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Future atmospheric carbon dioxide may increase tolerance to glyphosate

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We tested whether the efficacy of chemical weed control might change as atmospheric CO_2 concentration [CO_2] increases by determining if tolerance to a widely used, phloem mobile, postemergence herbicide, glyphosate, was altered by a doubling of [CO_2]. Tolerance was determined by following the growth of *Amaranthus retroflexus* L. (redroot pigweed), a C_4 species, and *Chenopodium album* L. (common lambsquarters), a C_3 species, grown at near ambient (360 μmol mol^{-1}) and twice ambient (720 μmol mol^{-1}) [CO_2] for 14 d following glyphosate application at rates of 0.00 (control), 0.112 kg ai ha^{-1} (0.1 × the commercial rate), and 1.12 kg ai ha^{-1} (1.0 × the commercial rate) in four separate trials. Irrespective of [CO_2], growth of the C_4 species, *A. retroflexus*, was significantly reduced and was eliminated altogether at glyphosate application rates of 0.112 and 1.12 kg ai ha^{-1}, respectively. However, in contrast to the ambient [CO_2] treatment, an application rate of 0.112 kg ai ha^{-1} had no effect on growth, and a 1.12-kg ai ha^{-1} rate reduced but did not eliminate growth in elevated [CO_2]-grown *C. album*. Although glyphosate tolerance does increase with plant size at the time of application, differences in glyphosate tolerance between CO_2 treatments in *C. album* cannot be explained by size alone. These data indicate that rising atmospheric [CO_2] could increase glyphosate tolerance in a C_3 weedy species. Changes in herbicide tolerance at elevated [CO_2] could limit chemical weed control efficacy and increase weed–crop competition.

Nomenclature: Glyphosate; *Chenopodium album* L. CHEAL, common lambsquarters; *Amaranthus retroflexus* L. AMARE, redroot pigweed.

Key words: Climate change, carbon dioxide, postemergence, CHEAL, AMARE

It is recognized that as a result of anthropogenic activities, the concentration of carbon dioxide [CO_2] is increasing in the earth's atmosphere and should reach 600 μmol mol^{-1} sometime during the middle of the 21st century (Conway et al. 1994; Houghton et al. 1996). It has long been known that CO_2 was the entry point for carbon in the biological cycle and that increasing [CO_2] resulted in a general stimulation of leaf photosynthesis and plant growth (Bowes 1996). The anticipated stimulation in growth should be greater in plants with the C_3 photosynthetic pathway (~95% of all plant species) than in those with the C_4 photosynthetic pathway, due, in part, to the amount of carbon lost through photorespiration in each pathway (Poorter 1993).

In agricultural systems, a number of studies have quantified the photosynthetic and growth responses of major crop and weedy species to future increases in atmospheric carbon dioxide (e.g., Bunce 1997; Patterson and Flint 1980; Tremmel and Patterson 1993, 1994; Ziska and Bunce 1997). It is clear that substantial increases in photosynthesis and growth can be anticipated with increasing [CO_2], although fewer studies have measured competitive interactions between weeds and crops directly (Patterson 1995; Patterson and Flint 1990).

Because increasing [CO_2] can stimulate weed growth and, presumably, alter weed–crop competition, how will [CO_2] alter future chemical weed control efforts in agricultural systems? This area of research remains primarily speculative (see Patterson 1995), but growth at elevated [CO_2] could result in anatomical, morphological, and physiological changes that could influence herbicide uptake rates, herbicide movement, and overall effectiveness. For example, increasing [CO_2] can increase leaf thickness and reduce stomatal number and conductance, possibly limiting uptake of foliar-applied herbicides. Decreased stomatal conductance with increasing [CO_2] could also reduce transpiration and uptake of soil-applied herbicides. The duration of time weeds are susceptible to herbicide treatment could also be affected because at elevated [CO_2], time spent in the seedling stage (usually the time of greatest effectiveness in postemergence, foliar-applied herbicides) could be reduced if increasing [CO_2] stimulates growth. In addition, for perennial weeds, increasing [CO_2] could stimulate greater rhizome and tuber growth, making herbicidal control of such weeds more difficult and costly.

Although there are empirical reasons for assuming that growth at future levels of [CO_2] could influence herbicide efficacy, we are aware of no study that has compared the relative effectiveness of herbicide control for susceptible weedy species as a function of carbon dioxide concentration. We chose glyphosate (formulation: "Roundup") as a widely used, phloem mobile, postemergence herbicide. Although new herbicides are likely in the future, the current monetary investment in glyphosate and its overall effectiveness suggest that its widespread use will coincide with the global rise in atmospheric [CO_2] well into the next century. In the current experiment, we used two weedy species, chosen among the world's worst weeds (Holm et al. 1977): *Amaranthus retroflexus* (redroot pigweed) and *Chenopodium album* (common lambsquarters), a C_4 and C_3 species, respectively, to determine the efficacy of glyphosate at two different plant growth stages (seedling and maturity) and two different CO_2 concentrations.

