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Rising Atmospheric Carbon Dioxide and Potential Impacts on the Growth and Toxicity of Poison Ivy (*Toxicodendron radicans*)

L. H. Ziska, R. C. Sicher, K. George, and J. E. Mohan

Because of its ability to induce contact dermatitis, the establishment and spread of poison ivy is recognized as a significant public health concern. In the current study, we quantified potential changes in the biomass and urushiol content of poison ivy as a function of incremental changes in global atmospheric carbon dioxide concentration (CO₂). We also examined the rate of new leaf development following leaf removal to simulate responses to herbivory as functions of both CO₂ and plant size. The experimental CO₂ values (300, 400, 500, and 600 μmol mol⁻¹) corresponded approximately to the concentration that existed during the middle of the 20th century, the current concentration and near and long-term projections for this century (2050 and 2090), respectively. Over 250 d, increasing CO₂ resulted in significant increases in leaf area, leaf and stem weight, and rhizome length relative to the 300 μmol mol⁻¹ baseline with the greatest relative increase occurring from 300 to 400 μmol mol⁻¹. There was a nonsignificant (P = 0.18) increase in urushiol concentration in response to CO₂; however, because of the stimulatory effect of CO₂ on leaf biomass, the amount of urushiol produced per plant increased significantly for all CO₂ above the 300 μmol mol⁻¹ baseline. Significant increases in the rate of leaf development following leaf removal were also observed with increasing CO₂. Overall, these data confirm earlier, field-based reports on the CO₂ sensitivity of poison ivy but emphasize its ability to respond to even small (~ 100 μmol mol⁻¹) changes in CO₂ above the mid-20th century carbon dioxide baseline and suggest that its rate of spread, its ability to recover from herbivory, and its production of urushiol, may be enhanced in a future, higher CO₂ environment.

Nomenclature: Poison ivy, *Toxicodendron radicans* (L.) Kuntze TOXRA.

Key words: Climate change, carbon dioxide, leaf development, toxicology.

Records of carbon dioxide concentration (CO₂) have shown an increase in ambient CO₂ of ~ 22% from 311 to 380 μmol mol⁻¹ since the late 1950s (IPCC 2001). The current annual rate of CO₂ increase (~ 0.5%) is expected to continue, with concentrations exceeding 600 μmol mol⁻¹ by the end of the 21st century (IPCC 2001). In addition to its indirect role as a greenhouse gas, rising carbon dioxide has a direct effect on plant systems because it represents the primary raw material (carbon) needed for plant growth. Because 95% of all plants currently lack optimal levels of carbon dioxide for photosynthesis (i.e., those with the C₃ photosynthetic pathway), the rapid increase in carbon availability is likely to stimulate the photosynthesis and growth of both anthropogenically important and deleterious plant species.

Potential differences in the response of weeds and crops to recent and projected increases in CO₂ have been the subject of a number of investigations at the agronomic level (See Ziska and Runion 2006, for a review). Although the role of weed biology in the context of agriculture remains a primary focus of inquiry by weed scientists, it should also be recognized that the role of weeds in altering human health can be significant (Ziska 2003a).

One of the most common weed-induced health effects experienced by the general populace is dermatitis. More than 100 plant species are associated with contact dermatitis, but perhaps the most recognized is the poison ivy group (*Toxicodendron* spp.). These species, ubiquitous throughout North America, can spread by seed or asexually through rhizome production. Rhizomes spread in all directions, both at, and below, the surface, rooting continuously (i.e., not at nodes like runners) and establish themselves over large

horizontal or vertical areas. Sensitivity to urushiol, the oil-like compound found in this species, can occur in about 2 of every 3 people upon initial exposure, with amounts as small as 1 ng sufficient to induce contact dermatitis in sensitive individuals (Tanner 2000). Clearly, potential changes in the growth or toxicity of *Toxicodendron* to rising levels of carbon dioxide will have significant consequences for human health on a broad scale.

