Density-dependent responses of reproductive allocation to elevated atmospheric CO_2 in *Phytolacca americana*

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Summary

• This study was conducted to determine whether elevated CO_2 alters patterns of plant reproduction, and whether density affects population- and individual-level responses to elevated CO_2.

• *Phytolacca americana* was grown in a glasshouse at three population densities under ambient and elevated CO_2 environments, and harvested at both vegetative and seed mature stages.

• CO_2 did not affect the observed or estimated minimum size required for reproduction. At the population-level, elevated CO_2 increased the total and aboveground biomass at both harvests. Density decreased both measurements at the second harvest. At the individual-level, elevated CO_2 increased reproductive mass but decreased seed size, and the responses of reproductive allocation were density-dependent. Net photosynthesis at saturating light (P_{max}) increased under elevated CO_2, but decreased with density, with a $CO_2 \times$ density interaction.

• These results indicate that CO_2 advances timing of flowering by changing growth rate rather than modifying minimum size required for reproduction, while density modifies the responses of reproductive allocations to elevated CO_2 in *P. americana*.

Key words: *Phytolacca americana*, reproductive allocation, reproduction, minimum size for flowering, density dependence, elevated CO_2.

Introduction

Under elevated atmospheric CO_2 concentrations, the future biodiversity of plant communities will depend on both changes in growth rates and reproductive success (Morison & Lawlor, 1999; Körner, 2000; LaDeau & Clark, 2001). If there is interspecific variation in the fitness under CO_2 enrichment, then advantages gained by some species over others could have dramatic consequences for future communities (Jackson *et al.*, 1994; LaDeau & Clark, 2001). Even if specific changes in reproduction are too small to detect, they may accumulate to large net effects (Gifford *et al.*, 1996).

Studies have recorded changes in the timing of flowering and fruit production under elevated CO_2, although the direction and magnitude of the changes are highly variable (Garbutt & Bazzaz, 1984; Garbutt *et al.*, 1990; Bazzaz *et al.*, 1992; Farnsworth & Bazzaz, 1995; Jackson *et al.*, 1995; Jablonski, 1997; Huxman *et al.*, 1999; Leishman *et al.*, 1999; Grünzweig & Körner, 2000; LaDeau & Clark, 2001). Plants within the same community may show very different phenological responses (Rusterholz & Erhardt, 1998). However, the data on the responses of plant reproduction patterns, that is the minimum size for reproduction, reproductive allocation and total reproductive biomass, of wild plant species to elevated CO_2 remain limited (Amthor, 2001; Saxe *et al.*, 2001).

The onset of reproduction is a key element in the life history of most organisms, and the age or size at maturity is a life history trait with high impacts on fitness (Stearns & Koella, 1986; Wesselingh *et al.*, 1997). In most perennials, size rather than age triggers the onset of reproduction (Harper, 1977; Lacey, 1986; Weiner, 1988). Because of the obvious link with fitness, many theoretical studies have explored the evolution of age and size at maturity (Rees *et al.*, 1999). Although studies showed that elevated CO_2 could cause plants to flower earlier (Farnsworth & Bazzaz, 1995; Rusterholz & Erhardt, 1998; Leishman *et al.*, 1999), little is known of whether the phenology was affected by changing growth rate or by modifying the flowering size.

Studies that have incorporated density–dependent interactions suggest that CO_2-induced growth enhancements are generally lower when individuals are grown in the presence of neighboring plants (Ackerly & Bazzaz, 1995; Wayne & Bazzaz, 1995; Retuerto *et al.*, 1996; Wayne *et al.*, 1999). When size hierarchies were compared between stands grown in ambient and elevated CO_2 environments, the variance in size hierarchies was reduced under elevated CO_2 (Wayne & Bazzaz, 1997) because elevated CO_2 can cause the suppressed understory individuals to be more productive in biomass (Wayne & Bazzaz, 1995; Kerstiens, 2001). This could lead to an alteration of the effective population size as more of the otherwise suppressed individuals produce seeds. Therefore, it is important to study CO_2 effect on populations rather than single individuals.

Several studies have distinguished between the expected responses of determinate and indeterminate plants to elevated CO_2 (Lawlor & Keys, 1993; Morison & Lawlor, 1999). Determinate plants are considered to offer only limited sink capacity for assimilates because their developmental regulation is strictly controlled and the number and size of organs are limited and fixed (Lawlor & Keys, 1993). Indeterminate plants, on the other hand, do not suffer from developmentally restricted capacity for growth or storage (Ho, 1988). For example, the biomass response to elevated CO_2 is greater in the plants that have nonfoliar starch storage organs, such as perennials and root crops (Pooter, 1993; Miglietta *et al.*, 1998). Thus, we expected that an increase in nonfoliar vegetative storage due to elevated CO_2 would promote reproductive output, if a positive linear relationship between reproductive output and plant size exists.

The objective of this study was to determine the effects of elevated CO_2 and density on the reproduction and biomass allocation in *Phytolacca americana*, an indeterminate perennial. In particular, we wanted to examine whether elevated CO_2 alters patterns of plant reproduction, that is the minimum size for reproduction, reproductive allocation and total reproductive biomass, whether stand-level and individual-level responses to CO_2 differ; and if population density modifies the effect of CO_2.

Materials and Methods

Study organism

Phytolacca americana L. (Phytolaccaceae), commonly known as pokeweed, is a polycarpic perennial herb common to much of the eastern United States, ranging from Quebec and Ontario south to northeastern Mexico (Caulkins & Wyatt, 1990). It is often abundant in open disturbed habitats, particularly in forest edges and canopy gaps (Sauer, 1952; Caulkins & Wyatt, 1990; Wilson & Shure, 1993). According to our investigation at Harvard University's Concord Field Station, MA, the first-year plants have an average height of 98 cm, with an average total biomass of 28 g, and the root depth of 15–25 cm, depending on soil conditions. The plant is a predominantly autogamous species (Armesto *et al.*, 1983). *P. americana* was selected for this study because it is an edge plant. As forest areas diminish in the future, edge habitats will become increasingly more common.

