

Article

Evidence of Adaptation to Recent Changes in Atmospheric CO₂ in Four Weedy Species

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Abstract: Seeds of three C₃ and one C₄ annual weedy species were collected from agricultural fields in Beltsville, Maryland in 1966 and 2006, when atmospheric CO₂ concentrations averaged about 320 and 380 mol mol⁻¹, respectively. Plants from each collection year were grown over a range of CO₂ concentrations to test for adaptation of these weedy species to recent changes in atmospheric CO₂. In all three of the C₃ species, the increase in CO₂ concentration from 320 mol mol⁻¹ to 380 mol mol⁻¹ increased total dry mass at 24 days in plants from seeds collected in 2006, but not in plants from seeds collected in 1966. Shoot and seed dry mass at maturity was greater at the higher growth CO₂ in plants collected in 2006 than in 1966 in two of the species. Down-regulation of photosynthetic carboxylation capacity during growth at high CO₂ was less in the newer seed lots than in the older in two of the species. Overall, the results indicate that adaptation to recent changes in atmospheric CO₂ has occurred in some of these weedy species.

Keywords: CO₂; adaptation; photosynthesis; growth; weeds; carboxylation efficiency

1. Introduction

Gene frequencies in genetically diverse populations respond to environmental change, and unidirectional environment change should lead to shifts in gene frequencies. Rising atmospheric carbon dioxide concentration is such a unidirectional change. Tests of adaptation to imposed elevated CO₂ concentrations have been rather inconclusive [1,2], but that could be because the elevated CO₂ concentrations tested may be stressful in some ways, as evidenced, for example, by photosynthetic down-regulation [3]. The concentration of carbon dioxide in the atmosphere has been gradually increasing since the beginning of the Industrial Revolution in Europe, from a concentration of about 280 mol mol⁻¹ [4]. Because C₃ photosynthesis usually remains limited by CO₂ availability, even at the current concentration of about 400 mol mol⁻¹, the past increase has represented an increase in a growth-limiting resource for many plants [3]. Bunce [5] found that four annual weedy C₃ species were better adapted to the current atmospheric CO₂ concentration in several aspects, including photosynthetic carboxylation capacity, than they were to the pre-industrial concentration. This suggested that adaptation to recent changes in the atmospheric CO₂ concentration had probably occurred in these species. Comparisons of growth and photosynthetic characteristics of older and newer crop cultivars have had variable results, sometimes with higher rates in newer cultivars [6,7], but no differences in other cases [8,9]. However, in all of those studies plants were only grown at the current ambient CO₂, not at the prior concentration, so possible adaptation to the increase in CO₂ was not evaluated. The few tests in crop species of whether growth at projected higher future CO₂ concentrations selected for plants with higher growth rate at elevated CO₂ have sometimes, but not always, found higher growth rates [10,11]. Some studies have found that exposure of populations of non-cultivated plants to elevated CO₂ resulted in adaptation to the elevated concentration, as shown by more rapid growth rates

and/or increased reproduction [12–17], but there are other cases in which this did not occur [18–20]. In this study, I compared the response of both growth rates and photosynthetic properties to growth CO₂ concentration in seeds of four annual weedy species collected in the same location about 40 years apart in order to more directly test for evidence of adaptation to changes in CO₂ concentration in the recent past in these species. The primary hypothesis was that the growth of seedlings from newer seed lots would show greater increase in growth from 320 mol mol⁻¹ to 380 mol mol⁻¹ CO₂ concentration than plants would from the older seed lots. A secondary hypothesis was that plants from more recent seed lots would have less down-regulation of photosynthesis when grown at elevated CO₂.

2. Results

2.1. Seedling Growth

In all three of the C₃ species, the increase in CO₂ concentration from 320 mol mol⁻¹ to 380 mol mol⁻¹ increased the total dry mass at 24 days after planting in plants grown from seeds collected in 2006, but not in plants grown from seeds collected in 1966 (Table 1). Mean leaf area ratios for days 20 and 24 did not differ between seed lots, or with growth CO₂ in *A. theophrasti* or *C. album*. In *D. stramonium* and *A. hybridus*, mean leaf area ratios were decreased at the higher growth CO₂. Relative growth rate from day 20 to 24 differed between 320 mol mol⁻¹ and 380 mol mol⁻¹ only in the cases of *C. album* and *D. stramonium*, for the newer seed lots (Table 1).

