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Changes in biomass and root:shoot ratio of field-grown Canada thistle (*Cirsium arvense*), a noxious, invasive weed, with elevated CO_2: implications for control with glyphosate

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Canada thistle was grown under field conditions in 2000 and 2003 at ambient and elevated (~ 350 μmol mol^{-1} above ambient) carbon dioxide [CO_2] to assess how rising [CO_2] alters growth, biomass allocation, and efficacy of the postemergent herbicide glyphosate. By the time of glyphosate application, approximately 2 mo after emergence, elevated CO_2 had resulted in significant increases in both root and shoot biomass. However, the relative positive effect of [CO_2] was much larger for root, relative to shoot growth, during this period (2.5- to 3.3-fold vs. 1.2- to 1.4-fold, respectively) with a subsequent increase in root to shoot ratio. Glyphosate was applied at 2.24 kg ae ha^{-1} in 2000 and 2003. Subjective classification of leaf damage in shoots after spraying indicated no significant difference in the extent of necrosis in aboveground tissue as a function of CO_2 concentration. After a 6-wk regrowth period, significant reductions in shoot and root biomass relative to unsprayed plots were observed under ambient [CO_2]. However, the decrease in the ratio of sprayed to unsprayed biomass was significantly less at elevated relative to ambient [CO_2] conditions for roots in both years, and no difference in shoot biomass was observed between sprayed and unsprayed plots for Canada thistle grown at elevated [CO_2] in either year. The observed reduction in glyphosate efficacy at the enriched [CO_2] treatment did not appear to be associated with differential herbicide uptake, suggesting that tolerance was simply a dilution effect, related to the large stimulation of root relative to shoot biomass at elevated [CO_2]. Overall, the study indicates that carbon dioxide–induced increases in root biomass could make Canada thistle and other perennial weeds that reproduce asexually from belowground organs harder to control in a higher [CO_2] world.

Nomenclature: Canada thistle, *Cirsium arvense* L. Scop. CIRAR.

Key words: Climate change, carbon dioxide levels, herbicide efficacy.

The concentration of atmospheric carbon dioxide [CO_2] has risen ~ 39% from a preindustrial concentration of 270 μmol mol^{-1} to a current estimate of 372 μmol mol^{-1}, with most of the increase occurring in the past 50 yr (McCarthy et al. 2001). The United Nations Intergovernmental Panel on Climate Change predicts that [CO_2] could exceed 700 μmol mol^{-1} by the end of the current century (McCarthy et al. 2001). Under present ambient [CO_2], plants with C_3-type photosynthesis (i.e., 96% of all plant species) are carbon limited. Numerous experiments and reviews have consistently reported increases in the rate of photosynthesis, growth, and reproductive response of agronomically important C_3 crop and tree species. A limited number of studies on annual weed species have shown that increased [CO_2] concentration may result in sustained growth stimulation (Patterson 1995a). However, the impact of elevated [CO_2] on the growth and development of perennial, noxious weeds under field conditions has never been examined.

An estimated 5,000 alien plant species have become established in North America, with economic costs exceeding $36 billion each year (Pimental et al. 2000). The noxious, invasive perennial weed Canada thistle has been widely recognized as one of the most serious weed pests known to agriculture. At present, it is the most frequently listed noxious weed species among farmers and growers in the continental United States and southern Canada (Skinner et al. 2000).

Although various methods have been used to control Canada thistle and other noxious weed species, one of the most effective methods continues to be chemical control (Salzman et al. 1997). Systemic, nonselective postemergent herbicides have proven to be among the most effective chemistries for the control of perennial weeds (Bradshaw et al. 1997). However, if increasing atmospheric CO_2 also stimulates the growth of Canada thistle, how will this alter future control efforts? In a seminal work of its kind, Patterson and Flint (1990) noted that chemical control could be exacerbated if additional CO_2 resulted in greater root, rhizome, or tuber growth, although they did not specifically test this hypothesis. To date, although additional CO_2 has resulted in dramatic increases in root growth in some studies (Bernston and Woodward 1992; Prior et al. 1994; Rogers et al. 1986), the role of CO_2 in enhancing belowground structures and subsequent effects on herbicide efficacy has not been specifically tested in noxious or invasive weeds under field conditions.