Materials and Methods

Experiments were conducted in two air-conditioned glasshouses located at the USDA-ARS Climate Stress Laboratory in Beltsville, MD. Each glasshouse was 13.5 m^2 in surface area and transmitted 65% of incoming photosynthetically active radiation (PAR), with temperature and CO_2 concentration maintained within preset limits. Glasshouses were designed to maintain maximum and minimum temperatures between 31 and 17 C, respectively. Temperature sensors in shaded, ventilated enclosures were placed near the top of plants in each greenhouse. PAR sensors were located near the top of each greenhouse. Dew-point temperatures were determined periodically near midday and closely approximated those of outside air. Carbon dioxide was maintained by a WMA2 infrared gas analyzer,[1] which injected CO_2 if levels fell below 350 and 700 $\mu mol\ mol^{-1}$, respectively, for each glasshouse. Blowers constantly circulated air in the glasshouses and provided an air speed of about 0.5 $m\ s^{-1}$ over leaves. A data logger[2] recorded PAR, temperature, and CO_2 concentration in both glasshouses at 30-s intervals.

Seed of *A. retroflexus*, a broad-leaved C_4 species, and seed of *C. album* a broad-leaved C_3 species, obtained from local populations, were sown on two different dates in the spring (March 20 and April 3) and again in the summer (June 26 and July 10) in 14-cm-diam (1.8 L) pots filled with vermiculite. CO_2 treatments were switched between glasshouses, and the experiment was repeated in the summer months, partly to confirm earlier findings and because the shorter spring days resulted in early anthesis and reduced vegetative growth for *A. retroflexus*. After emergence, seedlings were thinned to one per pot with 50 pots per species, and growth stage was randomly assigned to one of the two CO_2 treatments. Pots were flushed daily with a complete nutrient solution and rotated weekly to minimize microclimate effects within a glasshouse. CO_2 treatments assigned to a given glasshouse were switched between spring and summer experiments. Solutions of commercial glyphosate[3] (with no additional surfactant) were applied on April 16 (13 and 27 d after sowing, DAS) and again on July 27 (17 and 31 DAS) at spray rates of 0.00 (control), 0.112, or 1.12 kg ai ha^{-1} (0.1 × and 1.0 × of the recommended rate) using a pressurized backpack sprayer with TeeJet 8003-E nozzles.[4] Plants 13 and 17 DAS were still in the seedling stage (i.e., <10 cm in height and entirely vegetative), while those 27 and 31 DAS had flowered. Sixteen plants per species, CO_2 treatment, and growth stage were sprayed with each glyphosate concentration. To prevent short-term changes in stomatal conductance, herbicide applications were performed in the respective glasshouses to maintain near normal ambient and elevated CO_2 treatment levels (365 and 723 $\mu mol\ mol^{-1}$, respectively, when averaged for the April 16 and July 27 applications). No significant differences in 24-h average day/night temperatures or PAR were observed between glasshouses for a given experiment (Table 1). As might be expected, average day/night temperatures and PAR were higher for the July/August experiment compared to March/April (Table 1). No difference in CO_2 concentration (for a given CO_2 treatment) was observed between experiments. However, for both experiments, nighttime CO_2 concentrations rose to 440 and 520 $\mu mol\ mol^{-1}$ for the ambient glasshouse (Table 1).

Table 1. Average 24-h values for daytime and nighttime CO_2 concentrations, day and night temperatures, and PAR during the 14-d period following glyphosate application for the ambient and elevated CO_2 greenhouses. Greenhouses were switched between trials.

Time	$[CO_2]$	Day $[CO_2]$	Night $[CO_2]$	Day temp.	Night temp.	PAR
				C	C	mol m^{-2} d^{-1}
April 16–30	Amb.	390.7	517.4	25.1	19.8	19.1
	Elev.	720.7	730.7	24.9	19.4	
July 27–August 10	Amb.	381.0	443.0	26.4	21.2	31.9
	Elev.	727.9	732.1	26.0	20.8	

Single-leaf photosynthesis (measured as *A*, the rate of CO_2 assimilation) and stomatal conductance were obtained 1 d prior to and 7 d after spraying with glyphosate for postflowering plants of each weedy species for both the spring and summer experiments. For each sampling time, assimilation was determined on the uppermost fully expanded leaf for six plants of each species and CO_2 treatment. Measurements were made using a portable open-gas exchange system incorporating infrared CO_2 and water vapor analyzers for determining net photosynthetic CO_2 uptake rate and stomatal conductance.[1] PAR was supplied by a separate light unit that produced a constant PAR of 1,600 $\mu mol\ m^{-2}\ s^{-1}$ for all measurements of gas exchange. The water vapor pressure surrounding the leaf was approximately 1.5 kPa and did not vary between CO_2 treatments or species.

One hour prior to glyphosate application on April 16 and July 27 and 5, 10, and 14 d afterward (4, 9, and 14 d for the July 27 date), four to six plants of each species, growth stage, and CO_2 treatment were cut at ground level and separated into leaf laminae, stems (including petioles), and roots. At the time of spraying, plant height was also determined for six postflowering plants of each species. CO_2 treatment had no effect on plant height for *A. retroflexus* at the time of spraying, while *C. album* heights were 31/47 cm and 42/62 cm for the ambient and elevated $[CO_2]$ for the April 16 and July 27 applications, respectively. Leaf area was determined photometrically with a leaf area meter.[5] Leaf area was obtained only on upright green leaves. Dry weights were obtained separately for leaves, stems, and roots. Material was dried at 55 C for a minimum of 72 h (or until dry weight was constant) and then weighed. If dry weight did not significantly increase over the 14 d following spraying, plants were considered to have died.

The effect of elevated CO_2 on growth and gas exchange with and without glyphosate application was analyzed separately for each species, growth stage (seedling or postflowering), and application time (April 16 or July 27) for a given sampling date using a Student's unpaired *t* test assuming unequal variance. Unless otherwise stated, differences were determined to be significant at the $P < 0.05$ level.