A recent 6-yr in situ study of poison ivy at Duke University (Mohan et al. 2006) demonstrated that exposure to elevated CO₂ (~ 200 μmol mol⁻¹ above current background) stimulated photosynthesis and growth (mean plant biomass) with a more toxic form of urushiol when compared with current levels of atmospheric carbon dioxide. Although this study was significant in its scope, there were additional facets of the poison ivy response to CO₂ that required clarification, including (1) to what extent poison ivy has already responded to recent (20th century) changes in CO₂, (2) how rhizome length (a principle means of asexual reproduction) responds to CO₂, (3) how sensitive urushiol concentration or production is to small changes in CO₂, and (4) how CO₂ alters the rate of new leaf development if leaves are removed, as through herbivory. To address these questions, we initiated an 8-mo experiment to examine the growth and urushiol production of clonal poison ivy populations at 100 μmol mol⁻¹ intervals between 300 and 600 μmol mol⁻¹.

Materials and Methods

Experimental Conditions. Rhizome segments (4 to 5 cm in length, 1 to 2 cm in diameter) were collected from a single clonal population of poison ivy located near Buckeystown, MD. Rhizomes were collected in the spring of 2003 and 2004. After sizing, one to two segments were placed in pots filled with a 1:1 mixture of sand and vermiculite and placed on a greenhouse mist bench. Once leaf initiation occurred,

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plants were transferred to one of four CO₂. To avoid root binding and because of space considerations, different pot volumes, from 0.6 to 22.1 L were used, with the smaller pots being sampled first. Pot volumes at time of harvest were constant across experimental treatments. The height of all pots was made uniform to avoid differences in light (photosynthetically active radiation, PAR) intensity. For each CO₂ treatment, pots were watered to the drip point daily with a complete nutrient solution (Robinson 1984).

At present, no methodological system exists that is able to provide subambient CO₂ to plants under field conditions 24 h a day (see Mayeux et al. 1993); consequently, the study was conducted using controlled-environment chambers¹ at Beltsville, MD, with a given chamber set at one of four CO₂ set points (300, 400, 500, and 600 μmol mol⁻¹) for 24 h d⁻¹. The concentration used approximated the atmospheric CO₂ during the middle of the 20th century, the current ambient level, and that projected concentration for the years 2050 and 2090 (Schimel et al. 1996). Actual average 24 h CO₂ values (± SD) were 298.8 ± 15.2, 389 ± 12.1, 504 ± 18, and 589 ± 21 μmol mol⁻¹. For all chambers, day/night temperature was 25/20 C, with an average daily (24 h) value of 22.9 C. Daytime PAR (14 h) was supplied by a mixture of high-pressure sodium and metal halide lamps. To simulate the low-light in situ conditions of poison ivy, plants were placed under neutral-density shade cloth (i.e., reduced PAR by ~ 50% but did not alter the red:far red ratio) and received PAR of 180 μmol m⁻² s⁻¹. 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 infrared gas analyzer.²

Vegetative Measurements. After leaf initiation and assignment of a given CO₂, plants were further divided into five groups, four groups with leaves destructively harvested at periodic intervals and an additional group that was undisturbed for 250 to 260 d. For those plants destructively harvested, all leaves were removed at approximately weekly intervals (i.e., one group per week, four to five plants in a group) from ~ 60 to 90 d following exposure at a given CO₂ treatment. All leaves were removed from a given plant only once, and then leaf regrowth was followed for 30 to 45 d (i.e., until new leaves had fully expanded). Leaf area was determined photometrically for the first harvest; thereafter, the allometric relationship between leaf area and leaf weight ($r^2 > 0.95$) was used to estimate leaf area from leaf dry weight. Leaf area and dry weight were determined separately for regrown leaves. For plants grown at a given CO₂ for 250 to 260 d, nondestructive measurements of rhizome number and length (and total rhizome length per plant) were determined at periodic intervals over the period. Rhizomes grew at the soil surface and could be easily measured with a meter stick. After 250 to 260 d of exposure, that group was destructively sampled to determine leaf area, leaf biomass, and rhizome number, length, and biomass. All vegetative biomass for all destructive sampling was determined following drying at 65 C for a minimum of 48 h or until dry mass was constant.