Table 1. Seedling dry mass production for seed lots of four species collected in 1966 and 2006, grown at 320 and 380 mol mol⁻¹ CO₂ concentration. Total dry mass (DM, in grams) is for plants at 24 days after planting, and leaf area ratio (LAR, in cm² g⁻¹) and relative growth rate (RGR, in g g⁻¹ d⁻¹) are means for the period of 20 to 24 days after planting. Within species, values followed by different letters were different at $p = 0.05$, based on analysis of variance.

Species	Year of Collection	Growth CO ₂	Total DM	LAR	RGR
<i>A. theophrasti</i>	1966	320	4.9a	139a	0.29a
	1966	380	5.0a	134a	0.28a
	2006	320	4.0b	140a	0.21b
	2006	380	4.9a	136a	0.20b
<i>C. album</i>	1966	320	5.2a	127a	0.29a
	1966	380	5.3a	123a	0.31a
	2006	320	3.2b	122a	0.22b
	2006	380	5.6a	120a	0.29a
<i>D. stramonium</i>	1966	320	6.7b	154a	0.36a
	1966	380	7.1b	135b	0.32b
	2006	320	7.6b	145ab	0.32b
	2006	380	9.0a	116c	0.27c
<i>A. hybridus</i>	1966	320	4.1a	178b	0.30a
	1966	380	4.4a	134c	0.33a
	2006	320	3.7a	215a	0.32a
	2006	380	3.8a	165b	0.29a

2.2. Dry Mass at Maturity

In *C. album*, flowering did not occur in most of the individual plants from either era by the time of seed maturity of the other species, so for this species, only shoot biomass at 57 days after planting was obtained. The newer seed lot of *C. album* produced more shoot biomass, and more shoot biomass was produced at the higher than at the lower CO₂ for seed lots from both eras (Table 2). *A. theophrasti* had the same response pattern as in *C. album* for both total and seed biomass, with increases with growth CO₂ concentration, and higher mass for the newer seed lot at both CO₂

concentrations. In *D. stramonium*, total shoot and seed dry mass were increased by the growth CO₂ for both seed lots, but no differences occurred between seed lots. In *A. hybridus* total shoot and seed dry mass at maturity did not differ between 320 mol mol⁻¹ and 380 mol mol⁻¹ from either seed collection time (Table 2). Flowering occurred 6 days earlier (day 27 vs. day 33 after planting) in the newer seed lot in this species, which limited the final biomass accumulated in this determinate species.

Table 2. Total shoot dry mass (DM) and seed dry mass at seed maturity in four species from two years of seed collection, when grown at two CO₂ concentrations (mol mol⁻¹). Within species, values followed by different letters were different at $p = 0.05$, based on analysis of variance. na indicates not available.

Species	Year of Collection	Growth CO ₂	Total Shoot DM (g)	Seed DM (g)
<i>A. theophrasti</i>	1966	320	99c	30c
	1966	380	135b	41b
	2006	320	130b	40b
	2006	380	153a	62a
<i>C. album</i>	1966	320	46c	na
	1966	380	60b	na
	2006	320	59b	na
	2006	380	71a	na
<i>D. stramonium</i>	1966	320	315ab	172b
	1966	380	350a	193a
	2006	320	289b	172b
	2006	380	351a	205a
<i>A. hybridus</i>	1966	320	186a	78a
	1966	380	191a	82a
	2006	320	90b	62b
	2006	380	102b	60b

2.3. Photosynthesis

In the C₄ species, *A. hybridus*, the carboxylation efficiency of PEPcase was reduced by growth at the lowest and highest CO₂ concentrations (Figure 1). The reduction at the highest-growth CO₂ in the plants from 1966 was larger than that of plants from 2006 (Figure 1). Despite differences in carboxylation efficiency, rates of photosynthesis under the growth conditions were the same for all growth CO₂ concentrations in this species, and did not differ between seed lots, even at the highest-growth CO₂ (Figure 1).

In *A. theophrasti*, the carboxylation efficiency of Rubisco was also reduced at the highest-growth CO₂, but only in the plants from the 1960s (Figure 2). At the higher-growth CO₂, photosynthesis under the growth conditions was also lower in the older seed lot than in the newer. Photosynthesis under the growth conditions was also lower at the lowest growth CO₂ than at the intermediate CO₂ concentrations (Figure 2).

In *C. album*, carboxylation efficiency of Rubisco was unaffected by the growth CO₂, and never differed between old and new seed lots (Figure 3). Rates of photosynthesis under the growth conditions increased with growth CO₂ in both seed lots, and never differed between seed lots (Figure 3).