Experimental design and growth condition

Mature seeds of *P. americana* were collected from several individuals of a population in Lexington, MA, in October of 1999 and stored dry at 4°C for 6 months. On June 29, 2000, seeds were sown in $53 \times 40 \times 20$ cm plastic tubs (Consolidated Plastics Company, Inc, Twinsburg, OH, USA) filled with Pro-Mix general-purpose growing medium (Premier Horticultural Company, Red Hill, PA, USA). This soil has a background nutrient level of 70–150 mg L^{-1} NO_3^- N. To each tub, 9 g Osmocote (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) controlled release fertilizer (N: P: K = 14%: 14%: 14%), which released evenly over a 4-month period, was applied when seeds were sown. No further fertilizer was added during the experiment. Six holes were drilled into the bottom of each tub to provide drainage. Three density levels and two CO_2 levels (3 density $\times$ 2 CO_2) were applied in a complete factorial design. Tubs were randomly assigned to one of the 6 treatment combinations. There were 18 replicates per treatment (n = 108). Two CO_2 concentration levels (370 or 700 μmol CO_2 mol^{-1}) simulated atmospheric CO_2 concentrations of the present and the future. For the density treatments, 20, 100, and 500 seeds per tub were sown for the low, medium and high density, and produced an average of 8, 23 and 108 plants per tub (44, 126 and 592 plants m^{-2}) at the harvest (Table 1). These density levels were typical of seedlings as observed for *P. americana* growing in open habitats and forest edges. No thinning was carried out during the experiment.

Tubs were randomly assigned to an environmentally controlled (including CO_2) glasshouse at Harvard University (Cambridge, MA), which is divided into six separately controlled chambers. In three chambers, CO_2 concentration was maintained at 370 μmol CO_2 mol^{-1}, while CO_2 was maintained at 700 μmol CO_2 mol^{-1} in the other chambers. The

Table 1 Seeds sown and plants produced in the three density treatments. The mean number, standard error (SE), minimum and maximum of plants produced are listed

		Plants produced			
Density	Seeds sown	Mean	SE	Minimum	Maximum
Low	20	8.08	0.91	3	20
Medium	100	23.29	1.71	10	40
High	500	107.75	3.54	74	137

temperature in all chambers was kept at 25°C from 08.00 to 20.00 hours and at 19°C overnight. Lighting was provided by natural sunlight filtered through the roof of the glasshouse, which reduced light levels by about 28%. Tubs were arranged on one bench with the sides adjacent to form a population in each chamber, resulting in a rectangle of 3×6 tubs. Within each chamber, positions of the tubs were re-randomized once a week to reduce variation in growing conditions in the first 4 wk. From the fifth week, when the canopy was closed, a shade cloth wall was set around the rectangles to eliminate edge effects. The height of the shade cloth wall was set level with the top of the canopy and adjusted as the canopy height increased. Plants were watered daily throughout the experiment.

Harvesting

The first flowering occurred in an elevated CO_2 chamber on August 28. One week later, plants in ambient CO_2 began to flower. From September 2–4, after some plants in both treatments had started to flower, one third of the plants from both CO_2 treatments ($n = 36$) were harvested. To minimize edge effects, only 10 plants in the center of each tub of medium and high densities were used for further quantitative analysis. At low density, all plants in the tubs were used in order to maintain adequate and balanced sample size for quantitative analysis. However, the nontarget plants in medium and high density were used for the measurements of above-ground and below-ground biomass of the tubs. Leaf area was measured with a LI-3100 Area Meter (Li-Cor Inc., Lincoln, NE, USA). Roots were rinsed to remove soil particles. Plant material was dried to a constant weight at 65°C and weighed on an Acculab Lt-320 balance (Danvers, MA, USA). Dry weight was used to determine the biomass allocation. Specific leaf area ($cm^2\ g^{-1}$) was determined by dividing leaf area by the leaf dry weight of each plant.

The remainder of the plants ($n = 72$) reached the seed mature stage between November 15–22, at which point they were harvested, dried and weighed as above. In addition, fruit weight was determined for each plant and each tub. The total number of seeds produced per plant was determined by multiplying the mean number of seeds produced per 1 g of random fruits on that plant by the dry mass of fruits on that plant. Mean seed mass was determined by dividing seed weight, measured on an electronic semianalytical balance (Sartorius AG, Goettingen, Germany), by the seed number of 1 g of random fruits on each plant. Dry weights were used to calculate the percentage of total biomass allocated to roots, stems, leaves and reproductive structures for each plant.

Net photosynthesis at saturating light (P_{max})

P_{max} was measured at the full-flowering stage, which was developmentally determined for each treatment, using an open path gas-exchange system (Li-Cor 6400) with a red-blue light source and a CO_2 mixer (Li-Cor Inc., Lincoln, NE, USA). For each treatment, we measured two tubs, and three individuals in each tub. During all measurements, temperature in the leaf cuvette was maintained at 25°C and relative humidity was kept between 50 and 65%. Reference CO_2 concentrations were maintained during measurement at 370 μmol CO_2 mol^{-1} in ambient CO_2 and 700 μmol CO_2 mol^{-1} in elevated CO_2. The saturating photosynthetic photon flux density (PPFD) was 1500 μmol $m^{-2}\ s^{-1}$.