Urushiol Analyses. Averages of three leaves per plant (most recent, fully expanded) were analyzed for urushiol content

from each group and CO₂. Leaf samples were transferred to the laboratory on dry ice and were stored at -80 C before analysis. Frozen leaves were ground to a fine powder with mortar and pestle, and the tissue extracted three times with 2 ml of ethanol (El-Sohly et al. 1982). The combined extracts were centrifuged for 15 min at 8,000 × *g* in a centrifuge,³ and the supernatants were partitioned with CHCl₃ and H₂O (1:1). The organic fraction was evaporated to dryness under N₂ at 37 C, and the samples were resuspended in 1 ml of 95% ethanol. A 150-μl aliquot of each sample was dried overnight in a vacuum desiccator and was derivatized for 30 min at 37 C with 0.1 ml of *N*-methyl-*N*-(trimethylsilyl) fluoracetamide. Derivatized urushiols were separated by gas chromatography,⁴ using methods similar to those previously used for *Toxicodendron* (Baer et al. 1980; El-Sohy et al. 1982), and urushiol was detected with a mass selective detector coupled to Chemstation software.⁵ Separations were performed with a 30 m by 0.25 mm SPB-50 column⁶ by using high-purity helium as a carrier gas at 1.2 ml min⁻¹. The oven temperature was increased from 150 to 275 C at 5 C min⁻¹. Up to five individual congeners were detected in poison ivy leaf extracts, and trimethyl derivatives were identified by the presence of a base peak at mass-to-charge ratio (*m/z*) 179. Quantification was based on standard curves prepared with known concentrations of urushiols from a partially purified poison oak (*T. diversilobum*) sample, and standard recoveries by using 3-pentadecylphenol were > 90%.

Design and Data Analysis. Given the limited number of chambers and because pots do not represent valid replications, a randomized complete-block design was used with runs over time as replications (blocks). Each of two chambers was assigned one of the four CO₂ treatments with all CO₂ treatments occurring in each chamber over time (i.e., all four CO₂ occurred in each chamber). At the end of a given run, CO₂ treatments were reassigned to another chamber, and the entire experiment was repeated. PAR, humidity, and temperature were quantified before and at the end of each run to determine within-chamber and between-chamber variability. Temperature, PAR, humidity, and CO₂ were also recorded every 15 min throughout a given run, and daily averages were determined for each chamber. Temperature, PAR, and humidity did not differ between chambers during the experiment. Both individual plants (pots) as well as cohorts were rotated every 2 wk inside the chambers until the final destructive harvest. The experiment was run twice, with each run considered a replicate. All plant parameters and urushiol content data were analyzed using an ANOVA with CO₂ and cohort as the classification variables.⁷ Treatment comparisons were made using a Fisher's Protected LSD. Unless otherwise stated, differences for any measured parameter were considered significant at the $P \leq 0.05$ level.

Results and Discussion

Increasing CO₂ above levels present in the mid-20th century resulted in significant increases in leaf area, leaf and stem weight, and rhizome length (Table 1). The increase in average aboveground plant weight for poison ivy reported in the Duke study, ~ 67% from a CO₂ of 370 to 570 μmol mol⁻¹ (Mohan et al. 2006), is consistent with the observed

Table 1. Averages and P values of the ANOVA for CO₂ concentration for vegetative and qualitative characteristics of poison ivy after 250 d.^{a,b}

Variable	Averages (μmol mol ⁻¹)				P value ^c CO ₂ effect
	300	400	500	600	
Leaf area (cm ²)	2,169 c	4,779 b	5,663 ab	6,568 a	***
Leaf wt (g)	13.2 d	31.8 c	37.8 b	43.3 a	**
Stem wt (g)	27.8 d	52.1 c	63.8 b	87.1 a	**
Rhizome length (cm)	343 d	547 c	664 b	851 a	**
Rhizome (No.)	1.11 c	1.75 b	2.19 ab	2.25 a	**
Urushiol (mg g ⁻¹ FW)	0.23	0.26	0.28	0.36	0.18 (NS)
Urushiol (mg)	15.0 d	40.9 c	53.3 b	78.1 a	**

^a Unless otherwise specified, data are per plant, e.g., urushiol (mg) is the average production of urushiol in milligrams per plant.

