The impact of nitrogen supply on the potential response of a noxious, invasive weed, Canada thistle (*Cirsium arvense*) to recent increases in atmospheric carbon dioxide

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Received 6 November 2002; revised 26 February 2003

A recognized invasive weed, Canada thistle (*Cirsium arvense* L. Scop.) was grown at ambient and pre-ambient concentrations of atmospheric carbon dioxide [CO$_2$] (373 and 287 µmol mol$^{-1}$, respectively) at three levels of supplemental nitrogen (N) (3, 6 and 14.5 mM) from seeding until flowering [77 days after sowing (DAS)]. The primary objective of the study was to determine if N supply limited the potential photosynthetic and growth response of this species to the increase in atmospheric [CO$_2$] which occurred during the 20th century (i.e. approximately 290 to 370 µmol mol$^{-1}$ CO$_2$). Leaf photosynthesis increased both as a function of growth [CO$_2$] and N supply during the first 46 DAS. Although by 46 DAS photosynthetic acclimation was observed relative to a common measurement CO$_2$ concentration, there was no interaction with N supply. Both [CO$_2$] and N increased biomass, relative growth rates and leaf area whereas root: shoot ratio was increased by CO$_2$ and decreased by increasing N; however, N supply did not effect the relative response to [CO$_2$] for any measured vegetative parameter up to 77 DAS. Due to the relative stimulation of shoot biomass, total above-ground N increased at elevated [CO$_2$] for all levels of supplemental N, but nitrogen use efficiency (NUE) did not differ as a function of [CO$_2$]. Overall, these data suggest that any potential response to increased atmospheric [CO$_2$] in recent decades for this noxious weedy species was probably not limited by nitrogen supply.

Introduction

One factor which may play a role in early establishment and growth of some weedy species is atmospheric CO$_2$ (Patterson and Flint 1980, Ziska and Bunce 1993, Patterson 1995). Since the late 1950s, atmospheric [CO$_2$] has risen approximately 20% from 310 to 375 µmol mol$^{-1}$ (Houghton et al. 1996). Although a number of studies have examined the response of weeds to future, projected increases in atmospheric [CO$_2$], little data is available regarding the potential photosynthetic or growth response to recent changes in atmospheric [CO$_2$] for weedy species (cf. Sage 1995).

Characterization of these responses may be of particular interest to weed ecologists given the recent, rapid increase in atmospheric [CO$_2$]. Recent data for Canada thistle (*Cirsium arvense* L. Scop.), a noxious weed, indicated a strong potential growth response to the rise in [CO$_2$] during the last few decades (i.e. from 280 to 370 µmol mol$^{-1}$) (Ziska 2003). Because Canada thistle is widely recognized as one of the most serious invasive weeds within agriculture (e.g. Skinner et al. 2000), any environmental factor which may alter its ecological success is of obvious interest.

Although a strong response of Canada thistle to recent increases in atmospheric [CO$_2$] was observed, it is unclear how other environmental factors could modify this response. One key environmental factor is nitrogen availability. To date, a number of studies that have examined the interaction of nutrients and elevated CO$_2$ indicate that N tissue concentration is reduced at elevated [CO$_2$] for a wide range of native and cultivated plant species. Although the effect of N deficiency on the CO$_2$ response varies by species, the general trend as reported by Poorter et al. (1996) was a decline in the average growth response to future atmospheric [CO$_2$] as a function of nutrient availability.

**Abbreviations** – DAS, days after sowing; LAR, leaf area ratio; NUE, nitrogen use efficiency; RGR, relative growth rate.
Interestingly, although the effect of N on the response of C₃ plants to future, elevated [CO₂] has been extensively studied, I could find no data regarding the interaction of recent [CO₂] increases and N availability. Yet, it seems clear that N availability can significantly alter plant response to [CO₂], at least for projected elevated concentrations (e.g. Ghannoum and Conroy 1998). Therefore, the principle objective of the current study was to determine if N limited the potential photosynthetic and growth response of Canada thistle, a recognized invasive weed, to recent increases in atmospheric [CO₂]. A secondary objective was to quantify how recent changes in atmospheric [CO₂] may have altered N uptake and concentration for this species.

