68.38***	0.04	12.28
Conductance (g)	0.22	0.79	10.07
Respiration	0.10	0.03	0.10
Flowering stage:			
Photosynthesis (A_{sat})	8.70	0.17	3.12
Conductance (g)	11.32	0.07	2.87
Respiration	0.69	1.08	1.22
Fruiting stage:			
Photosynthesis (A_{sat})	11.56	0.26	6.78
Conductance (g)	0.02	0.01	1.35
Respiration	11.44	1.16	0.87

Note. Plants grown under two CO_2 (ambient = 370 μmol mol^{-1}; elevated = 740 μmol mol^{-1}) and two nocturnal temperature (low = 26°/20°C day/night; high = 26°/24°C) treatments. Measurements included net photosynthesis at saturating light (A_{sat}; μmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g; mol H_2O m^{-2} s^{-1}) measured during the day and leaf-level respiration (μmol CO_2 m^{-2} s^{-1}) measured at night. Measurements were taken three times during the growing season while plants were at the preflowering stage, flowering stage, and reproductive maturity or fruiting stage. Degrees of freedom for the various terms in the model were as follows: block (2), CO_2 (1), temperature (1), CO_2 × temperature (1), and error (6). At the fruiting stage, data from one experimental unit within one block were missing, so the error term degrees of freedom was corrected to 5. Data shown are F values.

*** $P < 0.001$.

Discussion

Physiological and Biomass Responses to Elevated CO_2 and Increased Nocturnal Temperature

Most plants, particularly C_3 plants, exhibit short-term increased photosynthetic rates when grown under conditions of elevated CO_2. Although there is some question about long-term photosynthetic downregulation (e.g., Moore et al. 1999; Paul and Foyer 2001), plants generally exhibit at least slightly elevated photosynthetic rates over the long term (Drake et al. 1997; Curtis and Wang 1998; Morison and Lawlor 1999; Norby et al. 1999; Körner 2000; Sholtis et al. 2004; He et al. 2005). Furthermore, although early research indicated that elevated CO_2 may have an inhibitory effect on respiration (e.g., Amthor et al. 1992), recent research has demonstrated that elevated CO_2 has little direct effect on plant respiration rate (Amthor 2000; Amthor et al. 2001; Jahnke 2001; Tjoelker et al. 2001*a*). Thus, the long-term increase in leaf-level carbon fixation generally leads to increased plant growth and biomass accumulation under elevated CO_2 (e.g., Jackson et al. 1995; Farnsworth et al. 1996; Jablonski 1997; Curtis and Wang 1998; Lilley et al. 2001; Urban 2003; He et al. 2005). As expected, *P. americana* responded to elevated CO_2 in this experiment, with an increased photosynthetic rate, at least in the preflowering stage of growth, and no change in nocturnal respiration rate. Likely as a direct result of the early photosynthesis enhancement, *P. americana* responded to elevated CO_2, with greater biomass production partway through the growing season. Interestingly, the enhanced photosynthetic rate was not maintained throughout the life of the plants, perhaps because