^b Abbreviations: No., number; FW, fresh weight; NS, not significant.

^c Different letters indicate significant differences as a function of [CO₂] treatments using a Fisher's Protected LSD.

* P < 0.05; **P < 0.01; ***P < 0.001.

response reported here from 400 to 600 μmol mol⁻¹ (~ 55%).

The stimulation of leaf or stem biomass was observed even with small (100 μmol mol⁻¹) increases in CO₂ (Table 1). Overall, although vegetative response to CO₂ remained significant for 100 μmol mol⁻¹ increases up to 600 μmol mol⁻¹ CO₂, the relative response diminished as CO₂ increased above the 400 μmol mol⁻¹ baseline. Similarly, although a significant increase in total rhizome length was observed as a function of treatment CO₂, the largest relative increase in rhizome length over time occurred between 300 and 400 μmol mol⁻¹ CO₂ (Figure 1). Data on the response of plant biomass to recent changes in atmospheric CO₂ are limited but suggest an average response of ~ 30% (Sage 1995; Ziska 2003b). The response observed here for poison ivy (300 to 400 μmol mol⁻¹ CO₂) is about three times that average (~ 105%), suggesting that the most rapid potential change in poison ivy growth in relation to CO₂ may have occurred in the later half of the 20th century.

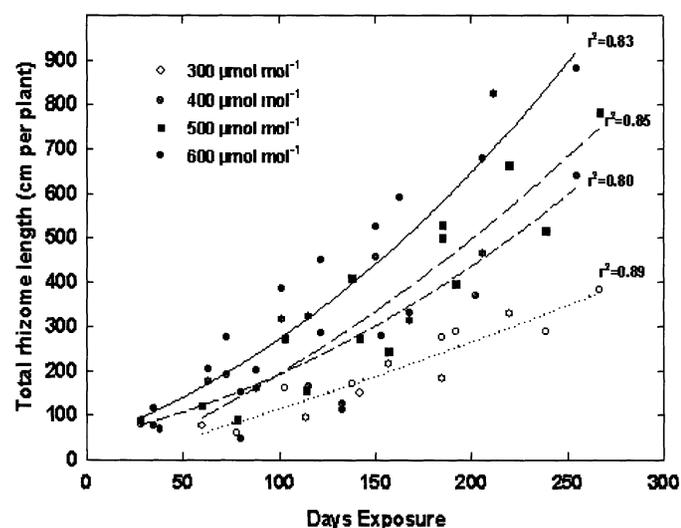


Figure 1. Nondestructive measurements of total rhizome length per plant (determined as the product of rhizome number and length) for poison ivy grown at four different concentrations of atmospheric carbon dioxide (CO₂). Carbon dioxide values corresponded roughly to the atmospheric concentration that existed during the middle of the 20th century, the current concentration, and the near and long-term projections for the current century (2050 and 2090), respectively. Lines are "best-fit." Equations for lines (from lowest to highest [CO₂]) are: $y = -16.001 + 1.204(x) + 0.000996(x^2)$; $y = 49.180 + 0.912(x) + 0.00512(x^2)$; $y = -24.167 + 1.736(x) + 0.00436(x^2)$; $y = 47.392 + 1.484(x) + 0.00758(x^2)$. Significant differences in slope were observed between the 300 μmol mol⁻¹ and all other [CO₂] treatments.