Materials and methods

Experimental conditions

Canada thistle (Cirsium arvense L. Scop.) was grown using two controlled environmental chambers (EGC Corp., Chagrin Falls, OH, USA) with each chamber kept at one of two constant 24 h carbon dioxide concentrations, 287 ± 11 or 373 ± 9 μmol mol⁻¹ [CO₂]. Two to four seeds (Herbiseed, Berkshire, United Kingdom) were sown in pots filled with vermiculite and thinned to one seedling 4–6 days after emergence. For each CO₂ treatment 60–75 pots were used (depending on seedling survival). To avoid root binding and because of space limitations 60–75 pots were used (depending on seedling survival). To avoid root binding and because of space considerations, different pot volumes, from 0.6 to 22.11 l were used, with the smaller pots being sampled first. The height of all pots was made uniform to avoid differences in light (PAR, photosynthetically active radiation) intensity. For each CO₂ concentration (i.e. each chamber), pots were watered to the drip point daily with one of three complete nutrient solutions that differed only in N concentration: 3, 6 or 14.5 mM (for a complete description and a listing of N sources, both NH₄ and NO₃, please see Robinson 1984 and Bunce 1995).

For all environmental chambers, temperature was altered in a diurnal fashion from an overnight low of 20°C to a maximum afternoon value of 30°C, with an average daily (24 h) value of 23.1°C. Similarly, PAR was also altered concurrently with temperature, with the highest PAR value (900–1000 μmol m⁻² s⁻¹) occurring during the afternoon (1200–1500 h). Daily PAR was 14 h, supplied by a mixture of high pressure sodium and metal halide lamps. The [CO₂] of the air within each chamber was controlled by adding either CO₂ or CO₂-free air to maintain the set concentration. Injection of CO₂ and CO₂-free air was controlled by a TC-2 controller using input from an absolute infra-red gas analyser (WMA-2; PP Systems, Haverhill, MA USA). Temperature, humidity and [CO₂] were recorded every 15 min, and daily averages determined for each chamber. Temperature, PAR and humidity did not differ between chambers. Typical examples of a diurnal temperature/PAR curve for these experimental chambers can be found in Ziska et al. (2001).

Gas exchange

Single leaf photosynthesis (A, the rate of net CO₂ assimilation) was determined at 32 and 46 days after sowing (DAS) on the uppermost, expanded leaf for each of three plants for each combination of [CO₂] and N supply. Measurements were made using a portable open gas exchange system (CIRAS-1; PP Systems) incorporating infra-red CO₂ and water vapour analysers for determining net photosynthetic CO₂ uptake and stomatal conductance. Measurements were determined during the early afternoon period (1200–1500 h) at air temperatures of 30.5°C, approximate PAR values of 1000 μmol m⁻² s⁻¹ and relative humidities of 60%. Photosynthetic response was determined at common CO₂ concentrations of 280 and 370 μmol mol⁻¹ for three levels of added N (3, 6 and 14.5 mM). Observed differences in CO₂ assimilation determined at a common CO₂ concentration were used to indicate photosynthetic acclimation at the single leaf level. In changing to a new [CO₂], sufficient time was given (5–10 min) to allow for a steady-state condition prior to the measurement of assimilation rate.