Data analysis

The reproductive mass of *P. americana* showed a highly positive linear relationship with plant total biomass ($R^2 > 0.84$, $P < 0.0001$) at all three densities. A simple model $F = a * (B - b)$ (Weiner, 1988) was used to estimate the flowering size, that is the minimum size required for reproduction, where F is the reproductive mass at ambient or elevated CO_2, B is the total biomass, a is slope of the relationship, and b is the flowering size. We used the method of Zar (1999) to compare simple linear regression equations under ambient and elevated CO_2. At the same time, the observed range of flowering size (height and total biomass) at ambient or elevated CO_2 was determined by connecting the size of the smallest flowering plant with the size of the largest non-flowering plant of the population (Wesselingh *et al.*, 1997). The differences in the size distributions of fruit biomass and total biomass for the flowering individuals at ambient and elevated CO_2 concentrations were tested using the Kolmogonov-Smimov nonparametric method (Sokal & Rohlf, 1995).

The data were analyzed separately at population (tub) and individual level using a two-factor multivariate analysis of variance (MANOVA) to test for the effects of CO_2 and density on the variables for population biomass and individual reproductive characteristics, because we measured a number of dependent variables and were interested in looking at the overall response across the suite of variables (Meekins & McCarthy, 2000; Scheiner & Gurevitch, 2001). The Wilks' Lambda was used to test for significance of each MANOVA. To determine which variable or variables was responsible for the difference in the CO_2 and density treatments, those variables that were analyzed in the MANOVA were also analyzed by subsequent ANOVA, using General Linear Model procedure, employing type III sums of squares. Significant results were explored using Scheffé *post hoc* tests for subsequent multiple comparisons. The MANOVAs and ANOVAs were performed using SAS version 8.01 (SAS Institute, 1999). All biomass variables were $\log_{10}$-transformed, and percentage data were arcsine transformed to meet assumptions of normality and homogeneity of variances.

Table 2 Results of multivariate analysis of variance (MANOVA) for the effects of CO_2 and density on total, above-ground, below-ground biomass, and root : shoot ratio (in both harvests), and fruit biomass and flowering ratio (Harvest 2) measured for populations 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

Source	df (H)	df (E)	Wilks' Lambda	*P*
Harvest 1				
CO_2	4	27	0.576	0.004
Density	8	54	0.715	0.299
$CO_2 \times$ Density	8	54	0.803	0.621
Harvest 2				
CO_2	6	61	0.643	< 0.001
Density	12	122	0.178	< 0.001
$CO_2 \times$ Density	12	122	0.758	0.128

Table 3 F values resulting from a two-factor analysis of variance (ANOVA) on total, above-ground, below-ground biomass, and root: shoot ratio (in both harvests), and fruit biomass and flowering ratio (Harvest 2) of *Phytolacca americana* populations grown under ambient and elevated CO_2 and at low, medium and high densities. Degrees of freedom in the model were as follows: CO_2 (1), density (2), and $CO_2 \times$ density (2). Significant symbols are as follows: $*P < 0.05$, $**P < 0.01$

Source	CO_2	Density	$CO_2 \times$ Density
Harvest 1			
Total biomass	14.33*	3.60	1.46
Above-ground biomass	17.27*	3.88	1.06
Below-ground biomass	5.53	2.20	0.26
Root : shoot ratio	0.24	0.21	1.28
Harvest 2			
Total biomass	21.66**	8.67**	0.71
Above-ground biomass	43.31**	18.39**	1.03
Below-ground biomass	2.54	0.67	0.13
Fruit biomass	15.59*	39.70**	0.32
Root : shoot ratio	10.06*	22.00**	0.21
Flowering ratio	2.69	112.98**	0.44

Results

Population level

For the first harvest (vegetative), results from the MANOVA indicated that only CO_2 had a significant effect (Table 2). Further ANOVA showed that CO_2 mainly affected total and above-ground biomass (Table 3). Elevated CO_2 significantly increased total biomass (Fig. 1a) and above-ground biomass (Fig. 1b). CO_2 showed the trend of increasing below-ground biomass (Fig. 1c), but the effect is statistically insignificant. RSR was not affected by the two factors (Table 3, Fig. 1d). The $CO_2 \times$ density interaction was insignificant.

Table 4 Parameter estimates for the relationship between fruit biomass and total biomass at ambient and elevated CO_2. The model $F = a * (B - b)$ was used, where F is the fruit mass at ambient or elevated CO_2, B is the total biomass, a is the slope parameter for the relationship, and b is the minimum size required for reproduction. n, number of samples. Because the relationship may depend on density, only samples of high density were used

	b (x-intercept) (± SE)	*a* (slope) (± SE)	*n*	R^2	*P*
Ambient CO_2	3.89 ± 0.403	0.293 ± 0.020	36	0.881	< 0.001
Elevated CO_2	3.71 ± 0.426	0.280 ± 0.017	61	0.847	< 0.001

At the second harvest (mature), results from the MANOVA indicated that both CO_2 and density had significant effects (Table 2). Further ANOVA showed that CO_2 and population density significantly affected total biomass, above-ground biomass, fruit production and RSR. Below-ground biomass was not affected by the two factors (Table 3). Elevated CO_2 significantly increased total biomass, above-ground biomass (Fig. 1e,f), and population fruit production (Fig. 2a). Medium and high density significantly increased RSR (Fig. 1h). There was no interaction between density and CO_2.

Only density influenced population flowering ratios (proportion of flowering plants which fruited) (Table 3). Flowering ratio declined from 79.7% at low density to 21.9% at high density (Fig. 2b). Elevated CO_2 had the trend to increase the flowering ratio (Fig. 2b), but the effect is statistically insignificant (Table 3).

The Kolmogonov-Smimov test indicated that the size distributions of flowering individuals between ambient and elevated CO_2 were different ($P < 0.05$). Flowering plants under elevated CO_2 had a larger proportion of smaller plants (total biomass under 10 g) than those of ambient CO_2, indicating that elevated CO_2 mainly increased the flowering ratio of smaller plants. Furthermore, there were larger size variations at elevated CO_2 than at ambient CO_2 (Fig. 3).