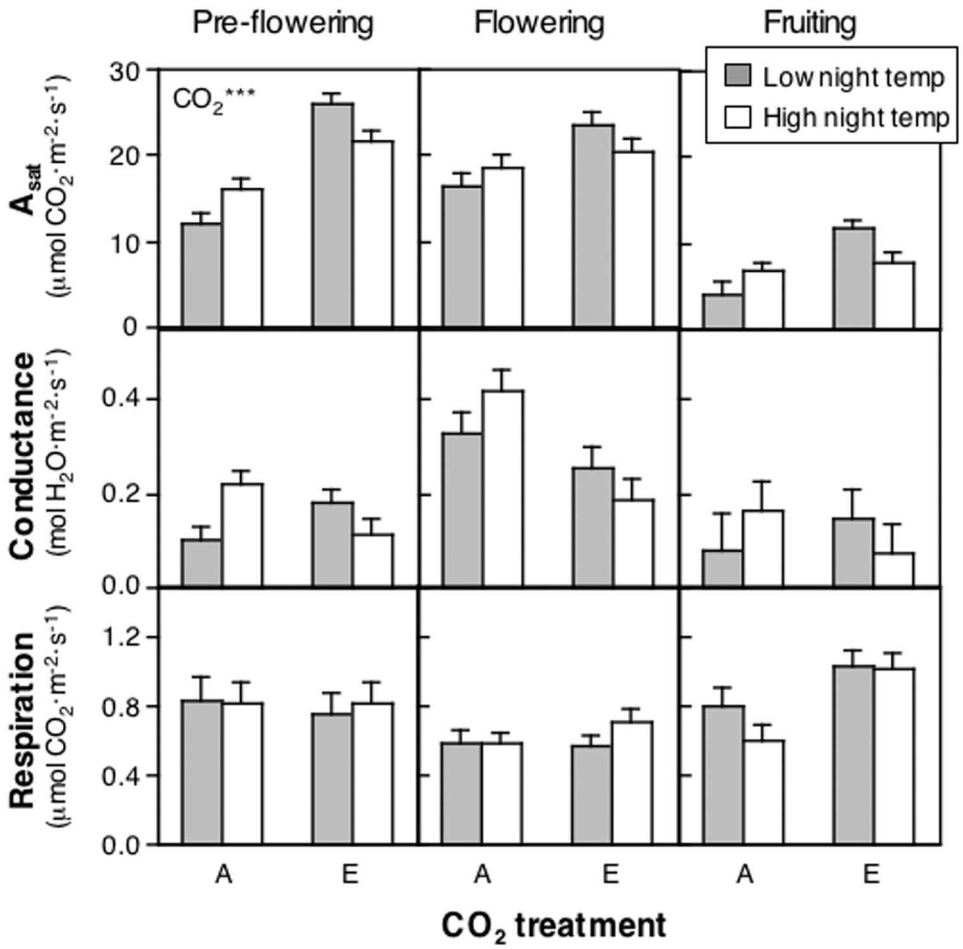

Fig. 1 *Phytolacca americana* leaf-level physiological responses to two CO_2 (ambient = 370 μmol mol^{-1}; elevated = 740 μmol mol^{-1}) and two nocturnal temperature (low = 26°/20°C day/night; high = 26°/24°C) treatments. Responses were measured at the following three stages of plant development: preflowering, flowering, and fruiting or reproductive maturity. Responses included net photosynthesis at saturating light (A_{sat}) and stomatal conductance (g) measured during the day and respiration measured at night. A_{sat} was measured as μmol CO_2 taken up m^{-2} s^{-1}, while respiration was measured as μmol CO_2 evolved m^{-2} s^{-1}. For clarity, both are shown as absolute values of CO_2 exchanged by the leaf. CO_2 treatments are abbreviated as ambient (A) and elevated (E). Mean ± 1 SE is shown for each treatment ($n = 12$; however, data were missing from one experimental unit within one block at reproductive maturity, resulting in $n = 11$ for the fruiting stage). Significant treatment effects (CO_2 or temperature, T) are indicated as follows: three asterisks, $P < 0.001$.

of carbohydrate accumulation in the leaves signaling photosynthetic downregulation (Moore et al. 1999; Paul and Foyer 2001), which could have diminished the whole-plant biomass response by the end of the growing season. It has also been reported that allocation of biomass can be affected by elevated CO_2, with plants exhibiting a greater ratio of belowground biomass to aboveground biomass (RSR; Farrar and Williams 1991; Gregory et al. 1997; Saxe et al. 1998; He et al. 2005) and increased LMA (Poorter and Navas 2003). Contrary to the predictions, however, the RSR in our experiment showed no response to elevated CO_2, although LMA at the intermediate harvest was enhanced. Perhaps the surplus carbohydrates produced by the upregulation of photosynthesis early in the growing season were stored in thicker leaves, rather than being translocated to roots for storage, and these accumulated leaf carbohydrates could then have contributed to photosynthetic downregulation.