Vegetative measurements

Plants were grown until 77 DAS by which time floral spikes were observed for all treatments (i.e. end of vegetative stage). Although [CO₂] per se did not affect floral times, N supply was proportional to reproductive development with the 14.5 mM treatment 6–7 days earlier than the 6 mM treatment which in turn, was 3–4 days ahead of the 3 mM treatment. Harvests of four to five plants for each level of [CO₂] and N supply were made at 20, 33, 47, 61 and 77 DAS. For all harvests leaf area was determined photometrically using a leaf area meter (Model 3100; Li-Cor Corporation, Lincoln, NE, USA). Because of the large leaf area after 33 DAS, subsamples of 15–20 leaves per [CO₂] and N level were placed in moistened paper towels to prevent desiccation and leaf area determined as described above. These leaf subsamples were then dried at 65°C and total leaf area per plant and specific leaf weight estimated by the linear regression of leaf area to leaf dry weight (r² > 0.90 for all treatments). In addition to leaf area, dry mass was determined separately for all leaves, stems, roots and floral spikes for each harvest for all treatments following drying at 65°C for a minimum of 48 h or until dry mass was constant. Relative growth rate (RGR) and leaf area ratio (LAR) were determined according to Jones (1983).

Carbon, hydrogen and nitrogen analysis

Determinations of shoot carbon, nitrogen and hydrogen were made for individual plants for the last three runs of the experiment for all treatments at 47, 61 and 77 DAS. Determinations were made by the University of Maryland, Soil Testing Laboratory by combustion on a Leco CHN 2000 instrument. Samples were combusted at 960°C and gases were passed through isolating reagents.
Carbon and hydrogen were measured using infrared detectors and nitrogen was measured using thermal conductivity. Final percentages of C, H and N are based on the dry weight of the sample.

**Statistics**

Because only two chambers were available, and pots do not represent valid replications, a randomized complete block was used with runs over time as replications (blocks). At the end of a given run (i.e. 77 DAS), \([\text{CO}_2]\) was switched between chambers and levels of N supply (3, 6 and 14.5 mM) randomly assigned within the chamber. The entire experiment was repeated four times with the mean value from each run used as a single repeat (photosynthetic values were determined on the last three replications). All measured parameters were analysed using a two way analysis of variance with N and \([\text{CO}_2]\) as the classification variables (Statview, Berkeley, CA, USA). Treatment comparisons were made using a Fisher protected least significant difference. Unless otherwise stated, significant differences for any measured parameter were determined as significant at the \(P < 0.05\) level.

**Results**

**Photosynthesis**

Single leaf photosynthesis was significantly increased from sub-ambient to current \([\text{CO}_2]\) at both 32 and 46 DAS, irrespective of N supplied (Table 1A). However, a significant reduction was observed in photosynthetic rate between leaves from plants grown at sub-ambient and current \([\text{CO}_2]\) when measured at a common concentration of 280 or 370\(\mu\text{mol}\text{mol}^{-1}\) by 46 DAS (Table 1B and C). No significant interaction between N and \([\text{CO}_2]\) was observed for leaf photosynthesis at either growth or measured concentrations for any sampling date.

**Growth characteristics**

Although absolute rates of leaf area were N dependent, increasing atmospheric \([\text{CO}_2]\) from sub-ambient to current levels significantly stimulated whole plant leaf area up to 61 DAS for all levels of N (i.e. no N–\([\text{CO}_2]\) interaction) (Fig. 1). Leaf area ratio (LAR) determinations (Table 2) were generally (but not significantly) lower as a function of \([\text{CO}_2]\) for all N-values and declined with time. Significant reductions as a function of N were observed for a given \([\text{CO}_2]\) treatment at 47 and at 61 DAS (Table 2).

Increasing atmospheric \([\text{CO}_2]\) to current levels significantly stimulated biomass in Canada thistle (Fig. 2). Although the absolute increase in biomass was N dependent, the relative stimulation of biomass by \([\text{CO}_2]\) was unaffected by N supply (Fig. 2). Relative growth rate (RGR) declined over time, but when averaged among N treatments, did not differ as a function of \([\text{CO}_2]\) (Table 2).

Net assimilation rate (determined from LAR and RGR), was significantly greater averaged among all N treatments at 33 DAS but at no other sampling date. Overall, NAR declined for both \([\text{CO}_2]\) treatments over time (Table 2).

An analysis of biomass characteristics at 77 DAS demonstrated significant increases in all parameters with increasing \([\text{CO}_2]\), with no additive effect between N supply and \([\text{CO}_2]\) (e.g. 81 and 76% increase in total biomass).