Our objective of the current study was to quantify changes in Canada thistle growth, particularly root:shoot ratio, and subsequent changes in chemical efficacy as a function of [CO_2] in situ. We chose glyphosate as a highly effective postemergent herbicide with associated genetically modified

crop lines, e.g., glyphosate-resistant soybean [*Glycine max* (L.) Merr]. Because of the large monetary investment, it is anticipated that long-term use (decades) of the associated herbicides will coincide with the continuing increase in atmospheric [CO_2] during the 21st century.

Materials and Methods

Experiments were conducted in 2000 and 2003 using six clear, acrylic open-top chambers located at the USDA experimental farm in Beltsville, MD. Each chamber was approximately 1.1 by 2.0 m (i.e., 2.1 m^2 of surface area) and was 1.8 m tall. A blower pulled air out of the base of each chamber at a rate of 6 m^3 min^{-1}. Carbon dioxide was introduced into three of the chambers at the inlets of mixing fans positioned above the canopies. Samples of air from each chamber were pumped sequentially through an absolute infrared analyzer in an adjacent air-conditioned shelter and [CO_2], air temperature, and photosynthetically active radiation (PAR) logged at 5-min intervals. Average ambient (24 h) [CO_2] was 421 ± 24 μmol mol^{-1} and 415 ± 18 μmol mol^{-1}, whereas elevated [CO_2] averaged 350 ± 50 μmol mol^{-1} and 346 ± 39 μmol mol^{-1} above this value for 2000 and 2003, respectively. Additional CO_2 was injected if elevated levels fell below the desired set point. Air temperature inside the chambers averaged 1 C above outside temperature, and chamber walls transmitted about 90% of the incident PAR. Air temperature and PAR were determined by an aspirated shaded thermocouple and quantum sensor,[1] respectively. A weather station at the site recorded standard meteorological variables for comparison with chamber values.

The soil at the site was a Cordurus silt-loam with pH 5.5 and high availability of potash, phosphate, and nitrate (Ziska 2000). No additional nutrients were added. For 2000 and 2003, root sections were obtained in a nearby fallow field from Canada thistle plants that had been tagged the previous year. In late May and early June, sections were sized to ~ 0.5 cm in diameter and 1 to 2 cm in length and planted to a depth of 5 cm in 2-m-long rows spaced 30 cm apart within each chamber (i.e., three rows per chamber). After emergence, plots were watered weekly, depending on rainfall, to replace estimated evapotranspirational loss. Canada thistle growing in this field had not received herbicide previously.

Recommended doses for Canada thistle control using commercial glyphosate, 'Round-up',[2] were applied on July 27, 2000, and August 6, 2003, at 62 and 65 d after planting, respectively. Each chamber was split (using a large plexiglass sheet to prevent drift during spraying) into two plots, with each split plot receiving 2.24 kg ae ha^{-1} glyphosate using a pressurized backpack sprayer. Top growth from the unsprayed plots was removed by hand at this time and dried to determine shoot biomass at the time of herbicide application. Plastic borders placed within the soil prevented root intrusion between split plots.

Sprayed aboveground material was assessed for visual damage on a 1 to 6 scale (1 = slight marginal leaf chlorosis, 5 = 90% necrosis of all aboveground material) once a day after spraying for a 1-wk period. Two soil subsamples (2.43 L in volume to a depth of 30 cm) were also taken at random within rows of each unsprayed plot immediately after shoot removal and were used to screen, wash, and collect root material. Because of the age of the plants, these soil subsamples were sufficient to remove ca. 90% of the belowground root material. Once roots were removed, the soil was added back to the subsample site and the site marked to prevent resampling. Both roots and shoots were dried at 65 C until a constant weight was obtained. For the herbicide-treated plots, dead shoot material (which was 100% of aboveground biomass, i.e., 5+ on the visual scale) for both [CO_2] treatments (data not shown) was removed over a 1-wk period after spraying.