TABLE 2. Changes in stomatal conductance (g, mmol m^{-2} s^{-1}) and single-leaf photosynthesis (*A*, μmol m^{-2} s^{-1}) for *Amaranthus retroflexus* (C_4) and *Chenopodium album* (C_3) grown at ambient (390 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) carbon dioxide (measured at 370 and 700 μmol mol^{-1}). C, no herbicide application; 0.1 ×, 10% of recommended rate; 1.0 ×, 100% of recommended rate. Measurements were taken for the last fully expanded leaf at full sunlight (~1,400 μmol m^{-2} s^{-1}) for postflowering plants only. No green leaves were available for *A. retroflexus* at the 1.0 × herbicide treatment for the April 16–30 experiment or for the ambient CO_2 treatment for the July 27–August 10 experiment. * indicates a significant difference in g or *A* between the elevated and ambient [CO_2] treatments for a given species and application rate (Student's unpaired *t* test, *n* = 6).

			April 16–30		July 27–August 10	
Species	[CO_2]	Herbicide	g	*A*	g	*A*
						μmol m^{-2} s^{-}
Prior to spraying						
A. retroflexus	Amb.		679.2	37.7	523.1	49.0
	Elev.		455.2*	40.2	221.5*	45.6
C. album	Amb.		4,383.8	31.8	6,251.0	40.3
	Elev.		1,422.0*	40.2*	1,285.2*	52.8*
Seven days after spraying						
A. retroflexus	Amb.	C	926.0	42.5	531.1	32.7
	Elev.	C	298.0*	42.2	177.7*	32.0
A. retroflexus	Amb.	0.1 ×	341.0	33.0	314.5	27.4
	Elev.	0.1 ×	197.2	35.5	259.0	34.5*
A. retroflexus	Amb.	1.0 ×				
	Elev.	1.0 ×			142.2	8.9
C. album	Amb.	C	5,463.4	35.9	1,490.0	29.3
	Elev.	C	2,216.5*	40.2*	512.3*	38.5*
C. album	Amb.	0.1 ×	4,350.6	33.6	1,290.1	30.5
	Elev.	0.1 ×	3,369.2*	46.6*	504.8	37.6*
C. album	Amb.	1.0 ×	2,068.8	20.0	620.0	19.2
	Elev.	1.0 ×	587.0*	25.6*	555.2	38.3*

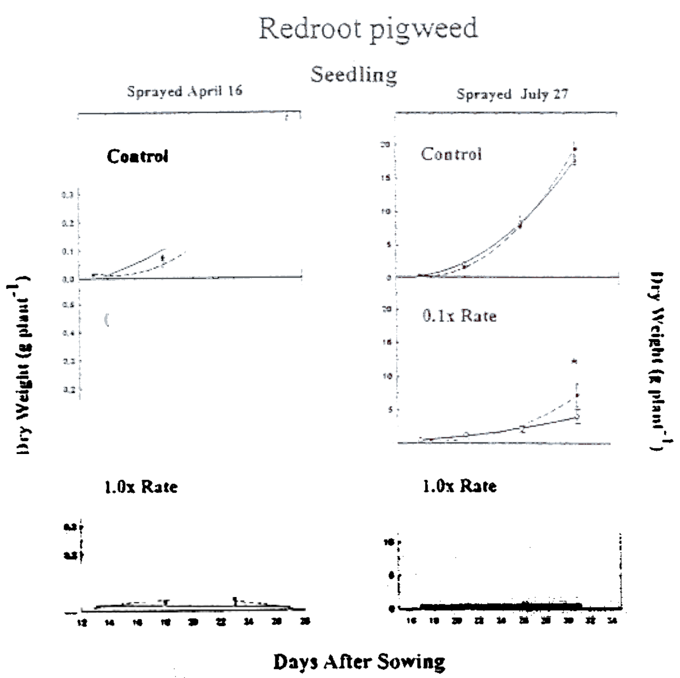

FIGURE 1. Change in whole-plant dry weight in seedlings of *Amaranthus retroflexus* for 14 d following application of glyphosate at either 0 (control), 10% (0.1 ×), or 100% (1.0 ×) the recommended commercial rate. Application dates for glyphosate were April 16 (spring) and July 27 (summer). Plants were grown at either ambient (○ solid line) or elevated (● dashed line) [CO_2]. Growth rate was considered positive if plant dry weights increased over 14 d. If dry weight was unchanged or decreased over this same period, plants were considered to have died. * indicates a significant difference between elevated and ambient CO_2 treatments for a given sampling time and application rate (Student's *t* test, *n* = 4 to 6). See Methods for additional details.

Results

Prior to application of glyphosate, growth at elevated [CO_2] resulted in a significant increase in photosynthesis (+26, +31%) and a decrease in stomatal conductance (−68, −79%) relative to the ambient [CO_2] in single leaves of the C_3 weed *C. album* for the April 16 and July 27 applications (Table 2). A similar pattern was observed for stomatal conductance in the C_4 weed, *A. retroflexus* (−33, −59%), but no effect of elevated [CO_2] on photosynthesis was observed (Table 2). For the April 16 application, postflowering *A. retroflexus* leaves had completely wilted at the 1.0 × rate for both CO_2 treatments within 7 d after spraying with glyphosate. Initial results were similar for the July 27 application; however, leaf regrowth from the main stem occurred at the elevated CO_2 treatment, allowing measurement of leaf photosynthesis (Table 2). For *C. album*, glyphosate had no effect on photosynthesis or conductance at the 0.1 × level for either application date relative to the photosynthetic performance at a given CO_2 level for the control (Table 2). At the 1.0 × glyphosate rate, photosynthesis was reduced for both [CO_2] treatments (April 16) and for the ambient [CO_2] treatment relative to the control (Table 2). However, significant stimulations in photosynthesis or decreased stomatal conductance at elevated relative to ambient [CO_2] were still observed for all three glyphosate treatments (Table 2).