Most experiments with increased nocturnal temperatures have measured an increase in dark respiration (Manunta and Kirkham 1996; Turnbull et al. 2002, 2004; Fu and Huang 2003). Some studies have shown that this leads to a subsequent increase in the ratio of dark respiration to photosynthesis and a decrease in plant growth (Manunta and Kirkham 1996; Fu and Huang 2003). Other studies, in contrast, report that increased dark respiration contributes to enhanced daytime photosynthesis because of a respiratory-driven reduction in leaf carbohydrate concentration (Turnbull et al. 2002, 2004), which has been hypothesized to contribute to a subsequent increase in plant growth with elevated nocturnal temperatures. In our experiment, we found no change in respiration or photosynthetic rates with increased nocturnal temperature. The lack of a respiration response may be due to the specific temperature treatments applied in this experiment (see discussion below), while the photosynthetic

Table 2

Two-Factor ANOVA Results for Whole-Plant Measurements of *Phytolacca americana*

Dependent variable	CO_2	Temperature	CO_2 × temperature
Intermediate harvest:			
Total biomass	63.06***	0.24	0.80
Root to shoot ratio	0.61	0.55	0.05
Total leaf area	0.11	20.11**	4.40
Leaf mass per area	19.81**	8.59	0.20
Final harvest:			
Total biomass	7.10	0.25	0.74
Root to shoot ratio	0.58	1.18	0.01
Total leaf area	1.23	0.97	0.07
Leaf mass per area	4.88	2.26	0.96
Reproductive biomass	1.98	7.70*	0.36
Reproductive/vegetative biomass	0.69	8.65*	0.03
Days to flowering	2.78	12.06*	0.16

Note. Plants grown under two CO_2 and two nocturnal temperature treatments. Measurements included total plant biomass, ratio of belowground to aboveground biomass (root to shoot ratio), total leaf area, and leaf mass per area at harvests conducted while plants were flowering (intermediate harvest) and when plants reached reproductive maturity (final harvest); reproductive biomass and the biomass allocated to reproduction (ratio of reproductive to vegetative biomass) at the final harvest; and days from seedling germination to appearance of the first flower (days to flowering). Degrees of freedom for the various terms in the model were as follows: block (2), CO_2 (1), temperature (1), CO_2 × temperature (1), and error (6). Data shown are *F* values.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

response is not surprising, given that elevated dark respiration is the hypothesized driving force behind potentially increased daytime photosynthesis rates (cf. Turnbull et al. 2002, 2004). The lack of a respiration or photosynthetic response likely caused the weak response in whole-plant biomass production under increased nocturnal temperatures: total plant biomass and RSR were unaffected, although allocation of biomass to reproduction increased under elevated nocturnal temperature and total leaf area significantly increased during the first part of the experiment.

The interactive effects of elevated CO_2 and elevated nocturnal temperatures on plant growth have been little studied. In the one other elevated CO_2 and elevated nocturnal temperature experiment in which physiological measurements were conducted, Turnbull et al. (2004) found that *Populus deltoides* saplings exhibited increased rates of both photosynthesis and respiration. They noted that the increase in photosynthetic rate was greater than that expected due to elevated CO_2 alone, however, because both elevated CO_2 and increased nocturnal temperature contributed to its enhancement. Thus, they speculated that saplings grown under elevated CO_2 and increased nocturnal temperature conditions should be larger than those exposed to either elevated CO_2 or increased nocturnal temperature alone. Another study reported enhanced plant productivity under elevated CO_2 but no productivity response to increased nocturnal temperature (Volder et al. 2004). In our experiment, we predicted that dark respiration rate would increase under conditions of elevated nocturnal temperature (Manunta and Kirkham 1996; Fu and Huang 2003), with no subsequent increase in photosynthetic rate during the day. Therefore, the ratio of dark respiration to gross photosynthesis would increase, leading to a decrease in biomass production and diminishment of the positive effects of elevated CO_2 on whole-plant growth. As expected, we found that *P. americana* plants responded to elevated CO_2 with elevated photosynthetic rate and enhanced whole-plant biomass, at least early in the growing season. But contrary to our predictions, we found that respiration rate did not increase and whole-plant biomass did not decrease under elevated nocturnal temperature. Interestingly, reproductive biomass and allocation of total biomass to reproduction did increase with elevated nocturnal temperature, indicating that allocation of total biomass to vegetative growth was reduced under elevated nocturnal temperature by the end of the experiment. However, we found no statistically significant interactive effect of CO_2 and nocturnal temperature on these plants. Perhaps the specific temperature treatments utilized in the experiment provide an explanation for the unexpected respiration response.