To evaluate the effects of [CO_2] on herbicide efficacy in Canada thistle, shoot regrowth and belowground biomass were determined for sprayed and unsprayed split plots. Shoot regrowth was determined by counting shoot numbers and removing aboveground biomass generated since herbicide application in both sprayed and unsprayed plots after a 6-wk period in 2000 and 2003. Simultaneously, two additional soil subsamples (2.43 L in volume to a depth of 30 cm) were obtained in each split plot to determine live root biomass. All samples were dried and weighed as described previously. Comparisons of sprayed with unsprayed biomass for both shoots and roots were used to determine herbicide efficacy. The experiment was conducted in nonsequential years (2000 and 2003) to prevent overwintering of root biomass.

The experiment was analyzed as a randomized complete split-plot design for each year using a one-way analysis of variance, with [CO_2] as the main effect (Statview[3]). To compare efficacy, the ratio of sprayed to unsprayed was evaluated as a function of either above- or belowground biomass for each CO_2 treatment relative to unity (i.e., no effect) using a *t* test, assuming unequal variance (Statview[3]). Unless otherwise stated, all differences are considered significant at the $P < 0.05$ level.

Results and Discussion

Canada thistle is widely regarded as a noxious or invasive weed in agricultural situations both within the United States and Canada (Skinner et al. 2000; White et al. 1993). One of the notable features contributing to the invasive ability of Canada thistle is its extensive root system (Robbins et al. 1970). Because any part of the root system can give rise to buds, which develop into leafy shoots, single plants can develop into dense clonal patches. Although it can be argued that other environmental factors (competition, water, nutrients) may limit the growth response of plant species to rising [CO_2] (DeLucia et al. 1985), Canada thistle typically occurs in monoclonal patches in agronomic situations (Donald 1990) where water or nutrients will probably not limit its response to [CO_2] in situ.

At the time of herbicide application, growth at elevated [CO_2] had resulted in a small but significant increase in shoot biomass in both years but a larger, significant increase in root biomass (2.5- to 3.3-fold) relative to ambient [CO_2] (Figure 1). For plots that were not sprayed, greater increases in root relative to shoot biomass were also observed for the elevated [CO_2] treatment at the time of regrowth assessment (Figures 2A and 3A) in both years. This was consistent with the greater stimulation of root biomass for the elevated [CO_2] treatment at the time of herbicide application.

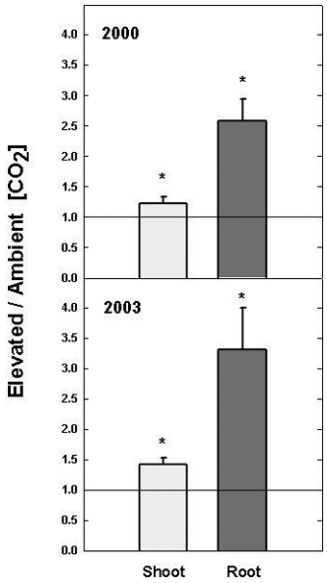

FIGURE 1. Canada thistle shoot and root biomass at elevated (~ 350 μmol mol^{-1} above ambient) relative to ambient (~ 420 μmol mol^{-1}) CO_2 concentration in 2000 and 2003 at the time of herbicide application (plants are approximately 2 mo old). Asterisk indicates a significant difference relative to unity (i.e., no [CO_2] treatment effect). Bars indicate ±SE.