Growth at elevated [CO_2] had no effect on final dry weights of *A. retroflexus* control seedlings from 13 to 27 DAS (April 16) or from 17 to 31 DAS (July 27) (Figure 1). Adding glyphosate at the 1.0 × rate resulted in seedling death for both application dates, with no effect of [CO_2] on susceptibility. However, for the July 27 application, gly-

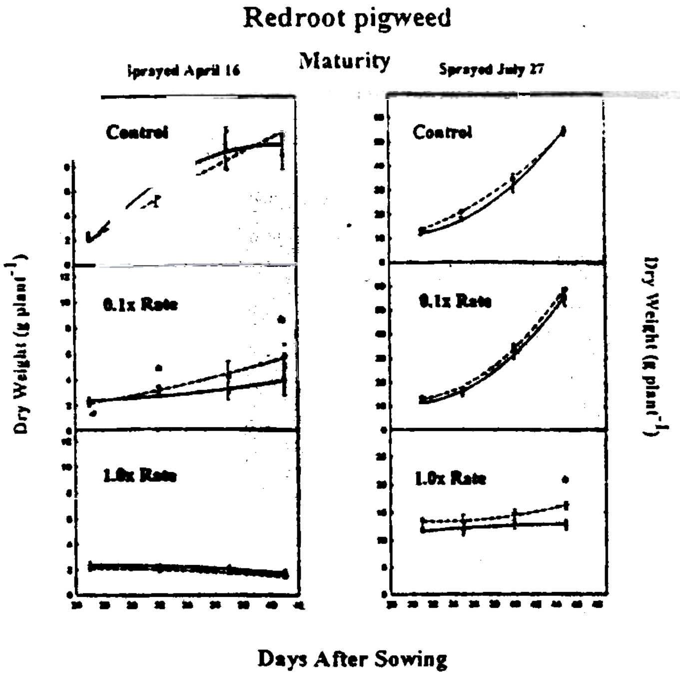

Figure 2. Same as Figure 1, but for postflowering plants of *Amaranthus retroflexus*.

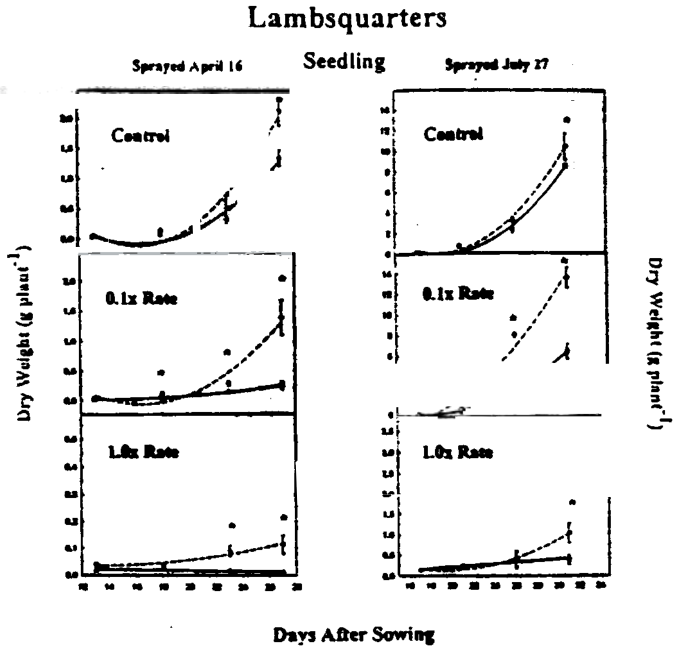

Figure 3. Same as Figure 1, but for seedlings of *Chenopodium album*. Note that the scale of the *y*-axis is reduced for the 1.0 × rate (bottom graph) to better illustrate differences between CO_2 concentrations.

phosate at the 0.1 × rate did not result in death for the elevated [CO_2]-grown *A. retroflexus* seedlings, although their growth rate was reduced relative to the control (Figure 1). For postflowering *A. retroflexus*, slightly greater tolerance to glyphosate at elevated [CO_2] was observed in some instances. For the April 16 application, the 1.0 × rate resulted in plant death with no effect of CO_2 concentration, whereas at the 0.1 × rate, ambient-grown *A. retroflexus* died, and elevated [CO_2]-grown *A. retroflexus* doubled in size 41 DAS (Figure 2). In contrast, for the July 27 application, a 0.1 × rate was ineffective in reducing the growth of *A. retroflexus* at either CO_2 concentration, whereas positive growth was still maintained for the elevated [CO_2]-grown plants, even with a 1.0 × glyphosate rate (Figure 2).