In *D. stramonium*, carboxylation efficiency of Rubisco was highest at the growth CO₂ concentration of 320 mol mol⁻¹ in both seed lots, and did not differ between old and new seed lots at any growth CO₂ (Figure 4). Photosynthesis under the growth conditions increased slightly with increasing growth CO₂, up to 380 mol mol⁻¹ in this species (Figure 4).

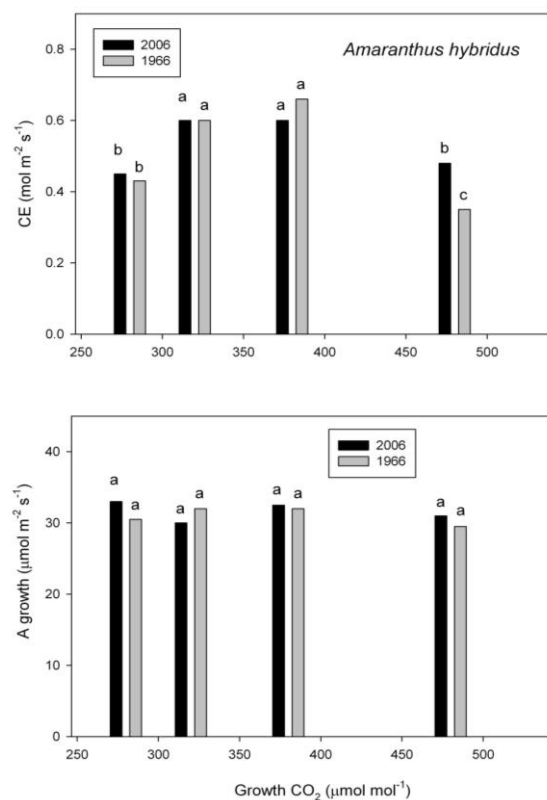


Figure 1. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Amaranthus hybridus* grown at four CO₂ concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.

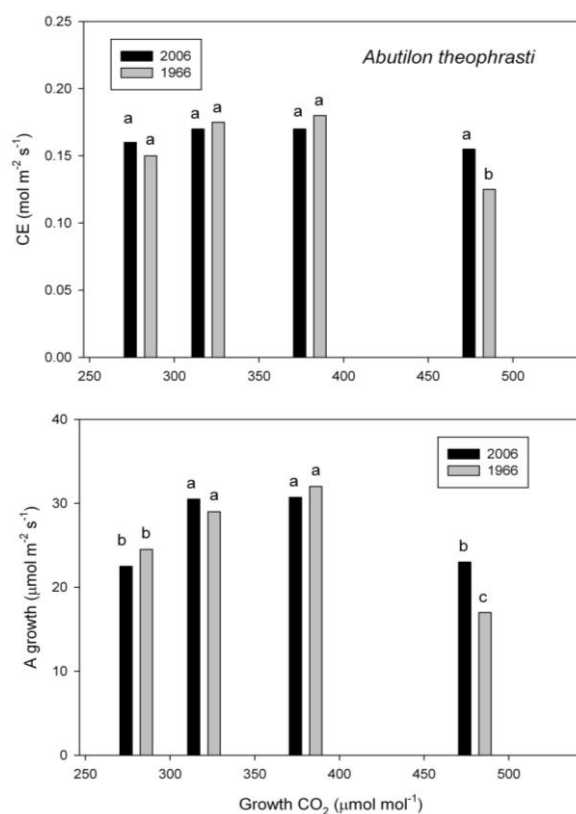


Figure 2. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Abutilon theophrasti* grown at four CO₂ concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.