Fruit production was significantly ($P < 0.001$) and strongly ($R^2 > 0.84$) correlated with total biomass of the plants at all three densities (Fig. 4a–c). Only plants from high density were used to calculate the flowering size because of their biomass range (from 2.15 g to 32.51 g). There was no difference in the estimated flowering sizes between plants grown at ambient (3.89 ± 0.403 g) and elevated CO_2 (3.71 ± 0.426 g) ($P > 0.05$) (Table 4). The observed ranges of flowering sizes of populations at ambient and elevated CO_2 were 2.15–7.62 g and 2.18–11.61 g, respectively. There was no difference in the observed minimum flowering size. We also found that plants under a height of 0.61 m at ambient CO_2 and 0.59 m at elevated CO_2 did not bear flowers.

Individual level

Results from the MANOVA indicated that elevated CO_2 and density significantly affected resource allocation and

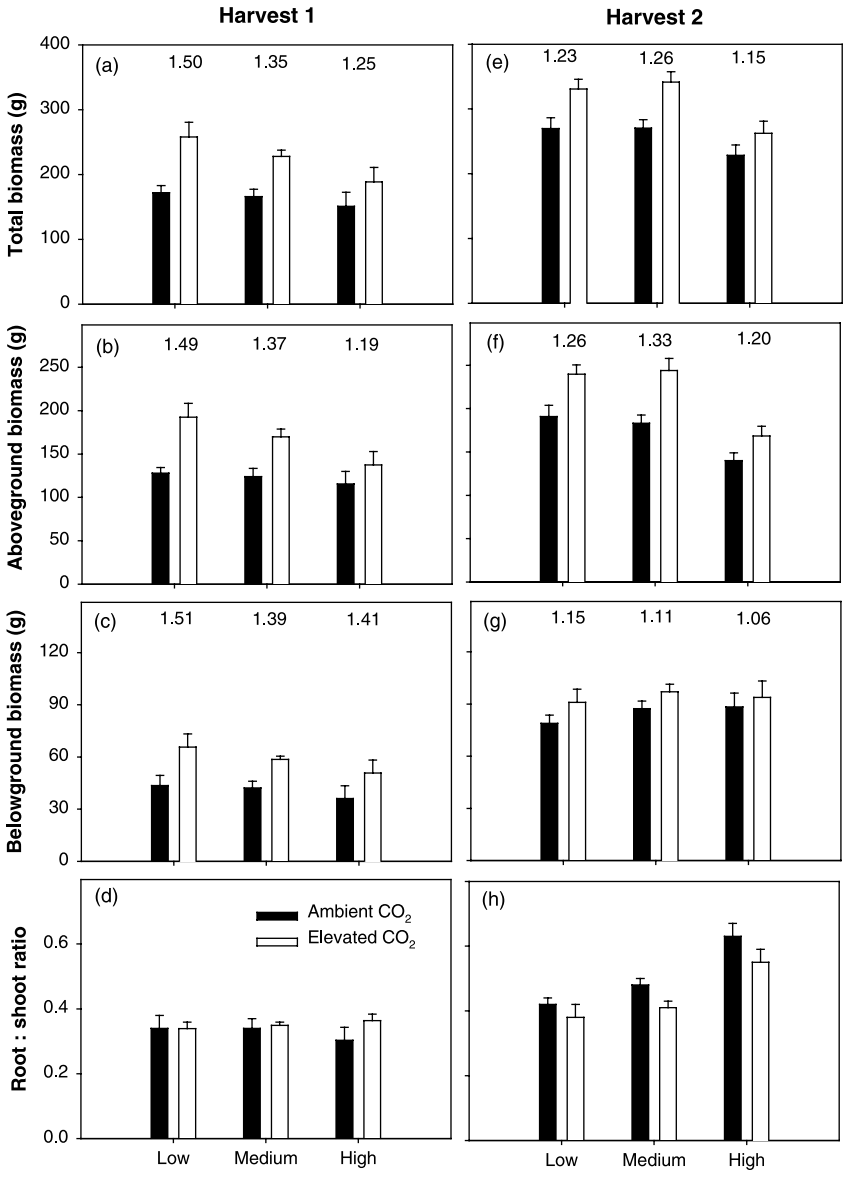

Fig. 1 Total population biomass, aboveground biomass, below-ground biomass, and root to shoot ratio (mean + 1 SE) of *P. americana* grown under ambient and elevated CO_2 and at low, medium and high densities in the first harvest (vegetative) and the second harvest (mature). CO_2 enhancement ratios (biomass at elevated CO_2/ambient CO_2) are shown above each bar pair. $n = 6$ in the first harvest and $n = 12$ in the second harvest.

Table 5 Results of multivariate analysis of variance (MANOVA) for the effects of CO_2 and density on the reproductive characteristics 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

Source	df (H)	df (E)	Wilks' Lambda	*P*
CO_2	5	92	0.365	< 0.001
Density	10	184	0.533	< 0.001
$CO_2 \times$ Density	10	184	0.665	< 0.001

reproductive characteristics at the individual level. The two–way interaction was also significant (Table 5). Further ANOVA indicated that elevated CO_2 significantly increased reproductive mass per unit leaf surface area (estimate of fecundity as a function of the potential for carbon gain of the vegetative structures, see Huxman *et al.*, 1999), and reproductive mass per plant (Table 6, Fig. 5b,d). Elevated CO_2 marginally increased seed number ($P = 0.077$) per plant. The effects of elevated CO_2 on reproductive allocation (reproductive mass per unit vegetative mass and reproductive mass per unit leaf surface area), were highly density-dependent (Fig. 5a,b). For example, the increase in reproductive mass per unit leaf surface area was observed only at low population density. The increase became less pronounced at high density (Fig. 5b). Elevated CO_2 can either decrease or increase the reproductive mass per unit vegetative mass in *P. americana* depending on population density. It decreased it at low density, but increased it at high density, while there was no effect at medium density, showing a significant $CO_2 \times$ density interaction (Table 6, Fig. 5a).