Experiments reporting an increase in respiration rate with increased nocturnal temperature have all been conducted with a range between the low and high temperature treatments of 5°C (Manunta and Kirkham 1996; Fu and Huang 2003) to 10°C (Turnbull et al. 2002, 2004), whereas our experiment was conducted with only a 4°C temperature differential. The temperature treatments in this experiment were chosen to represent realistic future scenarios for the northeastern United States (Cubasch et al. 2001; Dai et al. 2001; Wigley and Raper 2001), but it is possible that even higher temperatures may be required to elicit a detectable change in respiration rate for many plants. Moreover, populations of *P. americana* found in the northeastern United States, like those used in this experiment, tend to live at the northern end of the species' range (Caulkins and Wyatt 1990). Thus, perhaps the elevated temperature treatment in this experiment was actually closer to the optimal temperature for plants at the northern edge of the range than the nocturnal summertime temperatures they typically experience. In fact, generalizing plant responses to climate change can be difficult because many responses are known to be more sensitive to the specific temperatures applied experimentally than to the relative difference between high and low temperature treatments (Fuhrer 2003).

Reproductive Response to Elevated CO_2 and Increased Nocturnal Temperature

Some studies have shown that elevated CO_2 can advance plant phenological development (Garbutt and Bazzaz 1984; Ceulemans and Mousseau 1994; Farnsworth et al. 1996; LaDeau and Clark 2001; He et al. 2005) and increase reproductive biomass (Dhakhwa and Campbell 1998; Amthor 2001; LaDeau and Clark 2001; Jablonski et al. 2002; He and Bazzaz 2003; Prasad et al. 2003). However, some studies have reported little or even negative reproductive responses of grasses or short-day plants to elevated CO_2 (Huxman