Elevated [CO_2] per se resulted in a significant increase in plant dry weight of control *C. album* seedlings from 13 to 27 DAS (April 16) or from 17 to 31 DAS (July 27) (Figure 3). For either application date, glyphosate (0.1 ×, 1.0 × rate) applied to elevated [CO_2]-grown plants did not result in seedling death, although the increase in dry weight at the 1.0 × rate was significantly reduced relative to the elevated [CO_2] control (Figure 3). In contrast, the increase in dry weight of ambient [CO_2]-grown *C. album* seedlings was either severely reduced (0.1 ×) or eliminated (1.0 ×) with glyphosate (Figure 3). For postflowering control plants of *C. album*, elevated [CO_2] resulted in significantly higher dry weight from 27 to 41 DAS (April 16) or from 31 to 45 DAS (July 27) (Figure 4), although most of the stimulation in dry weight as a result of increasing [CO_2] probably occurred earlier during the seedling stage (see control graph, Figure 3). As with the seedling response, postflowering *C. album* plants grown at elevated [CO_2] were less sensitive to glyphosate applied at the 0.1 × rate (no significant change relative to the control at elevated [CO_2]), while dry weight of ambient-grown *C. album* was reduced for both application dates. Following treatment at the 1.0 × rate, dry weights of *C. album* grown at elevated [CO_2] were significantly less than the respective control but were still positive compared to ambient-grown *C. album* (Figure 4).

Although the absolute changes in dry weight varied as a function of application dates, *C. album* demonstrated increased tolerance to glyphosate at either growth stage at the elevated CO_2 concentration, as evidenced by significant increases in leaf area and leaf, stem, and root weights (as well as total dry weight) relative to the ambient CO_2-grown plants following spraying (Tables 3 and 4). Even at the commercially recommended rate (i.e., 1.0 ×), green leaves were still observed for postflowering *C. album* 14 DAS (Tables 3

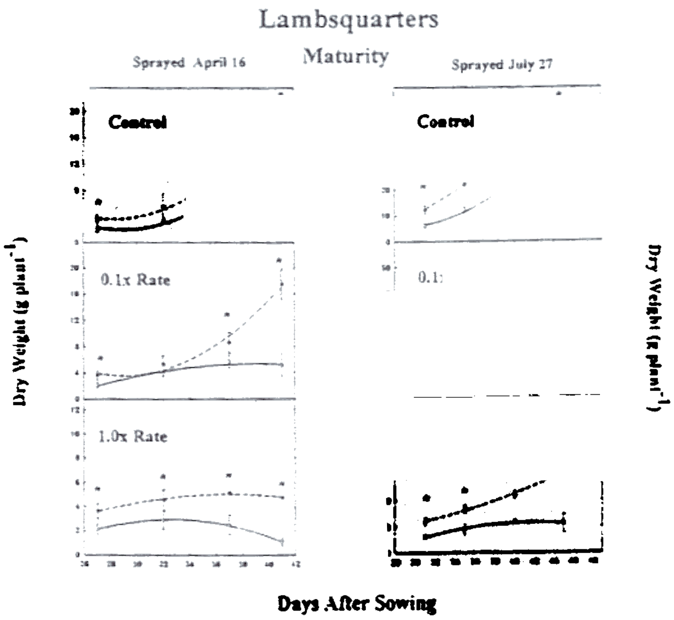

Figure 4. Same as Figure 3, but for postflowering plants of *Chenopodium album*.

TABLE 3. Vegetative and total dry weights (per plant) of *Amaranthus retroflexus* (C_4) and *Chenopodium album* (C_3) grown at ambient and elevated carbon dioxide 14 d after postemergent treatment with glyphosate on April 16. C, no herbicide application; 0.1 ×, 10% of recommended rate; 1.0 ×, 100% of recommended rate. Seedlings were sprayed 13 DAS, and postflowering plants were sprayed 27 DAS. $n = 4$. Dry weights for *A. retroflexus* seedlings at 0.1 and 1.0 × application rates at either [CO_2] are not available due to plant death. E/A is the ratio of total dry weight for the elevated : ambient CO_2 treatment for a given level of glyphosate applied. * indicates a significant difference between the elevated and ambient [CO_2] treatments for a given growth parameter for a given species and growth stage (Student's unpaired *t* test, $n = 4$).

Species	[CO_2]	Herb.	Leaf area	Leaf wt	Stem wt	Root wt	Total wt	E/A
			cm^2	g				
Seedling stage								
A. retroflexus	Amb.	C	65.8	0.21	0.14	0.12	0.46	
	Elev.	C	86.1	0.25	0.14	0.14	0.53	1.15
C. album	Amb.	C	229.5	0.69	0.38	0.31	1.38	
	Elev.	C	324.0*	1.06*	0.61*	0.49	2.16*	1.56*
C. album	Amb.	0.1 ×	45.4	0.12	0.06	0.06	0.25	
	Elev.	0.1 ×	210.0*	0.68*	0.39*	0.33*	1.39*	5.56*
C. album	Amb.	1.0 ×	0.0	0.00	0.01	0.00	0.01	
	Elev.	1.0 ×	3.5*	0.04*	0.04*	0.03*	0.11*	11.25*
Maturity								
A. retroflexus	Amb.	C	407.0	2.03	6.29	0.74	9.07	
	Elev.	C	508.0	2.18	7.64*	0.82	10.63	
A. retroflexus	Amb.	0.1 ×	205.0	0.66	2.94	0.37	3.96	
	Elev.	0.1 ×	362.5*	1.94*	3.35	0.49	5.78	
A. retroflexus	Amb.	1.0 ×	0.0	0.00	1.54	0.17	1.72	
	Elev.	1.0 ×	0.0	0.00	1.33	0.12	1.45	
C. album	Amb.	C	857.8	4.04	9.22	2.04	15.29	
	Elev.	C	1,384.3*	5.72*	11.80*	2.59	20.11	1.32*
C. album	Amb.	0.1 ×	466.5	2.17	3.49	1.12	5.15	
	Elev.	0.1 ×	1,229.0*	6.17*	9.36*	2.01*	17.53*	
C. album	Amb.	1.0 ×	0.0	0.00	0.93	0.15	1.08	
	Elev.	1.0 ×	44.5*[a]	1.47*[b]	3.08*	0.25	4.80*	4.44*