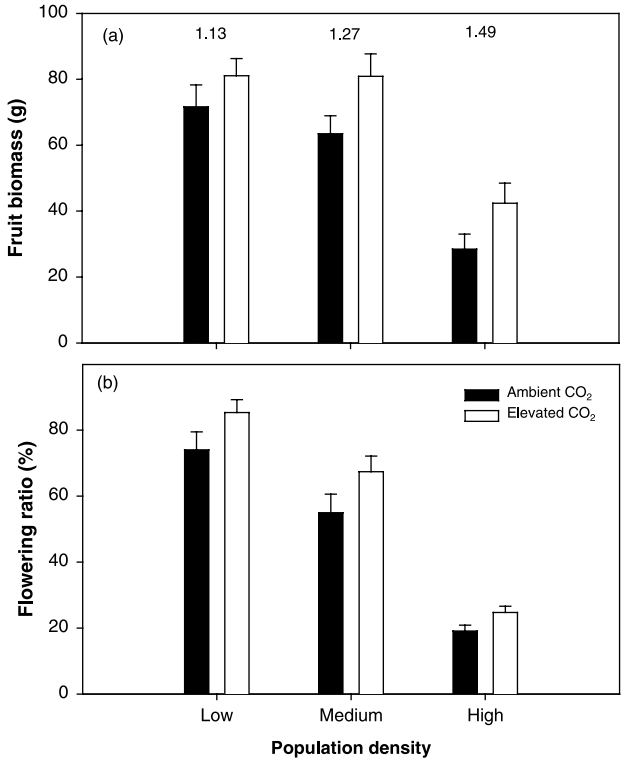

Fig. 2 Fruit biomass and flowering ratios (flowering plant number × 100/total plant number in each tub) of populations (mean + 1 SE, n = 12) of *P. americana* grown under ambient and elevated CO_2 and at low, medium and high densities in the second (mature) harvest. CO_2 enhancement ratios (fruit biomass at elevated CO_2/ambient CO_2) are shown above each bar pair in fruit biomass.

Density had large influences on reproductive allocation and fecundity (Table 6). High density significantly decreased reproductive mass per unit vegetative mass, reproductive mass per unit leaf surface area, seed number per plant and reproductive mass per plant (Fig. 5a–d). Elevated CO_2 significantly decreased seed size (Table 6).

Net photosynthesis at saturating light (P_{max}) and specific leaf area

Across all three density treatments, P_{max} increased 28% at elevated CO_2 ($P < 0.001$, ANOVA results not shown) (Fig. 6a). The effect of elevated CO_2 on P_{max} was also density-dependent, with increasing ratios 1.43, 1.19 and 1.16 at low, medium and high density, respectively, showing a significant CO_2 × density interaction ($P < 0.001$).

Elevated CO_2 significantly decreased specific leaf area (SLA) at all three density treatments (Fig. 6b). Across all density treatments, SLA decreased on average from 463 ± 30.7 $cm^2\ g^{-1}$ (Mean ± 1 SE) at ambient CO_2, to 270 ± 17.2 $cm^2\ g^{-1}$ at elevated CO_2, decreasing 42%. The density treatment did not affect SLA, but it significantly decreased total leaf area per plant. Elevated CO_2 slightly decreased total leaf area per plant (average total leaf area per plant was 3094 ± 373 and 2694 ± 325 cm^2 under ambient and elevated, respectively), but the effect was not statistically significant ($P = 0.344$). There was no CO_2 × density interaction in SLA or total leaf area per plant.

Discussion

Elevated CO_2 did not modify plant flowering size

There is little reason to expect increasing CO_2 to alter ontogenetic development. Temperature, interacting with environmental conditions such as photoperiod, is a key modifier of ontogenetic development rates (Morison & Lawlor, 1999). This is the case in some crops (Rawson, 1992), but in other species, larger effects of CO_2 on ontogenetic development have been observed. Depending on the species, elevated CO_2 can increase, decrease, or have no effect on flowering development. For example, elevated CO_2 caused plants to flower earlier in *Polygonum* spp., *Cassia* spp. (Farnsworth & Bazzaz, 1995), *Centaurea jacea*, *Betonica*

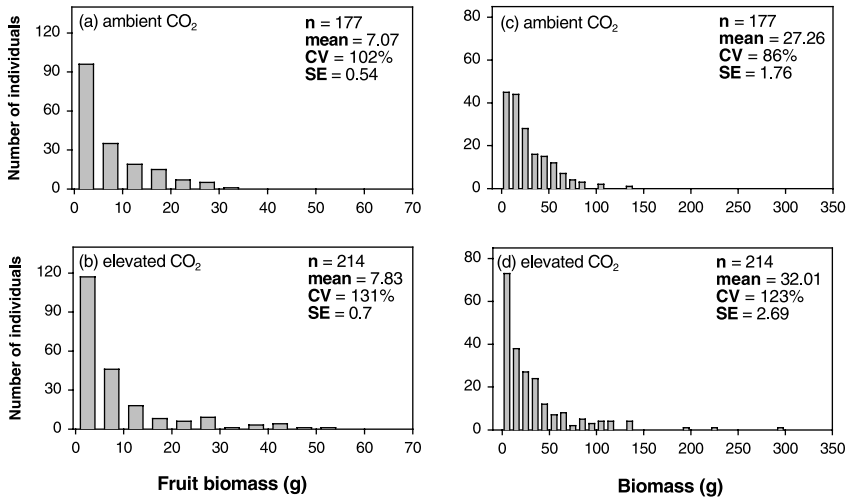

Fig. 3 Frequency distribution of fruit biomass and total biomass of flowering individuals of *P. americana* at ambient and elevated CO_2 concentrations across three densities in the second harvest (mature). Number (n), mean, coefficient of variance (CV) 1 SD × 100/mean, and standard error (SE) of the samples are shown in the figures.

