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Article

Evidence of Adaptation to Recent Changes in Atmospheric CO_2 in Four Weedy Species

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Abstract: Seeds of three C_3 and one C_4 annual weedy species were collected from agricultural fields in Beltsville, Maryland in 1966 and 2006, when atmospheric CO_2 concentrations averaged about 320 and 380 µmol mol^{-1}, respectively. Plants from each collection year were grown over a range of CO_2 concentrations to test for adaptation of these weedy species to recent changes in atmospheric CO_2. In all three of the C_3 species, the increase in CO_2 concentration from 320 to 380 µmol 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 CO_2 in plants collected in 2006 than in 1966 in two of the species. Down regulation of photosynthetic carboxylation capacity during growth at high CO_2 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 CO_2 has occurred in some of these weedy species.

Keywords: CO_2; adaptation; photosynthesis; growth; weeds; carboxylation efficiency

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1. Introduction

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 µmol mol^{-1} [1]. Because C_3 photosynthesis usually remains limited by CO_2 availability even at the current concentration of about 400 µmol mol^{-1}, the past increase has represented an increase in a growth-limiting resource for many plants. Bunce [2] found that four annual weedy C_3 species were better adapted to the current atmospheric CO_2 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 CO_2 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 [3,4], but no differences in other cases [5,6]. However, in all of those studies plants were only grown at the current ambient CO_2, not at the prior concentration. The few tests in crop species of whether growth at projected higher future CO_2 concentrations selected for plants with higher growth rate at elevated CO_2 have sometimes, but not always found higher growth rates [7,8]. Some studies have found that exposure of populations of non-cultivated plants to elevated CO_2 resulted in adaptation to the elevated concentration, as shown by more rapid growth rates and/or increased reproduction [9-14], but there are other cases in which this did not occur [15-17]. In this study, I compared the response of both growth rates and photosynthetic properties to growth CO_2 concentration in seeds of four annual weedy species collected in the same location about 40 years apart in in order to more directly test for evidence of adaptation to changes in CO_2 concentration in the recent past in these species.

2. Results

2.1 Seedling growth. In all three of the C_3 species, the increase in CO_2 concentration from 320 to 380 µmol 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 CO_2 in *A. theophrasti* or *C. album*. In *D. stramonium* and *A. hybridus*, mean leaf area ratios were decreased at the higher growth CO_2. Relative growth rate from day 20 to 24 differed between 320 and 380 µmol 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 µmol mol^{-1} CO_2 concentration. Total dry mass (DM, in grams) is for plants at 24 days after planting, and leaf area ratio (LAR, in cm^2 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
A. theophrasti	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
C. album	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
D. stramonium	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
A. hybridus	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 Photosynthesis. In the C_4 species, *A. hybridus*, the carboxylation efficiency of PEPcase was reduced by growth at the lowest and highest CO_2 concentrations (Fig. 1). The reduction at the highest growth CO_2 in the plants from the 1966 than was larger than that of plants from 2006 (Fig. 1). Despite differences in carboxylation efficiency, rates of photosynthesis under the growth conditions were the same for all growth CO_2 concentrations in this species, and did not differ between seed lots, even at the highest growth CO_2 (Fig. 1).