[a] Green, nonwilted leaves only.
[b] All leaf material.

and 4). For the July 27 application, no significant difference was observed between the ambient control and the elevated 1.0 × treatment 14 d after postemergent treatment with glyphosate (i.e., 40.6 vs. 30.7 g $plant^{-1}$) (Table 4). Because of the influence of elevated [CO_2] on dry weight after spraying, the ratio of total dry weight for the elevated : ambient CO_2 treatment increased significantly with increasing rates of glyphosate in *C. album* (Tables 3 and 4). For *A. retroflexus,* changes in leaf area and in leaf, stem, and root weights indicated that increased tolerance to glyphosate was not consistently observed (Tables 3 and 4).

Overall, herbicide tolerance can be defined as the extent to which growth rates are reduced by an herbicide. A determination of relative growth rate (RGR) averaged from all trials demonstrated that for *A. retroflexus,* on average, glyphosate applied at the 0.1 × rate reduced the RGR by half and, when given at the 1.0 × rate, reduced it to zero (i.e., plant death) (Figure 5). Growing *A. retroflexus* at either ambient or elevated [CO_2] did not alter glyphosate tolerance. In contrast, glyphosate applied to elevated [CO_2]-grown *C. album* either had no effect (0.1 × rate) or reduced but did not eliminate growth (1.0 × rate), whereas growth of ambient [CO_2]-grown *C. album* was either significantly reduced (0.1 × rate) or eliminated altogether (1.0 × rate) (Figure 5).

Discussion

Elevated [CO_2] per se resulted in a significant stimulation of leaf photosynthesis, plant growth, and height and a significant reduction in stomatal conductance in the C_3 species, *C. album.* Increasing CO_2 concentration had no effect on growth and plant height in the C_4 species, *A. retroflexus.* In general, these observed differences are consistent with the response of C_3 vs. C_4 plants to elevated CO_2 concentration (Kimball et al. 1993; Poorter 1993; Ziska and Bunce 1997).

If the growth of C_3 weeds such as *C. album* can be directly stimulated by future levels of atmospheric carbon dioxide, how will this affect chemical weed control efforts? In general, the larger a plant is when a postemergence herbicide is applied, the less effective the herbicide. If increased tolerance to glyphosate is observed at elevated [CO_2] in *C. album,* does this simply reflect a larger size of the plant (due to growth at elevated [CO_2]) at the time of glyphosate application? For example, in the present study, *C. album* plants spend about 5 d less in the seedling stage when grown at elevated [CO_2]. This illustrates a simple but important point—i.e., that the efficacy of glyphosate (and other postemergence herbicides) would be reduced at elevated [CO_2] because the time spent in the stage of greatest herbicide sensitivity would be shortened. Clearly, therefore, the size of the plant at the time of spraying would be an important consideration.

The relative influence of plant size to glyphosate tolerance can be determined by plotting the ratio of the dry weight of treated plants to that of their respective control 2 wk after glyphosate application as a function of the initial dry weight at the time of spraying. If increased tolerance is sim-

TABLE 4. Vegetative and total dry weights (per plant) of *Amaranthus retroflexus* (C_4) and *Chenopodium album* (C_3) grown at ambient and elevated carbon dioxide 14 d after postemergent treatment with glyphosate on July 27. Seedlings were sprayed 17 DAS, and postflowering plants were sprayed 31 DAS. n = 4. All other parameters are given in Table 3.

Species	[CO_2]	Herb.	Leaf area	Leaf wt	Stem wt	Root wt	Total wt	E/A
						g		
Seedling stage								
A. retroflexus	Amb.	C	1,720	6.68	7.09	3.83	17.60	
	Elev.	C	1,815	7.14	8.36	3.82	19.31	10
A. retroflexus	Amb.	0.1 ×	573	1.95	1.33	0.72	4.00	
	Elev.	0.1 ×	793*	2.98*	2.02*	2.19*	7.19*	.80*
A. retroflexus	Amb.	1.0 ×	0.0	0.00	0.47	0.04	0.52	
	Elev.	1.0 ×	0.0	0.00	0.44	0.09	0.46	0.89
C. album	Amb.	C	1,092	4.06	3.13	1.37	8.55	
	Elev.	C	1,188	4.94	3.43	2.05*	10.41*	.22*
C. album	Amb.	0.1 ×	1,076	3.17	2.13	1.05	6.35	
	Elev.	0.1 ×	1,614*	6.42*	4.74*	2.37*	13.52*	2.13*
C. album	Amb.	1.0 ×	0.0	0.00	0.33	0.05	0.38	
	Elev.	1.0 ×	0.0	0.00	0.92*	0.12*	1.03*	2.71*
Maturity								
A. retroflexus	Amb.	C	3,394	13.94	33.36	7.63	54.92	
	Elev.	C	3,382	14.20	32.85	7.06	54.10	0.99
A. retroflexus	Amb.	0.1 ×	3,167	13.74	33.39	8.25	55.37	
	Elev.	0.1 ×	3,930*	16.33*	34.46	8.01	58.80	1.06
A. retroflexus	Amb	1.0 ×	0.0	0.00	10.34	2.38	12.71	
	Elev.	1.0 ×	177*a	0.88*b	13.10*	2.99	16.25*	1.28*
C. album	Amb.	C	3,615	14.42	19.66	6.52	40.60	
	Elev.	C	3,675	16.58	27.34*	7.30	51.20	
C. album	Amb.	0.1 ×	2,696	10.17	17.79	3.89	31.86	
	Elev.	0.1 ×	3,547*	15.87*	30.38*	6.35*	52.60*	.65*
C. album	Amb.	1.0 ×	538a	4.70b	5.98	1.65	12.33	
	Elev.	1.0 ×	1,421*a	9.94*b	17.18*	3.56*	30.68*	2.49*

[a] Green, nonwilted leaves only.
[b] All leaf material.

ply a function of plant size, then ambient and elevated [CO_2]-grown plants should have a similar response.