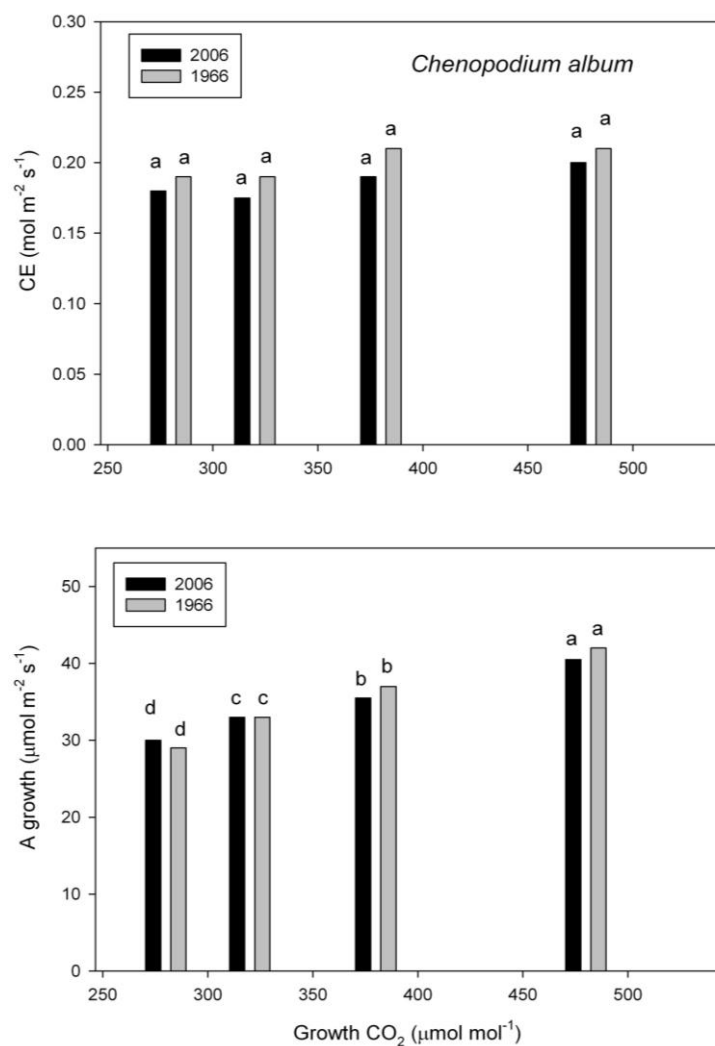
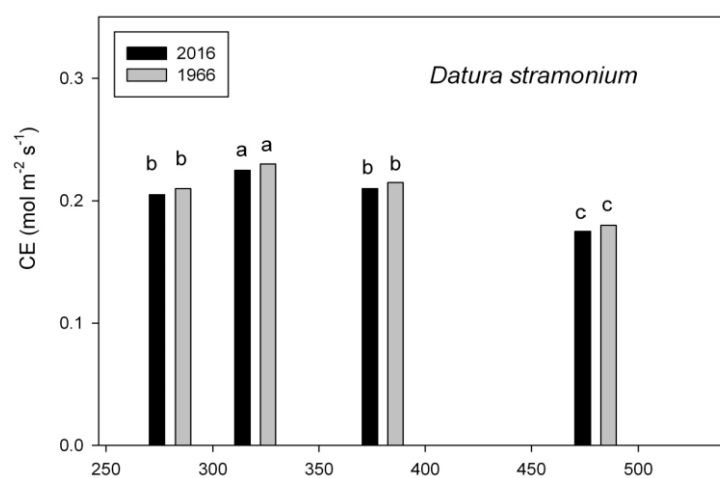


Figure 3. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Chenopodium album* grown at four CO₂ concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.



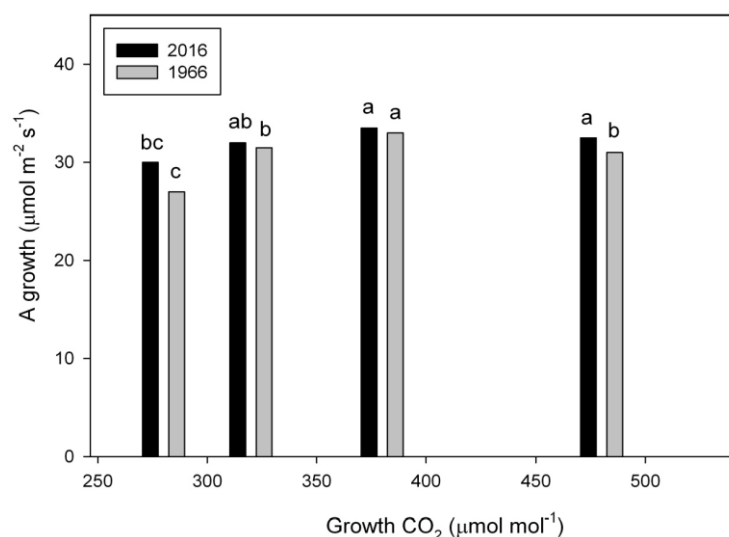


Figure 4. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Datura stramonium* grown at four CO₂ concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.