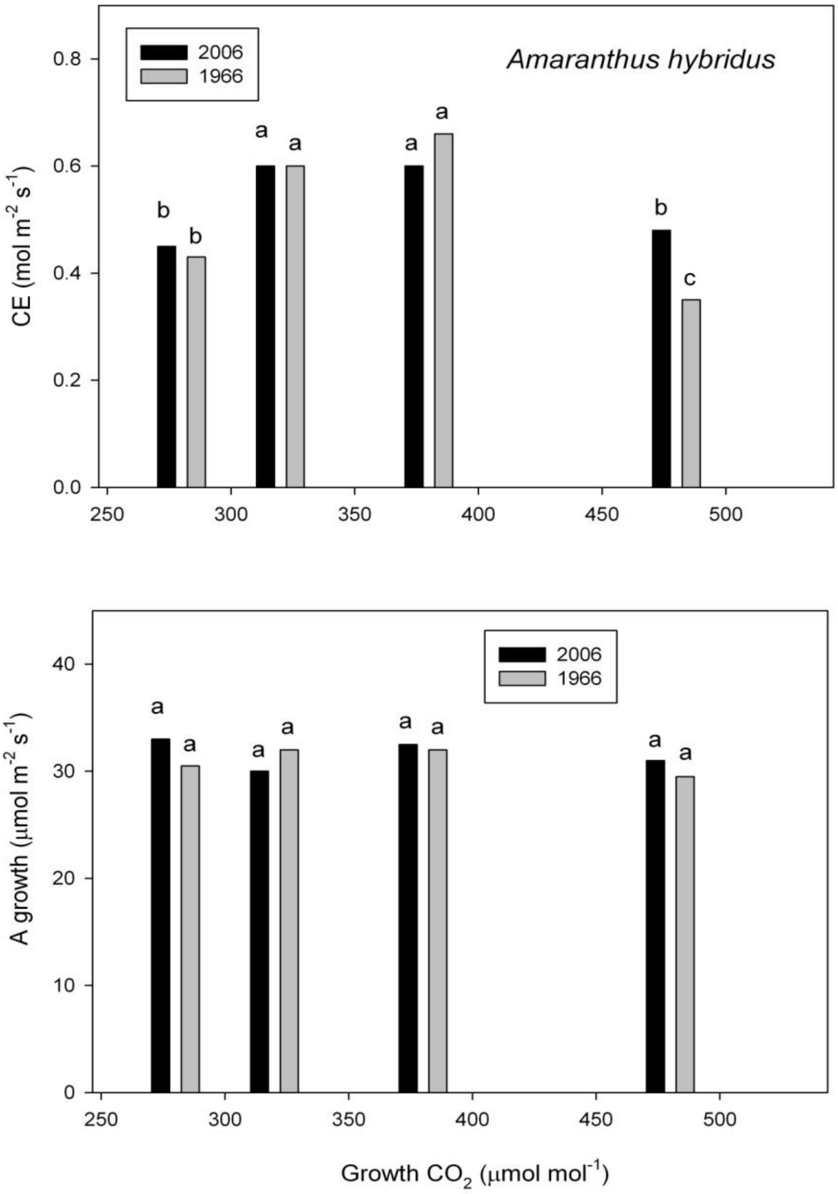

Figure 1. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Amaranthus hybridus* grown at four CO_2 concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.

In *A. theophrasti*, the carboxylation efficiency of Rubisco was also reduced at the highest growth CO_2, but only in the plants from the 1960s (Fig. 2). At the higher growth CO_2, 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 CO_2 than at the intermediate CO_2 concentrations (Fig. 2).