The four separate trials described previously provided a range of plant sizes (dry weights) at the time of glyphosate application. *Amaranthus retroflexus* treated at the 1.0 × rate showed enhanced tolerance (i.e., the dry weight ratio of sprayed to unsprayed plant increased) as the initial plant weight at the time of spraying increased, but no difference between ambient and elevated [CO_2]-grown plants was observed (Figure 6). *Chenopodium album* plants also demonstrated increased glyphosate tolerance with size. However, a distinct response was observed for each [CO_2] treatment at the 1.0 × rate. That is, glyphosate tolerance was increased as the size of the plant to be sprayed increased, but for any size plant, growth at elevated [CO_2] resulted in enhanced tolerance relative to the ambient [CO_2] condition (Figure 6).

If the differences are not only a reflection of plant size at the time of spraying, what is the basis for increased tolerance to glyphosate at elevated [CO_2] for *C. album?* It is reasonable to suggest that a [CO_2]-induced reduction in stomatal conductance may have limited glyphosate uptake. Indeed, in this study for postflowering *C. album* plants, stomatal conductance was reduced by ~73% at elevated [CO_2]. However, *A. retroflexus* also showed a reduction in conductance with elevated [CO_2] (~45%) and a much lower conductance relative to *C. album* overall, but it did not exhibit increased tolerance to glyphosate as determined by changes in RGR. This does not rule out [CO_2]-induced changes in foliar absorption and a subsequent reduction in glyphosate uptake; however, glyphosate absorption is not necessarily through stomates, and actual concentrations of glyphosate in the plants following application were not determined.

Even if glyphosate uptake rates did not differ as a function of elevated [CO_2], there are still a number of physiological changes commonly observed in elevated [CO_2] plants that could influence glyphosate effectiveness. In general, protein content per gram of tissue can be reduced at elevated [CO_2] (Bowes 1996), which could result in less demand for aromatic amino acids (glyphosate is the principal inhibitor of the shikimic acid pathway that is involved in the production of aromatic amino acids [Vaughn and Duke 1991]). Alternatively, high leaf starch concentrations that commonly occur in C_3 plants under CO_2 enrichment could interfere with herbicide activity (Patterson 1993). For *Beta vulgaris* L. (sugarbeet), short-term increases in CO_2 concentration restored net carbon exchange in glyphosate-treated plants (Geiger et al. 1986). However, it is clear that additional experiments are needed to determine whether elevated CO_2 results in reduced glyphosate uptake or alteration of glyphosate activity for *A. retroflexus* and *C. album*.

Irrespective of the mechanism, the present study demonstrates that growth at elevated [CO_2] can increase tolerance to glyphosate. In *C. album*, for example, although the dry weight at either stage of growth (for both experiments) was greatly reduced at elevated [CO_2] at the 1.0 × appli-

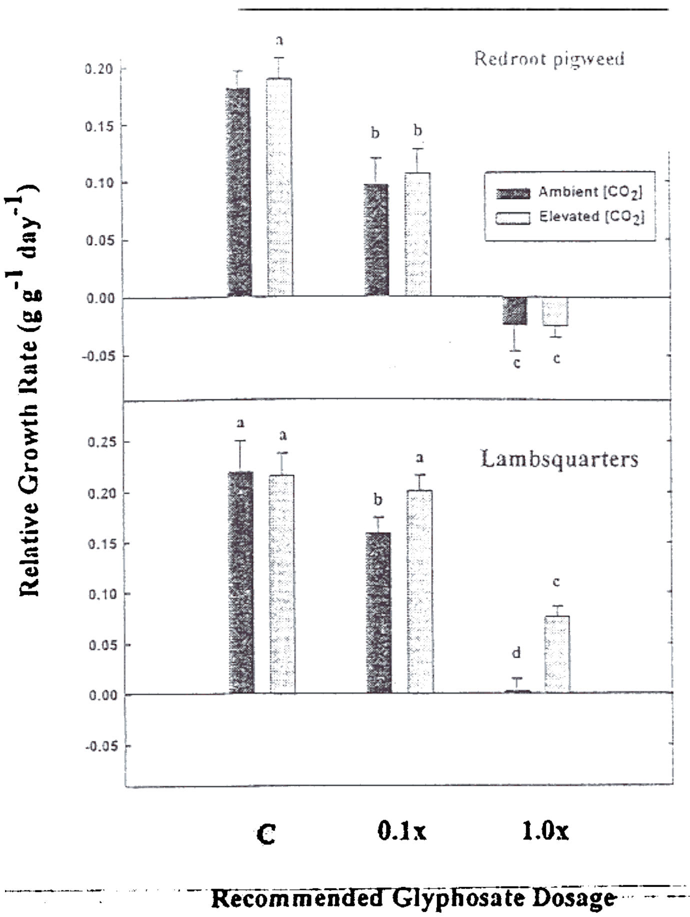

FIGURE 5. Average change in RGR (g g^{-1} d^{-1}) as a function of treatment [CO_2] for the 14-d period following glyphosate application in *Amaranthus retroflexus* and *Chenopodium album*. C = no herbicide, 0.1 × = 10% of recommended rate, and 1.0 × = 100% of recommended rate. Common means separated by different letters are statistically different ($P < 0.05$) relative to the control (LSD). Bars are ± SE.