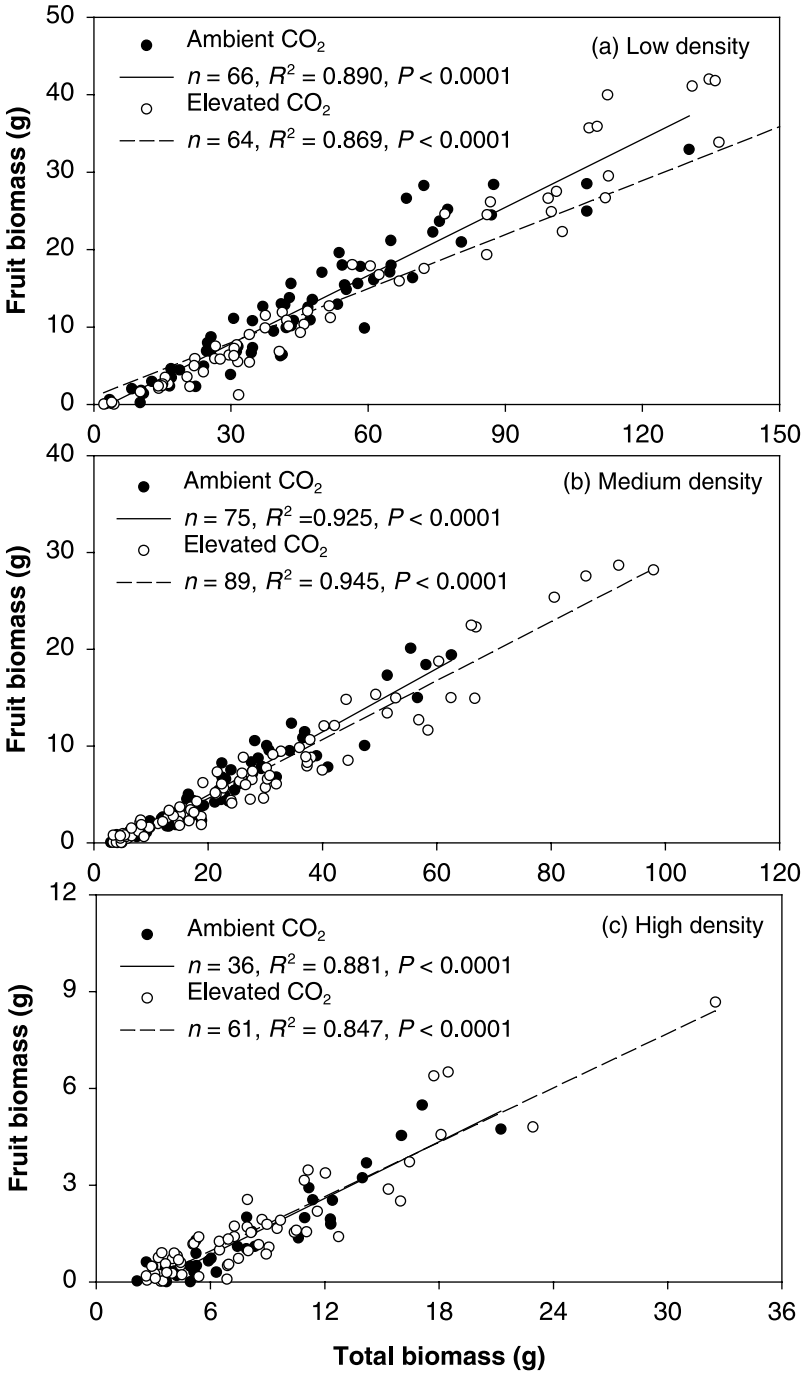

Fig. 4 The relationships between fruit biomass and total biomass in *P. americana* grown under ambient and elevated CO_2 and at low, medium and high densities. The linear regressions were not significantly different between ambient and elevated CO_2, or with density treatment. See also Fig. 5a. There was a significant interaction between CO_2 and density in fruit to vegetative biomass ratio.

Table 6 *F* values resulting from a two-factor analysis of variance (ANOVA) on reproductive characteristics of *Phytolacca americana* grown under ambient and elevated CO_2 and at low, medium and high densities. Degrees of freedom in the model were as follows: CO_2 (1), density (2), and $CO_2 \times$ density (2). Significant symbols are as follows: $^*P < 0.05$, $^{**}P < 0.01$

Source	CO_2	Density	$CO_2 \times$ Density
Seed number (no. plant^{-1})	3.19	31.83**	2.79
Seed size (mg)	9.33**	0.34	2.45
Reproductive mass per plant (g plant^{-1})	3.84*	73.29**	0.98
Reproductive mass per unit leaf surface area (mg cm^{-2})	16.45**	74.68**	5.91**
Reproductive mass per unit vegetative mass (g g^{-1})	0.52	28.10**	3.26*