3. Discussion

There are three results from this experiment indicating that the seeds collected 40 years apart differed in adaptation to the CO₂ environment. One of these results was that seedlings grown from seeds collected about 1966 did not increase in biomass at 20 or 24 days after planting when grown at 380 mol mol⁻¹ vs. 320 mol mol⁻¹ CO₂, whereas seedlings collected from seeds in 2006 were larger when grown at the higher CO₂ concentration. This pattern occurred in all three of the C₃ species. Because all of the experimental plants were grown together simultaneously in the same chambers, yet responded differently, environmental differences among chambers can be eliminated as causing the contrasting results between seed lots. The second result indicating adaptation rising atmospheric CO₂ was the larger final seed mass and/or shoot biomass in plants from the newer than from the older seed lot when plants grown at the higher CO₂ concentration, which occurred in two of the species studied here. Again, the lack of differentiation in *D. stramonium* grown at the same time eliminates other environmental differences as a cause of the observed differential response. The differential flowering times in the two seed lots of *A. hybridus* indicates that genetic change occurred over time in that species, but any relationship to changes in atmospheric CO₂ is unclear, although CO₂ effects on flowering time are well known [21]. These two results partially support our primary hypothesis of greater growth stimulation from 320 mol mol⁻¹ to 380 mol mol⁻¹ CO₂ in newer than in older seed lots.

The third result indicating that adaptation to rising atmospheric CO₂ occurred was the difference in photosynthetic acclimation to growth at elevated CO₂ between the older and newer seed lots, which occurred in two of the species studied. Growth at an elevated CO₂ concentration resulted in more down-regulation of photosynthesis in plants from the older seed lots, which partially supports our secondary hypothesis. While the growth CO₂ concentration of 480 mol mol⁻¹ may seem unreasonably high for a treatment, concentrations of CO₂ in the field at Beltsville are often at least 100 mol mol⁻¹ above the midday concentration for several hours in the morning, when wind speed is low [22]. Prior experiments with *C. album* also had found no evidence of down-regulation of photosynthesis during growth at elevated CO₂ in this species [5]. For the C₄ species *A. hybridus*, it is not surprising that photosynthetic rates under the growth conditions did not reflect the observed down-regulation of carboxylation efficiency, because rates of photosynthesis in C₄ species are generally only limited by carboxylation efficiency during periods of soil or atmospheric water stress.

Studies comparing photosynthesis of old and new crop cultivars [6–9] have only measured photosynthesis under the current growth CO₂ concentration, not the CO₂ concentration at the time of cultivar release or at projected higher concentrations. The photosynthetic characteristics of the

weeds studied here measured only at the mean CO₂ concentration of 2006 did not indicate any differences between the seed lots from different years, similar to the results found in wheat [8] and one study of soybean [9].

4. Materials and Methods

Seeds of four annual weedy species, *Abutilon theophrasti* (Medikus), *Amaranthus hybridus* (L.), *Chenopodium album* (L.), and *Datura stramonium* (L.) were collected in 1966 and again in 2006 from agricultural fields at the Beltsville Agricultural Research Center, Beltsville, Maryland (39°02' N, 76°94' W, elevation 30 m). Seeds were collected from multiple individual plants of each species and pooled within species. Seeds were air dried, and then stored at about 4 °C in sealed containers. Seed germination rate was measured in 2015 by planting 20 seeds from each of four 15 cm diameter pots filled with moist vermiculite per species in a growth chamber at 26/20 °C with 14 hours of light at 1000 mol m⁻² s⁻¹ and a dew point temperature of 18 °C, monitoring emergence daily. Germination rate remained high (>70%) even in the older seed lots.

4.1. Seedling Growth Rates

Seeds of each species from both collection periods (mid-1960s and 2006) were grown together in two controlled-environment chambers with day/night temperatures of 26/20 °C, with 14 hours of light at 1000 mol m⁻² s⁻¹ photosynthetic photon flux density (PPFD) from a mixture of high-pressure sodium and metal halide lamps, and a dew point temperature of 18 °C. The chambers had a growing area of 1 m², and a growing height of 1 m. The temperature, dew point temperature, and light regimes were chosen as typical of mean values for summer days in Beltsville, Maryland. The CO₂ concentrations of chamber air of the two chambers were 320 and 380 mol mol⁻¹ each ± 10 mol mol⁻¹ controlled by the injection of pure CO₂ or CO₂-free air under the control of absolute infrared CO₂ analyzers that sampled the chamber air continuously. The mean atmospheric CO₂ concentration was approximately 320 mol mol⁻¹ in 1966, and 380 mol mol⁻¹ in 2006 [4]. Two chambers were used in all of these experiments, with CO₂ treatments randomly assigned to chambers in sequential trials. There were three repetitions over time of each chamber CO₂ condition, with 10 pots per seed lot in each chamber run, with seedlings thinned randomly to one plant per pot two days after emergence. Plastic pots 15 cm in diameter were filled with 1.8 liters of medium grade vermiculite, and were flushed daily with a complete nutrient solution containing 14.5 mM nitrogen. Destructive harvests were made on days 20 and 24 after planting, in which whole plant leaf area, and leaf, stem and root dry mass were determined on 5 plants per species on each date. Two harvests a few days apart were used such that growth parameters such as relative growth and leaf area ratio could be calculated. The final harvest date of 24 days after planting was chosen such that flowering had not yet begun in any seed lot for any growth condition, because flowering slows growth rates. Analysis of variance was used to test separately for each species for differences between collection eras and growth CO₂ concentrations, using mean values for the three chamber replications in two-way analysis of variance.