<table>
<thead>
<tr>
<th>Nitrogen</th>
<th>32 DAS</th>
<th>46 DAS</th>
<th>32 DAS</th>
<th>46 DAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>13.9</td>
<td>16.5*</td>
<td>9.3</td>
<td>14.0*</td>
</tr>
<tr>
<td>6</td>
<td>15.3</td>
<td>21.1*</td>
<td>11.6</td>
<td>15.7*</td>
</tr>
<tr>
<td>14.5</td>
<td>16.6</td>
<td>22.7*</td>
<td>16.9</td>
<td>19.7*</td>
</tr>
</tbody>
</table>

Table 1. Photosynthetic response (determined as \(A\), the rate of \(\text{CO}_2\) assimilation, \(\mu\text{mol}\text{CO}_2\text{m}^{-2}\text{s}^{-1}\)) for Canada thistle leaves grown at 287 and 373\(\mu\text{mol}\text{mol}^{-1}\) \([\text{CO}_2]\) (A) and measured at common \([\text{CO}_2]\) concentrations of 280 and 370\(\mu\text{mol}\text{mol}^{-1}\) at 32 and 46 DAS (B, C) for three levels of added N (3, 6 and 14.5 mM). *Indicates a significant difference as a function of \([\text{CO}_2]\). Differences in \([\text{CO}_2]\) assimilation determined at a common \([\text{CO}_2]\) concentration (B, C) indicate photosynthetic acclimation of single leaves at a given level of N.
biodiverse for the 3 and 14.5 mM N treatments, respectively, with increased [CO2]. Table 3). Averaged among N treatments, leaf area and root weight showed the least and greatest relative response to increased [CO2] (Table 3). The root : shoot ratio increased with increasing [CO2] but only for the 3 and 6 mM treatments. When averaged for all sampling dates, root : shoot was inversely proportional to N supply (0.56, 0.54 and 0.47 for 3, 6 and 14.5 mM N, respectively).

Plant nitrogen

Leaf N percentage did not differ significantly for the high N treatment (14.5 mM) as a function of [CO2]; however, significant reductions were observed for the 3 and 6 mM treatments for some, but not all, sampling times (significant at 77 DAS and 47 and 61 DAS, respectively) (Table 4). Irrespective of [CO2], the relative rate of N uptake (g N g⁻¹ day⁻¹, based on above-ground N) declined with time for all treatments with significant declines observed for the ambient but not sub-ambient [CO2] treatment (Table 4). However, the total above-ground N was significantly higher as a function of [CO2] for all treatments due to the greater relative stimulation of above-ground dry matter (Fig. 3). As a consequence, the amount of N contained within the shoot biomass did not vary as a function of [CO2] (Fig. 4). Overall, the ratio of carbon to nitrogen declined with increasing N supply (8.8, 8.1 and 7.3 for 3, 6 and 14.5 mM N, respectively), but was greater with increasing [CO2] (7.7 versus 8.3).

Discussion

In the current experiment, increasing [CO2] to ambient levels significantly increased single leaf photosynthesis up to 46 DAS. Although the absolute values of photosynthesis declined between sampling dates, the relative stimulation in response to increased [CO2] was maintained. However, by 46 DAS, short-term (minutes) exposure to a common [CO2] indicated photosynthetic acclimation for the ambient [CO2] treatment (Table 4). Physiological basis for acclimation has been suggested to occur, in part, with sink under-utilization, carbohydrate accumu-
The occurrence of acclimation at the single leaf level at 46 DAS does not necessarily mean that carbon uptake is diminished with increased [CO₂]. Even if leaf photosynthetic rates had completely acclimated (i.e. no difference in leaf photosynthetic rates between 287 and 373 μmol mol⁻¹), the relative stimulation in leaf area observed for all levels of N in response to increasing [CO₂] by 46 DAS would still result in significant increases in carbon uptake at the whole plant level. This may be of critical importance since carbon acquisition during early development (i.e. the vegetative stage of growth) directly relates to leaf area and competitive success (e.g. Kropff and Spitters 1991). In the current experiment, leaf area was stimulated overall by both N and [CO₂]. Resources devoted to leaf area (i.e. leaf area per unit of dry biomass) were similar for [CO₂] treatments at a given N level.