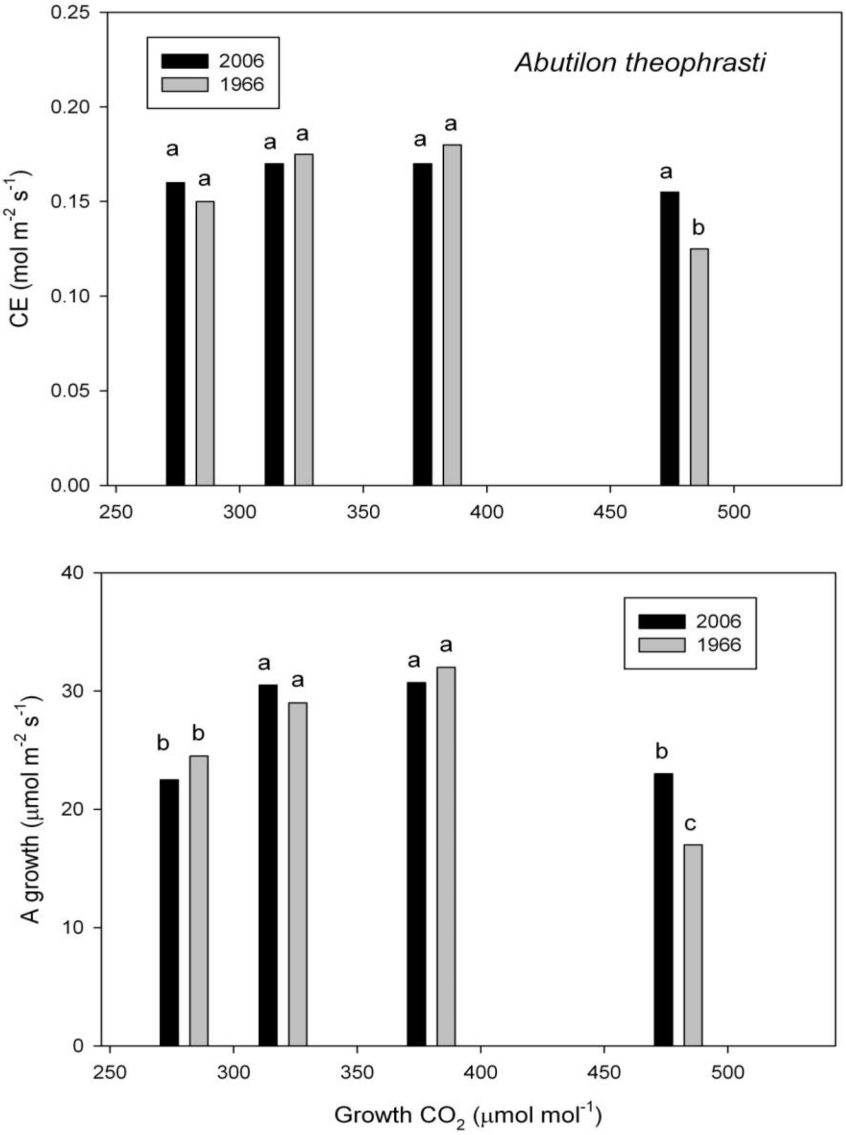

Figure 2. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Abutilon theophrasti* grown at four CO_2 concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.

In *C. album*, carboxylation efficiency of Rubisco was unaffected by the growth CO_2, and never differed between old and new seed lots (Fig. 3). Rates of photosynthesis under the growth conditions increased with growth CO_2 in both seed lots, and never differed between seed lots (Fig. 3).