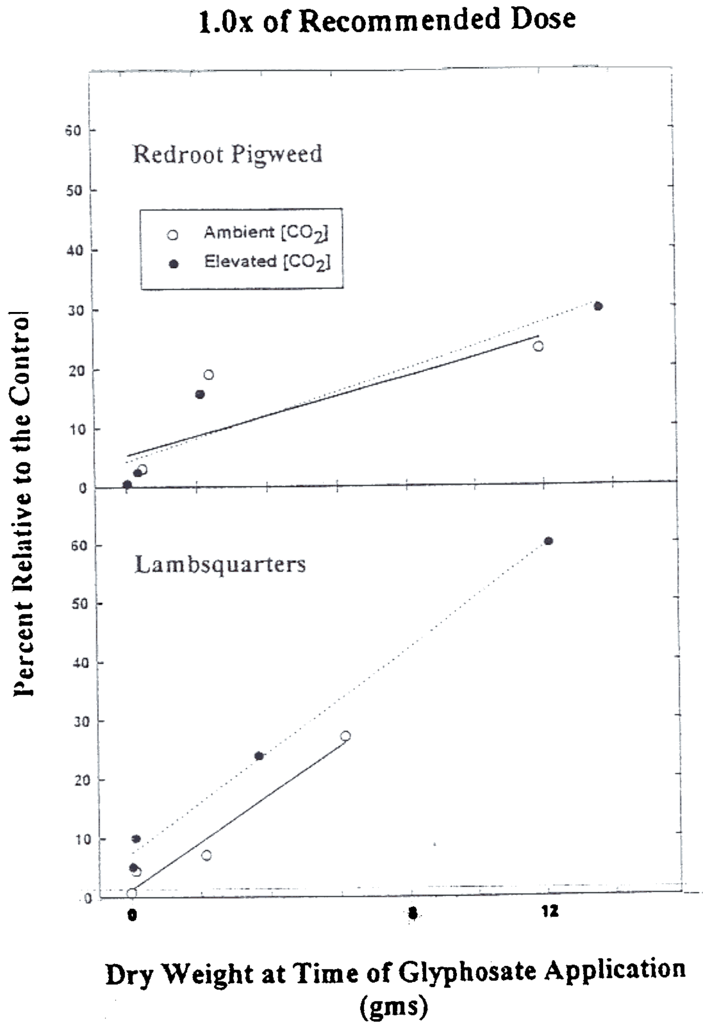

FIGURE 6. Relationship between the initial weight of *Amaranthus retroflexus* and *Chenopodium album* plants at the time of glyphosate application (1.0 × rate) and the ratio of the dry weight of the sprayed plants to the ambient or elevated [CO_2] control (nonsprayed) plants after 14 d (as a percentage). Increases in the "percent relative to the control" indicate decreasing effectiveness of glyphosate in controlling growth. For ratios of dry weight < 5%, plants were considered to have died. Data were obtained from separate trials on seedling and postflowering plants done in the spring (March/April) and summer (July/August).

cation rate, growth (as determined from the observed changes in whole-plant dry weight) was still positive, and green leaves were still present 14 DAS. No ambient *C. album* plants survived (i.e., no significant increase in dry weight was observed) during this period. Survival suggests regrowth and seed production of elevated [CO_2]-grown *C. album* plants over time. For *A. retroflexus*, the results are less clear. Regrowth of green leaves for postflowering, elevated [CO_2]-grown plants in the July trial treated at the 1.0 × rate suggests enhanced tolerance. However, regrowth, while significant, was small. Longer observation periods would be needed in future trials to confirm regrowth for this species.

One of the few positive benefits associated with the ongoing rise in atmospheric [CO_2] is the anticipated increase in crop growth and productivity. However, the data presented here show that postemergence application of glyphosate at commercial rates may be inadequate to control such common and troublesome C_3 weeds as *C. album*. Further applications, or additional glyphosate, could presumably control such weeds but would add to the economic cost of weed control. Consequently, one unintended consequence of higher [CO_2] levels may be to hinder chemical weed control efforts with subsequent effects on crop–weed competition. While additional data are needed to confirm the ubiquity of glyphosate tolerance for elevated [CO_2]-grown C_3 plants, if chemical weed control does become less effective, any potential increases in agricultural productivity with increasing atmospheric [CO_2] could be adversely affected.

Sources of Materials

[1] WMA2 infrared gas analyzer, CIRAS-1, PP Systems, 241 Winter Street, Haverhill, MA 01830.

[2] Data logger, Campbell Scientific, 815 West 1800 North, Logan, UT 84321-1784.

[3] "Roundup" commercial glyphosate, Monsanto Agricultural Products, 800 North Lindbergh Boulevard, St. Louis, MO 63167.

[4] TeeJet 8003E nozzles, Spraying Systems Corp., Wheaton, IL 60187.

[5] Leaf area meter, model 3100, Li-Cor Corporation, Lincoln, NE 68504.

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