These observed changes in carbon assimilation and leaf area are consistent with the observed stimulation of plant biomass for Canada thistle. That is, total biomass was dependent on N supply, but the ability to respond vegetatively to [CO₂] was not dependent on N. Among vegetative characteristics, it is worth noting that the greatest relative stimulation was observed for root biomass with significant increases in root:shoot ratio observed for the 3 and 6 mM N treatments. Typically in field situations, Canada thistle reproduces new shoots from below-ground root buds and can form large clonal patches (Donald 1990). Hence, alterations in root biomass and root:shoot ratio could, presumably, alter the establishment and spread of this species.

The lack of any significant [CO₂] by N interaction in Canada thistle is in contrast to the interaction of N supply and future, elevated [CO₂] observed for some species (e.g. cotton, Rogers et al. 1996; rice, Ziska et al. 1996; Panicum, Ghannoum and Conroy 1998). Although the magnitude of this interaction does vary (e.g. Wong and Osmond 1991), the general trend is that the average growth response to future, elevated [CO₂] decreases with a decrease in nutrient availability (Poorter et al. 1996).

The basis for N determination of [CO₂] sensitivity is unclear. A reduction in the foliage concentration of N in response to future [CO₂] has been observed in a number of studies (e.g. Hocking and Meyer 1991, Rogers et al. 1993). However, low N per se does not prevent a photosynthetic or growth response to enriched [CO₂] in many plant systems (e.g. Cure et al. 1988, Israel et al. 1990, Hocking and Meyer 1991). This is because at the biochemical level, elevated [CO₂] may result in a reduction in Rubisco content and activity, with a subsequent reduction in N allocation to leaves and greater nitrogen use efficiency (e.g. Sage et al. 1989). In contrast, for Canada thistle, increasing [CO₂] from 287 to 373 μmol mol⁻¹ did result in reduced foliar N for some, but not all, sampling dates. Consequently, above-ground N per plant was consistently higher for the ambient [CO₂] treatment and shoot biomass per g of N did not change as [CO₂] increased (i.e. no change in nitrogen use...
efficiency, NUE). However, the relative rate of N uptake diminished by 77 DAS for the 3 and 6 mM N treatments at ambient [CO2], and it is unclear if NUE would still be unchanged over a longer time period.

Aside from any biochemical restrictions, the dependence of the CO2 response to nitrogen may be related to limitations in new sink production. For example in rice, the formation of new tillers (and subsequent panicles), both strong carbon sinks, is N dependent. If N is limiting, photosynthetic and growth responses to [CO2] are transitory (i.e. acclimation occurs, Ziska et al. 1996). However, as mentioned previously, Canada thistle roots represent a large carbon sink utilized in asexual reproduction. Canada thistle can regenerate from root fragments as small as 3–6 mm thick and 8 mm long (Prentiss 1889). Since Canada thistle is perennial, a single plant over time can form a root mat over 1 m deep, extending for several metres in any direction and effectively crowding out other species (Robbins et al. 1970, Donald 1990).

In the current study, decreasing N resulted in significant increases in the root : shoot ratio. Such a shift in allocation could favour the ratio of sinks to sources, allowing a continued response to rising [CO2] even at low N. Interestingly, at the higher (373 μmol mol⁻¹ [CO2]) root : shoot ratio did not diminish, indicating that roots remain a strong sink for carbon even if additional CO2 is made available.

Table 3. Final biomass characteristics at 77 DAS for Canada thistle grown at sub-ambient and current levels of atmospheric CO2 at three different levels of supplied N. *For a given N level indicates a significant difference in response to [CO2] for that characteristic.