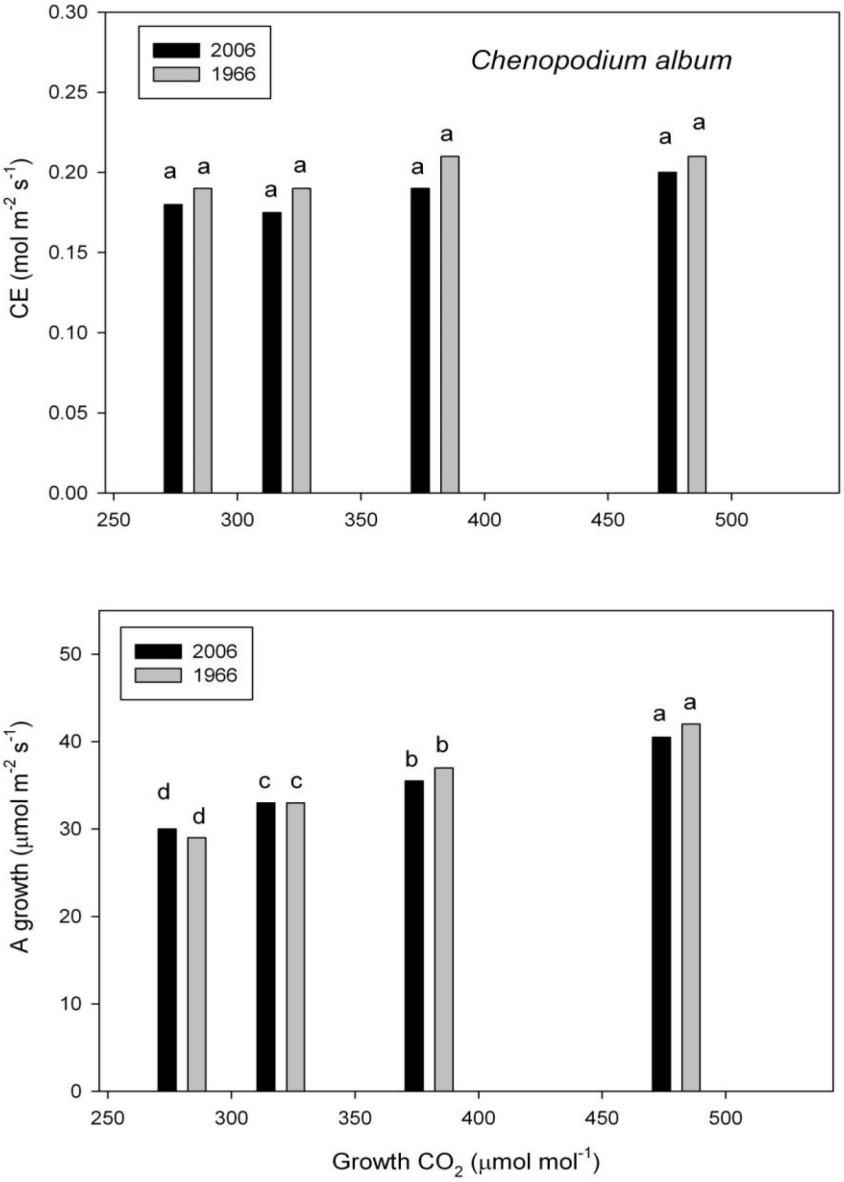

Figure 3. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Chenopodium album* grown at four CO_2 concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.

In *D. stramonium*, carboxylation efficiency of Rubisco was highest at the growth CO_2 concentration of 320 µmol mol^{-1} in both seed lots, and did not differ between old and new seed lots at any growth CO_2 (Fig. 4). Photosynthesis under the growth conditions increased slightly with increasing growth CO_2, up to 380 μmol mol^{-1} in this species (Fig. 4).