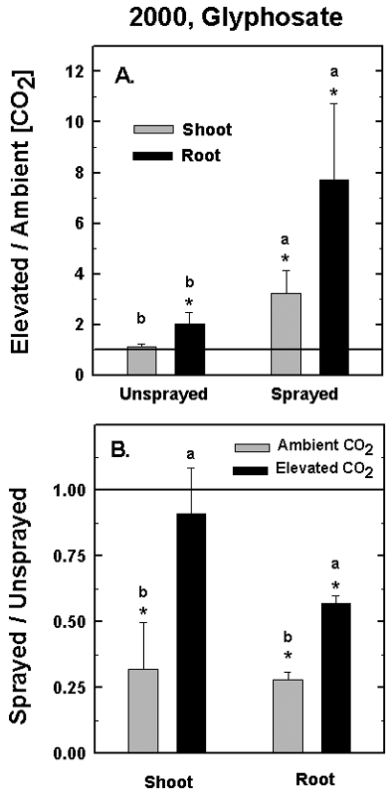

FIGURE 2. Canada thistle biomass allocation as a function of [CO_2], 6 wk after glyphosate application in 2000. Canada thistle was grown at either ambient or elevated [CO_2] (~ 350 μmol mol^{-1} above ambient) and sprayed with glyphosate or not. (A) Ratio of shoot or root biomass produced at elevated to that produced at ambient [CO_2] for plants either sprayed or not sprayed with glyphosate 6 wk after treatment. (B) Ratio of sprayed to unsprayed biomass for shoots and roots grown at ambient and elevated CO_2 6 wk after treatment. Asterisk indicates a significant difference relative to unity (i.e., no effect of the herbicide). Different letters indicate a significant effect of [CO_2] on either the ratio of elevated to ambient [CO_2] biomass (A) for roots and shoots or the ratio of sprayed to unsprayed biomass (B). Ratios were obtained from data in Table 1; however, means were obtained by averaging the ratios obtained from each replication and therefore are not necessarily equivalent to the values obtained if one calculates the ratio from the biomass means in Table 1.

Given the ability of Canada thistle to regenerate from root fragments as small as 3 to 6 mm thick and 8 mm long (Prentiss 1889), dispersal of roots by mechanical means (hoes, shallow cultivation) can result in widespread distribution of this species in agronomic situations. In the current experiment, the ability of Canada thistle to sequester additional biomass in roots relative to shoots with increasing [CO_2] suggests that Canada thistle could, potentially, be a more aggressive competitor in agronomic situations in a future, higher [CO_2] environment.

If rising [CO_2] increases Canada thistle growth and root allocation, how will this modify the efficacy of chemical control? In both years, glyphosate resulted in necrosis of all aboveground tissues with no difference in observable damage between ambient and elevated [CO_2] treatments (i.e., all ratings were above 5 for the sprayed plots, irrespective of [CO_2]). This suggested no significant change in herbicide uptake. However, because of Canada thistle's ability to regrow from belowground structures, any effective postemergence control must rely not only on foliar uptake but also on translocation of the herbicide to the roots in sufficient dosage to inhibit root bud formation. In the current experiment, we used a relative comparison of root bud formation (new shoot growth) between sprayed and unsprayed plots after herbicide application as a means of determining herbicide effectiveness. For unsprayed plots, shoot growth was removed by hand concurrently with herbicide application. Therefore, regrowth from each split plot reflects the difference between potential root bud formation (unsprayed) and root bud formation after herbicide application (sprayed).

Comparison of regrowth for the sprayed vs. unsprayed plots for glyphosate application in 2000 indicated significant reductions in sprayed biomass (either as shoots or roots) relative to unsprayed biomass when grown at ambient [CO_2] (Figure 2B; Table 1). Similarly, the ratio of sprayed to unsprayed biomass declined for roots at elevated [CO_2]. However, the reduction in this ratio was significantly less at elevated [CO_2], and for shoot biomass, no significant difference in the sprayed to unsprayed ratio was observed (Figure 2B). This differential response suggested [CO_2]-induced tolerance to this herbicide. The degree of tolerance is indicated both by the shoot:root biomass at elevated relative to ambient [CO_2] when sprayed vs. unsprayed plants were compared (Figure 2A) as well as the number of regrowth shoots for the elevated relative to the ambient [CO_2] sprayed plots (71 vs. 36, respectively).