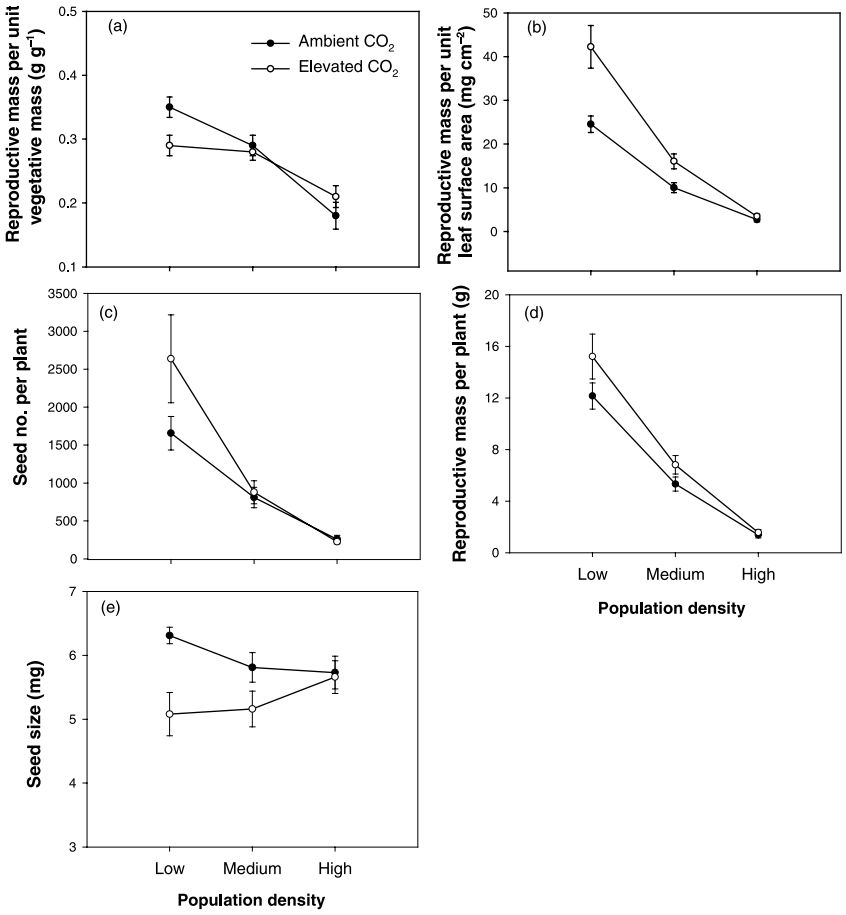

Fig. 5 Reproductive characteristics of *P. americana* grown under ambient and elevated CO_2 and at low, medium and high densities. Means and standard errors (mean ± 1 SE) are shown (n = 12 for each point).

officinalis (Rusterholz & Erhardt, 1998), *Datura stramonium* (Garbutt & Bazzaz, 1984), *Amaranthus retroflexus* (Garbutt *et al.*, 1990), *Senecio vulgaris* and *Poa annua* (Leishman *et al.*, 1999). In contrast, it caused other plants to flower later, such as *Setaria retroflexus* (Garbutt *et al.*, 1990). Reekie & Hicklenton (1994) reported that CO_2 advanced flower opening in four long-day species, but delayed flowering in four short-day species.

In the present experiment, we found that plants of *P. americana* at elevated CO_2 begin flowering 1 wk earlier than at ambient CO_2. Earlier flowering can be achieved through two mechanisms: modification of the flowering size, or alteration of the relative growth rate. Rather than modifying flowering size at which plants switch from vegetative growth to reproductive growth, CO_2 appears to affect phenology by changing growth rate in *P. americana*. In a previous study, it was found that elevated CO_2 decreased the number of leaves at flowering in *Guara brachycarpa* and *Oenothera laciniata* (Reekie & Bazzaz, 1991). We also noticed that if the number of leaves was measured as the critical size for flowering, two factors needed to be taken into consideration: the differences in specific leaf area (SLA), and the leaf size. SLA decreased with elevated CO_2 in this study, as well as in the others (Bazzaz, 1990; Huxman *et al.*, 1999). When flowering size was measured by total biomass, it is obvious that elevated CO_2 did not modify the flowering size of *P. americana*.

The density dependence of plant responses in reproductive allocation to elevated CO_2

Studies to date indicate that reproductive allocation varies widely in species and community types in response to climate change variables (Jackson *et al.*, 1995; Farnsworth *et al.*, 1996; Schappi, 1996; Jablonski, 1997; Leishman *et al.*, 1999). For example, it was found that high CO_2 caused significantly larger fruits in *Datura stramonium* (Garbutt & Bazzaz, 1984), more seeds per plant for the annual grassland species *Avena barbata* (Jackson *et al.*, 1994) and wild radish *Raphanus raphanistrum* (Curtis *et al.*, 1994), increased seed size (mass) (Garbutt & Bazzaz, 1984; Leishman *et al.*, 1999; Steinger *et al.*, 2000), unaffected seed size (Leishman *et al.*, 1999), or even decreased seed size in *Bromus rubens* (Huxman *et al.*, 1998; Huxman *et al.*, 1999). However, most of these results were based on plants grown individually, or at one density level.

Plant density is one of the important determinants of plant growth and reproduction (Harper, 1977). In a densely occupied habitat, there may be a decrease in individual plant biomass due to competition for resources (Grace & Tilman, 1990).

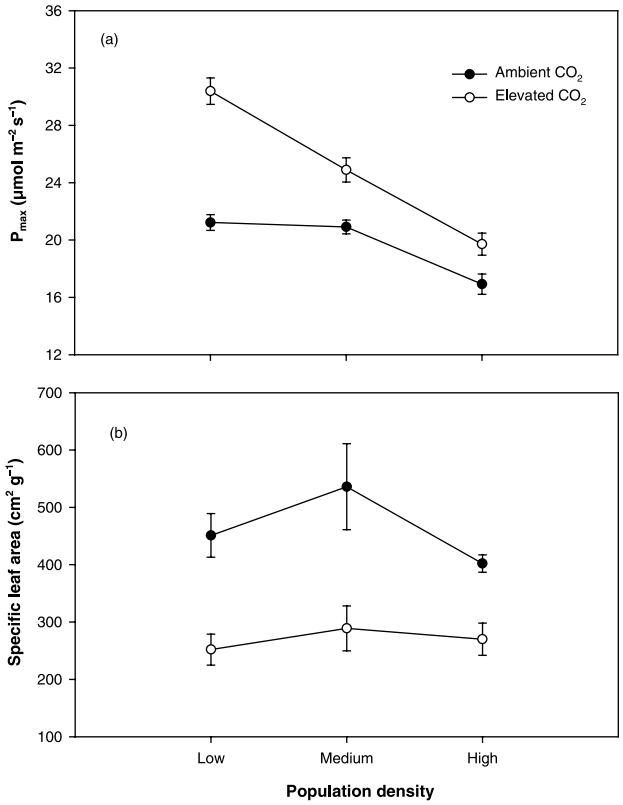

Fig. 6 *In situ* leaf maximum photosynthesis (P_{max}) (a) and specific leaf area (b) of *P. americana* at elevated and ambient CO_2 concentrations. Means and standard errors (Mean ± 1 SEM, n = 12) are shown.