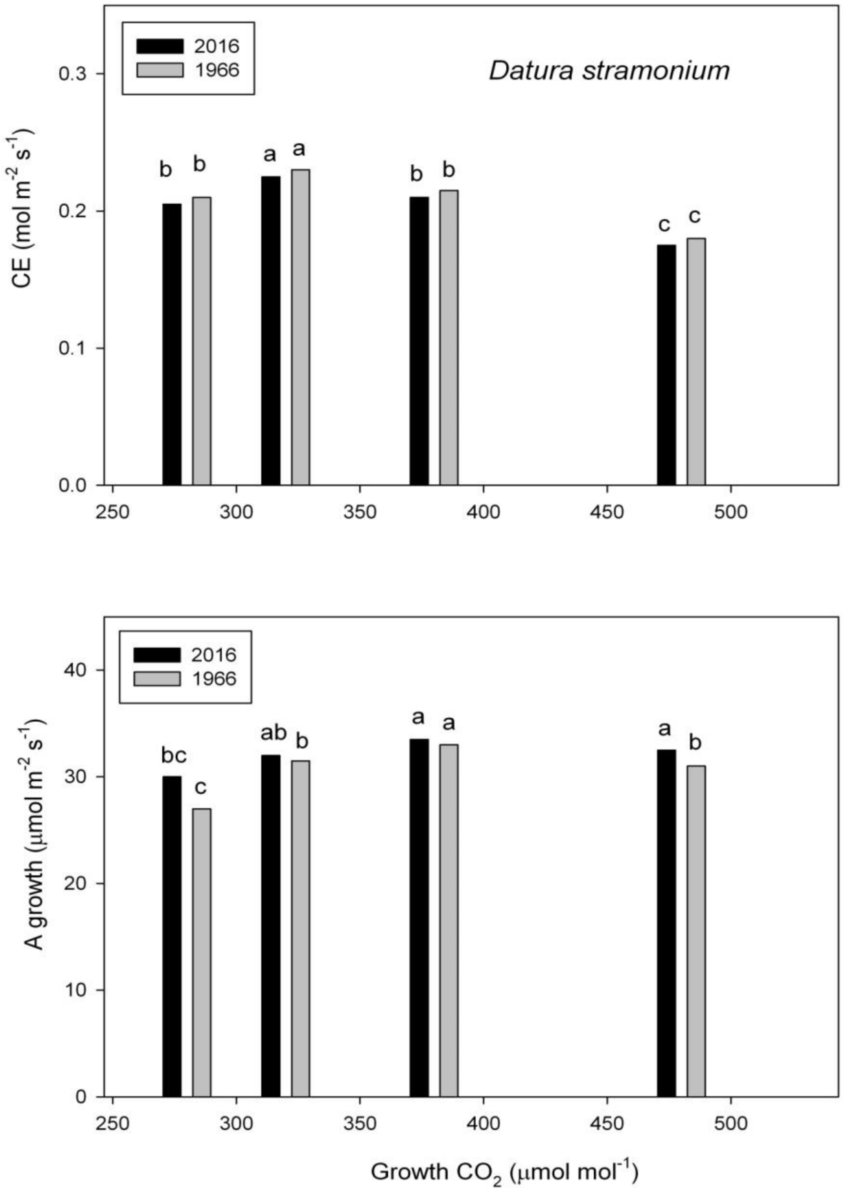

Figure 4. Carboxylation efficiency (CE) and assimilation rate (A) under the growth conditions in *Datura stramonium* grown at four CO_2 concentrations from seeds collected in 1966 and 2006. Different letters indicate significant differences, based on analysis of variance.

2.3 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 CO_2 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 CO_2 concentration, and higher mass for the newer seed lot at both CO_2 concentrations. In *D. stramonium*, total shoot and seed dry mass were increased by the growth CO_2 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 and 380 μmol mol^{-1} from either seed collection time (Table 2). Flowering occurred 6 days earlier 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 CO_2 concentrations (μmol mol^{-1}). 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 shoot DM (g)	Seed DM (g)
A. theophrasti	1966	320	99c	30c
	1966	380	135b	41b
	2006	320	130b	40b
	2006	380	153a	62a
C. album	1966	320	46c	na
	1966	380	60b	na
	2006	320	59b	na
	2006	380	71a	na
D. stramonium	1966	320	315ab	172b
	1966	380	350a	193a
	2006	320	289b	172b
	2006	380	351a	205a
A. hybridus	1966	320	186a	78a
	1966	380	191a	82a
	2006	320	90b	62b
	2006	380	102b	60b

3. Discussion

There are three results from this experiment which indicate that the seeds collected 40 years apart differed in adaptation to the CO_2 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 vs. 320 μmol mol^{-1} CO_2, whereas seedlings collected from seeds in 2006 were larger when grown at the higher CO_2 concentration. This pattern occurred in all three of the C_3 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 that adaptation to rising atmospheric CO_2 occurred was the difference in photosynthetic acclimation to growth at elevated CO_2 between the older and newer seed lots, which occurred in two of the species studied. Growth at an elevated CO_2 concentration resulted in more down-regulation of photosynthesis in plants from the older seed lots. While the growth CO_2 concentration of 480 μmol mol^{-1} may seem unreasonably high for a treatment, concentrations of CO_2 in the field at Beltsville are often at least 100 μmol mol^{-1} above the midday concentration for several hours in the morning, when wind speed is low [18]. Prior experiments with *C. album* also had found no evidence of down-regulation of photosynthesis during growth at elevated CO_2 in this species [2]. For the C_4 species *A. hybridus*, it is not surprising that photosynthetic rates under the growth conditions did not reflect the down-regulation of carboxylation efficiency, because rates of photosynthesis in C_4 species are generally only limited by carboxylation efficiency during periods of soil or atmospheric water stress.