In 2003, reapplication of glyphosate resulted in similar reductions in sprayed to unsprayed biomass for Canada thistle grown at ambient [CO_2]. As observed in 2000, the reduction in shoot or root biomass after regrowth was significantly less at the elevated [CO_2] treatment, and again, no difference in the sprayed to unsprayed ratio was observed for shoot biomass (Figure 3B; Table 1). As before, the [CO_2]-induced change in tolerance resulted in a significant increase in the ratio of elevated to ambient [CO_2] biomass for the sprayed relative to the unsprayed condition (Figure 3A). As previously observed, the elevated [CO_2] sprayed plots produced a greater number of shoots (52) relative to

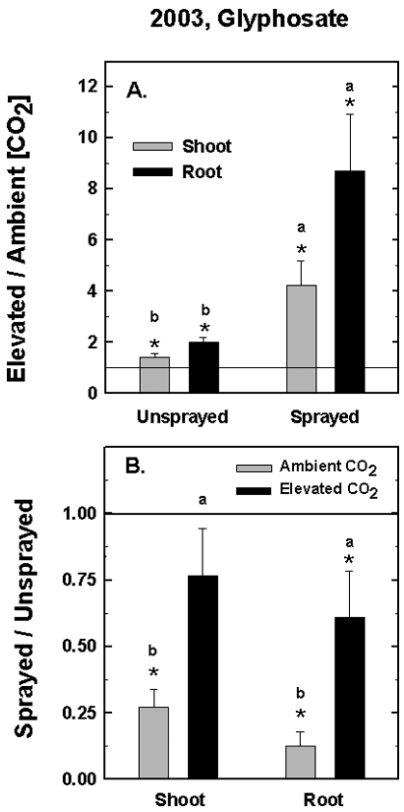

FIGURE 3. Canada thistle biomass allocation as a function of $[CO_2]$, 6 wk after glyphosate application in 2003. Canada thistle was grown at either ambient or elevated $[CO_2]$ (~ 350 μmol mol^{-1} above ambient) and sprayed with glyphosate or not. (A) Ratio of shoot or root biomass produced at elevated to that produced at ambient $[CO_2]$ for plants either sprayed or not sprayed with glyphosate 6 wk after treatment. (B) Ratio of sprayed to unsprayed biomass for shoots and roots grown at ambient and elevated CO_2 6 wk after treatment. Asterisk indicates a significant difference relative to unity (i.e., no effect of the herbicide). Different letters indicate a significant effect of $[CO_2]$ on either the ratio of elevated to ambient $[CO_2]$ biomass (A) for roots and shoots or the ratio of sprayed to unsprayed biomass (B). Ratios were obtained from data in Table 1; however, means were obtained by averaging the ratios obtained from each replication and therefore are not necessarily equivalent to the values obtained if one calculates the ratio from the biomass means in Table 1.

the ambient $[CO_2]$ sprayed plots (21) after herbicide application.

What is the basis for the observed change in herbicide efficacy with elevated $[CO_2]$? Patterson (1995b) and Patterson and Flint (1990) have suggested that $[CO_2]$ could induce anatomical changes, including changes in leaf thickness and stomatal number and aperture, that would reduce foliar absorption. Previous work on glyphosate and common lambsquarters (*Chenopodium album* L.) indicated significantly less necrotic leaf tissue at elevated relative to ambient $[CO_2]$ after spraying (Ziska et al. 1999), consistent with this supposition. However, in the current experiment, complete desiccation and necrosis of all aboveground shoot biomass was observed for both $[CO_2]$ treatments in 2000 and 2003 after spraying, suggesting that uptake did not differ as a function of $[CO_2]$. Interestingly, the use of new and improved commercial surfactants may allow penetration independent of thickness and stomatal number and aperture (Hiroyoshi et al. 1993; Zabkiewicz 2000) overcoming any $[CO_2]$-induced anatomical effects. However, because uptake was not measured directly, it is possible that there may have been less uptake (and subsequently less translocation to roots), although still sufficient uptake to kill the aboveground tissue.