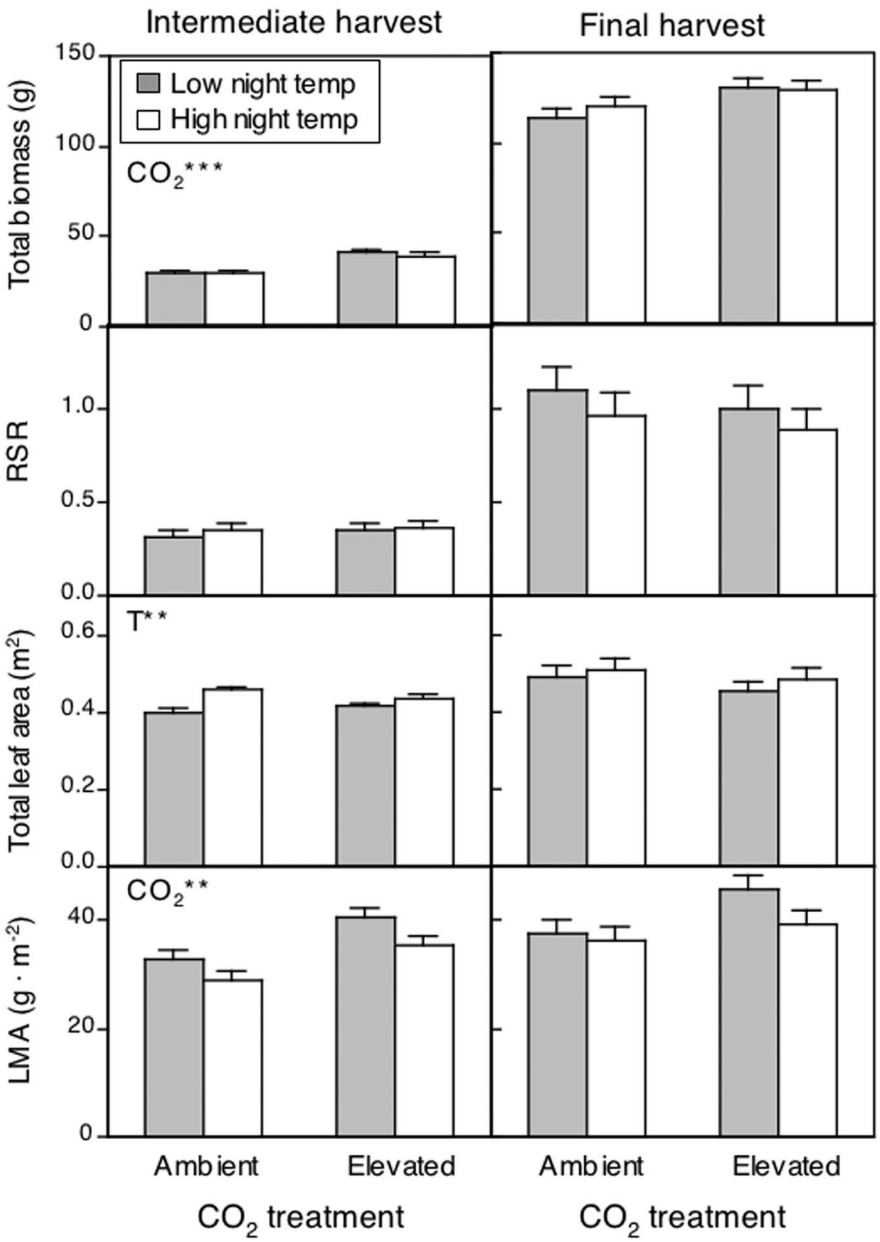

Fig. 2 *Phytolacca americana* responses at the whole-plant level to the two CO_2 and two nocturnal temperature treatments. Responses were measured during harvests conducted while plants were flowering (intermediate harvest) and when plants had reached reproductive maturity (final harvest). The responses included total plant biomass, ratio of belowground to aboveground biomass (*RSR*), total leaf area per plant, and leaf mass per area (*LMA*). Mean ± 1 SE is shown for each treatment ($n = 12$). Significant treatment effects are indicated as follows: two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

et al. 1999; Grünzweig and Körner 2000; Wagner et al. 2001; Lewis et al. 2003; He et al. 2005). In this experiment, we found that *P. americana* responded like other short-day plants, with no reproductive response to elevated CO_2; phenology, reproductive biomass, and biomass allocation to reproduction were all unchanged.

Elevated temperatures in general have been shown in many studies to advance plant phenological development (Mitchell et al. 1993; Moot et al. 1996; Dhakhwa and Campbell 1998; Fuhrer 2003; He et al. 2005). Accelerating plant development stages, however, can leave less time for accumulating resources before flower and fruit formation, and reproductive biomass can be reduced (Mitchell et al. 1993; Moot et al. 1996; Dhakhwa and Campbell 1998; Fuhrer 2003; Prasad et al. 2003). In our earlier experiment (He et al. 2005), however, reproductive biomass of *P. americana* and allocation of total biomass to reproduction were enhanced under elevated diurnal and nocturnal temperatures. Two studies focusing