The third result indicating adaptation rising atmospheric CO_2 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 CO_2 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 CO_2 is unclear, although CO_2 effects on flowering time are well known [19].

Studies which compared photosynthesis of old and new crop cultivars [3-6] have only measured photosynthesis under the current growth CO_2 concentration, not the CO_2 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 CO_2 concentration of 2006 did not indicate any differences between the seed lots from different years, similar to the results found in wheat [5] and one study of soybean [6].

The results presented here provide evidence that adaptation to rising atmospheric CO_2 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 CO_2 conditions in populations of wild species cf. [13]. We can expect weed adaptation to climate change conditions to occur alongside any improvements in crop responses to climate change.

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 (39º 02' N, 76º 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 measured in 2015 remained high (>70%) even in the older seed lots.

4.1 Seedling growth rates. Seeds of each species from both collection periods (mid-1960's, and 2006) were grown together in controlled environment chambers with day/night temperatures of 26/20 °C, with 14 hours of light at 1000 μmol 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 temperature, dew point temperature, and light regimes were chosen as typical of mean values for summer days in Beltsville, Maryland. The CO_2 concentrations of chamber air were 320 and 380 μmol mol^{-1} each $\pm$ 10 μmol mol^{-1} controlled by the injection of pure CO_2 or CO_2-free air under the

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control of absolute infrared CO_2 analyzers which sampled chamber air continuously. The mean atmospheric CO_2 concentration was approximately 320 μmol mol^{-1} in 1966, and 380 μmol mol^{-1} in 2006 (NOAA). Two chambers were used in all of these experiments, with CO_2 treatments randomly assigned to chambers in sequential trials. There were three repetitions over time of each chamber CO_2 condition, with 10 pots per seed lot in each chamber run, with seedlings thinned randomly to one plant per pot shortly after emergence. Plastic pots 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. Analysis of variance was used to test separately for each species for differences between collection eras and growth CO_2 concentrations, using mean values for the three chamber replications in two-way analysis of variance.

4.2 Photosynthetic acclimation. Plants were grown with the same temperature, dew point temperature, and light conditions as for the seedling growth rate experiments. In addition to the growth CO2 concentrations of 320 and 380 μmol mol^{-1}, plants were also grown at 280 and at 480 μmol 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 CO_2 treatments randomly assigned to chambers in sequential trials. There were three repetitions over time of each chamber CO_2 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). The gas exchange system controlled leaf temperature, light, CO_2, and water vapor pressure surrounding 2.5 cm^2 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 ^oC, 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 CO_2 concentration: at the growth PPFD of 1000 μmol m^{-2} s^{-1} at 320 and 380 μmol mol^{-1} CO_2, and at a PPFD of 2000 μmol m^{-2} s^{-1} at CO_2 concentrations of 100 and 200 μmol mol^{-1}. The slope of the response of photosynthesis to substomatal CO_2 concentration from measurements at 100 to 200 μmol mol^{-1} external CO_2 measured at the high PPFD was taken to indicate photosynthetic carboxylation efficiency. In the C_3 species, this was taken to indicate the maximum carboxylation capacity of Rubisco [20], and the in the C_4 species, it was taken to indicate the maximum carboxylation capacity of PEPcase [21]. 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 CO_2 concentrations for each species, using mean values for the three chamber replications in two-way analysis of variance.

4.3 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 μmol mol_{-1} CO_2 concentration. 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.

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

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