A simpler explanation for reduced efficacy may be related to CO_2-induced changes in biomass allocation. It is striking that in both years increased $[CO_2]$ preferentially increased root over shoot biomass by the time of herbicide application. This suggests that the $[CO_2]$ stimulation of belowground biomass could have resulted in a dilution effect as initially suggested by Patterson and Flint (1990). Thus, the efficacy of herbicide control of regrowth may be reduced at elevated $[CO_2]$ because of increased allocation to belowground root biomass. However, additional data, particularly on changes in herbicide uptake as a function of $[CO_2]$, are needed to confirm this.

It has been generally recognized that the rise in atmospheric CO_2 since the industrial revolution and the anticipated increase for the 21st century could result in increases in crop productivity; however, quantification of the response of noxious or invasive perennial weeds to $[CO_2]$ under field conditions is unavailable. Data from the current field study indicated that the growth and root:shoot ratio of Canada thistle may be improved in a future, elevated $[CO_2]$ environment. This is consistent with $[CO_2]$-induced increases

TABLE 1. Canada thistle shoot and root dry weight (±SE) 43 and 42 d after glyphosate application in 2000 and 2003 for plants grown at ambient and elevated CO_2 concentrations.

	Year	$[CO_2]$	Sprayed	Unsprayed
		(μmol mol^{-1})		
Shoots (g m^{-2})	2000	421	24.4 ± 7.2	60.3 ± 16.7[a]
		771	58.7 ± 3.0[b]	64.5 ± 7.1
	2003	417	18.1 ± 6.7	79.1 ± 11.1[a]
		753	85.7 ± 7.2[b]	116.5 ± 17.3[b]
Roots (g 2.43 L soil)	2000	421	0.08 ± 0.03	0.43 ± 0.04[a]
		771	0.45 ± 0.03[b]	0.70 ± 0.10[a,b]
	2003	417	0.09 ± 0.04	0.74 ± 0.26[a]
		753	0.69 ± 0.25[b]	1.46 ± 0.30[a,b]

[a] A significant difference between sprayed and unsprayed plants at a given $[CO_2]$.
[b] A significant difference as a function of $[CO_2]$ for either sprayed or unsprayed plants. Significance was determined at $P < 0.05$ (*t* test assuming unequal variances).

in root:shoot ratio that were observed for Canada thistle and other perennial weeds under growth chamber conditions (Ziska 2003). However, the increase in root:shoot ratio may, in turn, result in a subsequent decrease in herbicide efficacy with respect to regrowth for Canada thistle and other perennial noxious weeds. Although effective control of Canada thistle does not rely solely on chemical means, chemical control does play a significant role in the management and control of Canada thistle (Donald 1990).

It can be argued that [CO_2]-induced changes in efficacy are irrelevant given the rate of atmospheric [CO_2] increase (i.e., other herbicides will be developed in the future). However, herbicide use can persist over decades (e.g., 2,4-D has been in continuous use since 1950), coinciding with significant increases in atmospheric [CO_2] (i.e., 310 to 375 μmol mol^{-1} from 1950 to 2003). At present, many commercial ventures have invested in genetically modified crops specific for a given herbicide; consequently, it is likely that the use of these associated herbicides (e.g., glyphosate) would persist for decades. Overall, chemical control of Canada thistle will still be possible with rising [CO_2], but additional applications or increasing doses may be necessary. How these potential changes will alter future economic and environmental costs, however, is unclear.

Sources of Materials

[1] Thermocouple and quantum sensor, Li-Cor Corporation, 4808 Progressive Avenue, Lincoln, NE 68504.

[2] Glyphosate as isopropylamine salt, Monsanto Company, P.O. Box 1750, Columbus, OH 43216-1750.

[3] Statview, SAS Campus Drive, Cary, NC 27513-2414.

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