4.2. Growth to Maturity

For determination of plant dry mass at maturity, plants were grown in chambers in which daily changes in photoperiod were automatically programmed based on the latitude of Beltsville, Maryland, and a starting date of May 30. Air temperatures, the dew point temperature, and PPFD were set as described for the seedling growth experiments. There were 2 chambers each at 320 and 380 mol mol⁻¹ CO₂ concentration. Chamber interiors were 2 m × 3 m, with an interior height of 2 m. The lamp canopy was adjustable in height. Lamp output was controlled automatically based on a sensor held just above the tops of the plants in the center of the chamber. Plants were grown in 30 cm diameter plastic pots filled with vermiculite and watered daily or twice daily with nutrient solution. There were 5 pots of each seed lot for each species in each chamber.

4.3. Photosynthetic Acclimation

Plants were grown with the same air temperature, dew point temperature, CO₂ concentration, and light conditions as for the seedling growth rate experiments, in the same two chambers as were used for seedling growth rate determinations. Because no differences among seed lots in photosynthetic characteristics were obtained at the growth CO₂ concentrations of 320 and 380 mol mol⁻¹, (see results), comparisons were also made on plants were grown at 280 and at 480 mol mol⁻¹. The lower concentration approximated the atmospheric concentration just before the industrial revolution in Europe, and the highest concentration is that anticipated for about 50 years in the future. Two chambers were used in all of these experiments, with CO₂ treatments randomly assigned to chambers in sequential trials. There were three repetitions over time of each chamber CO₂ condition, with 5 pots per seed lot in each chamber run, with seedlings thinned randomly to one plant per pot shortly after emergence. Leaf gas exchange measurements were made on recently fully expanded upper leaves, at 22 or 23 days from planting, using a CIRAS-3 portable photosynthesis system (PP-Systems, Amesbury, MA, USA). The gas exchange system controlled leaf temperature, light, CO₂, and water vapor pressure surrounding 2.5 cm² intact sections of leaves, using an open measurement system. All measurements were made with leaf temperature controlled to the daytime growth air temperature of 26 °C, and at leaf-to-air water vapor pressure differences of 1 to 1.5 kPa. Each leaf was measured under four combinations of PPFD and CO₂ concentration: at the growth PPFD of 1000 mol m⁻² s⁻¹ at 320 and 380 mol mol⁻¹ CO₂, and at a PPFD of 2000 mol m⁻² s⁻¹ at CO₂ concentrations of 100 and 200 mol mol⁻¹. The slope of the response of photosynthesis to substomatal CO₂ concentration from measurements at 100 to 200 mol mol⁻¹ external CO₂ measured at the high PPFD was taken to indicate photosynthetic carboxylation efficiency. In the C₃ species, this was taken to indicate the maximum carboxylation capacity of Rubisco [23], and the in the C₄ species, it was taken to indicate the maximum carboxylation capacity of PEPcase [24]. Leaf gas exchange measurements were made on 3 or 4 plants of each species and collection era from each chamber run. Analysis of variance was used to test differences between collection eras and growth CO₂ concentrations for each species, using mean values for the three chamber replications in two-way analysis of variance.

5. Conclusions

The results presented here provide evidence that adaptation to rising atmospheric CO₂ concentration has occurred in three of the four weed species studied. This result is consistent with several observations of rapid physiological adaptation to imposed elevated CO₂ conditions in populations of wild species cf. [16]. We can expect weed adaptation to climate change conditions to occur alongside any improvements in crop responses to climate change.

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Conflicts of Interest: The author declares no conflict of interest.

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