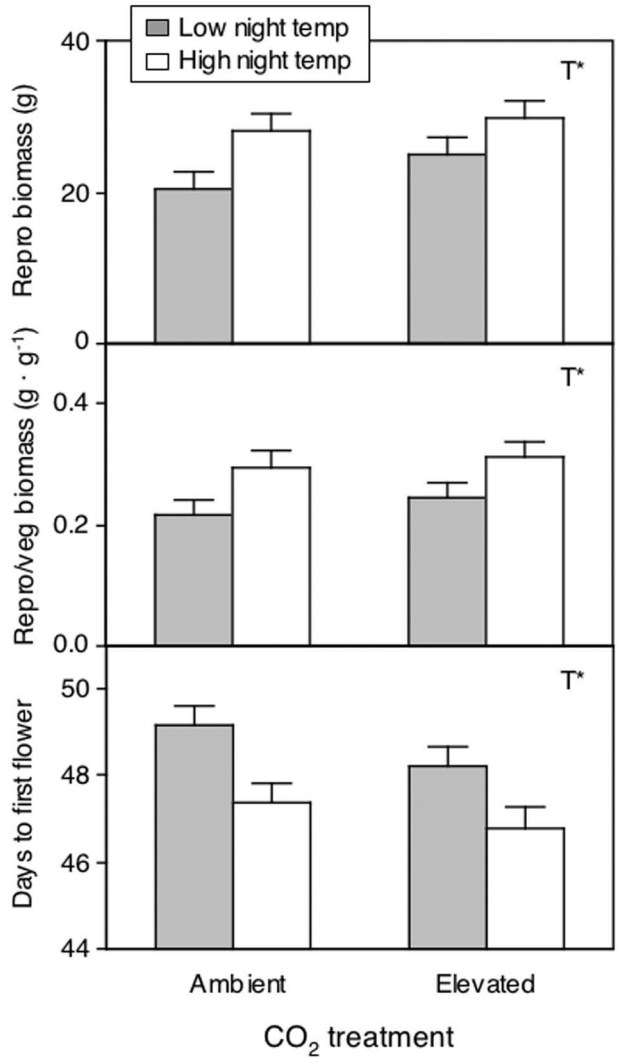

Fig. 3 *Phytolacca americana* reproductive and phenological responses to the two CO_2 and two nocturnal temperature treatments. Reproductive responses included total reproductive biomass and allocation of biomass to reproduction (repro/veg biomass) at the final harvest. The phenological response was measured as days elapsed between seedling germination and appearance of the first flower (days to first flower). Mean ± 1 SE is shown for each treatment ($n = 12$). Significant treatment effects are indicated as follows: asterisk, $P < 0.05$.

exclusively on increasing nocturnal temperature also reported that phenological development advanced (Seddigh et al. 1989; Manunta and Kirkham 1996), but neither study reported the effects on overall reproductive output. In this experiment, we found that *P. americana* time to flowering advanced under elevated nocturnal temperature and that, similar to its response to both elevated diurnal and nocturnal temperatures (He et al. 2005), reproductive biomass and allocation of total biomass to reproduction also increased. These results could be due to the fact that *P. americana* is a perennial plant that exhibits indeterminate growth, whereas most studies in which reproductive biomass decreased in response to elevated temperatures were conducted on agricultural species exhibiting determinate growth (e.g., Mitchell et al. 1993; Moot et al. 1996; Dhakhwa and Campbell 1998; Fuhrer 2003; Prasad et al. 2003). We speculate that the advanced phenological development of this indeterminate species likely contributed to the increase in reproductive biomass at the time of harvest, since plants exhibiting indeterminate growth, by definition, will grow indefinitely and must be harvested before they reach maximum size.

The advance in *P. americana* time to flowering and increase in reproductive output with elevated nocturnal temperature in this experiment lead us to conclude that development rate and reproduction for individual plants of this species could increase with future increasing nocturnal temperature, even potentially increasing population sizes, at least for plants growing at the northern edge of the species' range.

Conclusions

At the whole-plant level, *P. americana* grew larger in response to elevated atmospheric CO_2 and exhibited no negative plant size response to increased nocturnal temperature. These results directly reflect the physiology of the plants: photosynthetic rate was greater under elevated CO_2, while dark respiration rate, predicted to increase under higher nocturnal temperatures, exhibited no response to the nocturnal temperature treatment. Thus, as predicted, *P. americana* growing under these experimental conditions exhibited an increase in plant size in response to elevated CO_2 but, in contrast to our prediction, exhibited no diminishment of total plant size in response to elevated nocturnal temperature. Interestingly, time to flowering decreased and biomass allocation to reproduction increased under conditions of elevated nocturnal temperatures, indicating that increasing nocturnal temperatures could have a positive effect on *P. americana* population growth, at least at the northern end of its range. Thus, higher nocturnal temperatures did not diminish the generally positive effects of elevated CO_2 on *P. americana* growth in this experiment. In fact, since elevated CO_2 increased total plant biomass and higher nocturnal temperatures increased allocation to reproduction, the results indicate that elevated CO_2 and high nocturnal temperatures of the future could have a neutral or even positive effect on the growth of northern *P. americana* populations.

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