Studies showed that the survivorship, the proportion of plants flowering and fruiting, the number of seeds per individual, the total seed production per population, and the mean seed mass, all declined with increasing density (Harper, 1977; Bazzaz *et al.*, 1992). At the population level, *Abutilon theophrasti* grown under higher density had lower total biomass (Casper & Cahill, 1998). In *P. americana*, we observed that at population level the interaction between CO_2 and density was insignificant, but at individual level, the effects of elevated CO_2 on reproductive allocation were density-dependent. For example, elevated CO_2 decreased the reproductive mass per unit vegetative mass at low density, but increased it at high density. Furthermore, elevated CO_2 mainly increased the flowering ratio of smaller plants. These results support the hypothesis that elevated CO_2 can lead to an alteration of the effective population size and population viability, as those suppressed species become more competitive in biomass production.

Farnsworth & Bazzaz (1995) showed that vegetative characteristics alone are poor predictors of fitness and future population dynamics. In their study, early vegetative growth responses to elevated CO_2 were not a strong predictor of subsequent reproduction in nine herbaceous annual species. Leishman *et al.* (1999) arrived at the same conclusion. However, the vast majority of agricultural predictive models use growth parameters alone to estimate future levels of carbon sequestration (Goudriaan *et al.*, 1999). In the present study, although total biomass is a good predictor for the reproductive responses to elevated CO_2, it was accompanied by production of smaller seeds, especially at low density. From an evolutionary perspective, the smaller seeds produced under elevated CO_2 may have a far-reaching impact on reproductive success or fitness, as experiments have shown that larger-seeded species may have advantages under hazards like drought, mineral nutrient deficiency, dense shade, clipping, and burial under litter and soil (Leishman *et al.*, 2000). The density-dependent effects of CO_2 enrichment on relative reproductive success and fitness can significantly influence the dynamics and microevolution of natural populations in the future environments (Curtis *et al.*, 1994; Bazzaz *et al.*, 1995).

Only a few researchers have evaluated the quality of seeds produced under elevated CO_2 conditions. For example, smaller seeds produced from elevated parental CO_2 growth conditions lead to seedlings that produce smaller leaves that are delayed in development and have smaller roots (Huxman *et al.*, 1999). However, Steinger *et al.* (2000) found that although elevated CO_2 increased seed mass, parental CO_2 growth conditions had no significant effect on seedling size. They argued that the advantage of increased seed mass at elevated CO_2 may be offset by the reduced concentration of nitrogen. The McGinley & Charnov (1988) model suggested that the optimal seed size should be positively correlated with the ratio of the carbon and nitrogen pools available for investment to offspring, and that there should be a negative correlation between seed size and absolute seed nitrogen content. Because low nitrogen content in seeds has been shown a consistent trend across other studies (Parrish & Bazzaz, 1985; Huxman *et al.*, 1999; Leishman *et al.*, 1999; Steinger *et al.*, 2000). More but smaller seeds in *P. americana* might be caused by the trade-off between seed number and seed size (Bazzaz *et al.*, 2000).

Indeterminate plants and RSR

The partitioning of resources to plant organs is the outcome of many processes (Reynolds & Thornley, 1982; Bazzaz, 1997). Models of root : shoot partitioning have been proposed which consider carbon and nitrogen supply and utilization as the driving variables controlling allocation and growth (Reynolds & Thornley, 1982; Levin *et al.*, 1989; Grace, 1997). Optimal partitioning models predict that plants respond to environmental variation by partitioning biomass among various organs or structures to optimize resource acquisition and maximize growth (Thornley, 1969; Hirose, 1987; Levin *et al.*, 1989; Bernacchi *et al.*, 2000). When plants are exposed to elevated CO_2, increased carbon acquisition results in a shift in allocation toward roots until root activity is proportionally enhanced. RSR is also influenced

by the development of reproductive structures, which represent competing sinks for carbohydrates (Ho, 1988; Johnson & Lincoln, 2000). It is expected that indeterminate plants grown under elevated CO_2 will have a proportionately greater allocation of assimilate to roots than plants grown under ambient CO_2, and that they will have a larger response (Johnson & Lincoln, 2000). Apparently, this is not the case for *P. americana*, in which elevated CO_2 had no effect on RSR at vegetative stage, but decreased it at seed maturation. Also contrary to prediction, three cultivars of *Raphanus sativus* and the wild, *R. raphanistrum*, differing in root to shoot ratios, did not differ in total biomass at mature stage under two levels of CO_2 (Jablonski, 1997). Other studies also found that responses of biomass allocation to elevated CO_2 were not consistent with optimal partitioning predictions (Bernacchi *et al.*, 2000). However, our study supports the hypothesis that allocation adjustments in response to CO_2 should be less pronounced or absent when soil resources are nonlimiting (Rogers *et al.*, 1996; Curtis & Wang, 1998).

Conclusion

Early successional species such as *P. americana* often occur in monospecific patches of varying density. It is therefore crucial to study CO_2 effects on populations of such plants rather than on single individuals. In all experimental populations, high CO_2 caused *P. americana* to flower earlier. Changes in plant growth rate appear to contribute more to the CO_2 effect than modifications in the size at which plants switch from vegetative growth to reproduction. Our study demonstrates that the responses to elevated CO_2 differ between the stand-level and the individual-level, with the latter showing a density-dependent response. It is unclear whether these density-dependent responses are species-specific. If so, the species-specific responses could significantly influence the dynamics of natural populations and community composition in future elevated CO_2 environments.

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