<table>
<thead>
<tr>
<th>Nitrogen (mM)</th>
<th>[CO2] (μmol mol⁻¹)</th>
<th>Leaf area (m²)</th>
<th>Leaf weight (g plant⁻¹)</th>
<th>Stem weight (g plant⁻¹)</th>
<th>Root weight (g plant⁻¹)</th>
<th>Total weight (g plant⁻¹)</th>
<th>Root : shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>287</td>
<td>0.186</td>
<td>13.1</td>
<td>3.1</td>
<td>9.1</td>
<td>25.3</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>373</td>
<td>0.286*</td>
<td>22.2</td>
<td>5.0*</td>
<td>18.6*</td>
<td>45.9*</td>
<td>0.67*</td>
</tr>
<tr>
<td>6.0</td>
<td>287</td>
<td>0.331</td>
<td>28.5</td>
<td>7.3</td>
<td>18.9</td>
<td>54.8</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>373</td>
<td>0.361</td>
<td>32.4</td>
<td>10.9*</td>
<td>34.6*</td>
<td>75.8*</td>
<td>0.85*</td>
</tr>
<tr>
<td>14.5</td>
<td>287</td>
<td>0.677</td>
<td>47.6</td>
<td>12.9</td>
<td>33.5</td>
<td>89.2</td>
<td>0.56</td>
</tr>
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<td></td>
<td>373</td>
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<td>68.3</td>
<td>26.3*</td>
<td>56.3*</td>
<td>157.7*</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Table 4. Change in leaf N (%) from 33 to 77 DAS and relative N uptake (g N g⁻¹ day⁻¹, above-ground) from 47 to 77 DAS for Canada thistle grown at sub-ambient and current atmospheric CO2 at three different levels of supplied N. *For a given N level indicates a significant difference in response to [CO2] for given sampling date.

<table>
<thead>
<tr>
<th>Nitrogen (mM)</th>
<th>[CO2] (μmol mol⁻¹)</th>
<th>Percentage leaf N</th>
<th>Relative N uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>287</td>
<td>NA</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>373</td>
<td>5.0</td>
<td>0.019</td>
</tr>
<tr>
<td>6.0</td>
<td>287</td>
<td>5.0</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
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<td>0.004*</td>
</tr>
<tr>
<td>14.5</td>
<td>287</td>
<td>5.5</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>373</td>
<td>5.4</td>
<td>0.0101</td>
</tr>
</tbody>
</table>

Canada thistle is the most frequently listed noxious weed species in surveys of the continental United States.
and southern Canada, and is responsible for millions in economic damage (Skinner et al. 2000). Data in the current experiment suggest that increasing atmospheric carbon dioxide levels over the last century may have resulted in significant increases in leaf photosynthesis, leaf area, biomass, and root:shoot ratio for this species. These data also suggest that while nitrogen availability may determine the absolute photosynthetic and growth response, the relative response to $[\text{CO}_2]$ during the vegetative period (i.e. the period of critical establishment) was independent of $N$.

At present, however, it is difficult to assess to what extent the growth of Canada thistle has actually been altered in situ. Although field evaluations are needed to address such issues, technical limitations prevent exposure of plants to sub-ambient CO$_2$ under realistic conditions (see Mayeux et al. 1993). This does not, of course, lessen the importance of understanding how recent increases in atmospheric $[\text{CO}_2]$ may have affected plant species, particularly those that have been identified as invasive or noxious. The current study then, while limited to growth chamber conditions, does provide the first evaluation of the interaction between nitrogen availability and recent carbon dioxide increases for a recognized invasive weed. Such evaluations will be needed to address the potential agronomic and environmental consequences posed by other invasive weeds as atmospheric CO$_2$ continues to increase.

Acknowledgements – I wish to thank Ernie Goins and Shaun Faulkner for expert technical assistance.

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Woodrow IE (1994) Optimal acclimation of the C\textsubscript{3} photosynthetic system under enhanced CO\textsubscript{2}. Photosynthesis Res 39: 401–412


Edited by J. K. Schjørring