Although poison ivy can show a strong response to recent and projected changes in CO₂ how might that response be mediated by herbivory? Herbivory is a recognized check on the size and survivorship of plants, and leaves of poison ivy are browsed frequently by white-tailed deer, muskrat, and other herbivores (Pederson and Wallis 2004). To simulate herbivory, all poison ivy leaves were removed from different cohorts at weekly intervals between 60 and 90 d after exposure to a given CO₂. The rate of leaf redevelopment (i.e., leaf weight and area) was independent of plant age at the time of leaf removal (i.e., between 60 to 90 d, data not shown); however, the rate of development was strongly influenced by the treatment CO₂ (Figure 2). For example, the time to mature

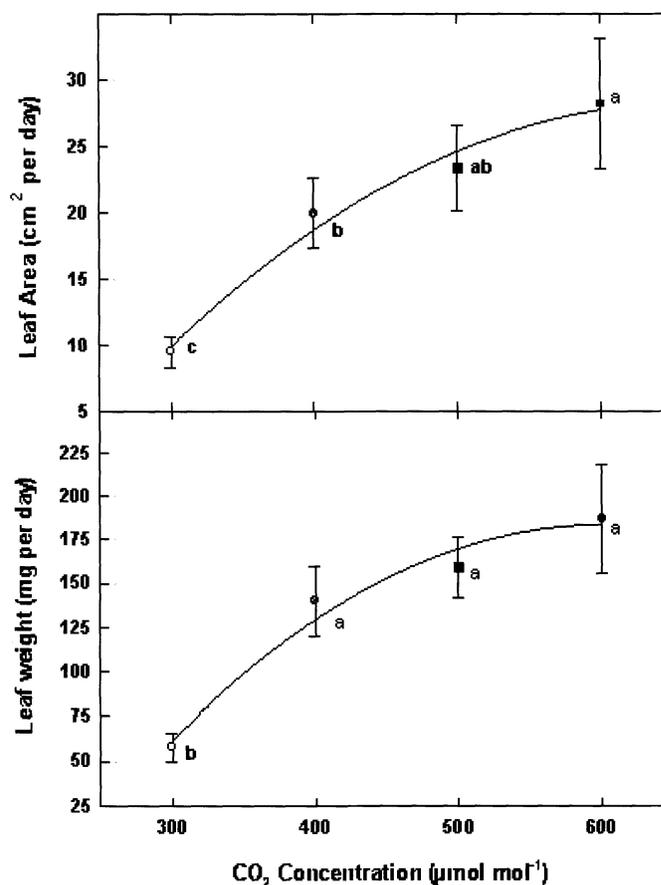


Figure 2. Changes in leaf area and dry weight as a function of time during regrowth as a function of [CO₂] following leaf removal. Different letters indicate significant differences as a function of [CO₂] treatments using a Fisher's Protected LSD. Symbols are the same as in Figure 1. Additional details are provided in the Materials and Methods section of the text.

trifoliate leaf development was approximately three times faster at 600 compared with 300 $\mu\text{mol mol}^{-1}$ CO_2 . The rapidity of leaf development likely reflects carbohydrate status of the whole plant, which generally increases in response to elevated CO_2 (e.g., Stitt 1991).

Will potential changes in growth of poison ivy with increasing CO_2 alter the concentration or production of urushiol, the oil associated with contact dermatitis? Urushiol is a mixture of phenolic catechols that can readily penetrate the skin and induce a rapid allergic response. It is the immune system, specifically the destruction of skin cells by white blood cells that, in turn, can lead to contact dermatitis. At present, it is estimated that 80 to 90% of the U.S. population can become sensitive to poison ivy with repeated exposures to urushiol (Krenzelok and Provost 1995). In the current study, no change in the ratio of unsaturated to saturated forms of urushiol (i.e., the more toxic form of urushiol) was observed (data not shown). There was a nonsignificant ($P = 0.18$) increase in urushiol concentration in response to increasing CO_2 , in part because of considerable leaf-to-leaf variation. However, given the stimulatory effect of CO_2 on leaf and plant growth, the total amount of urushiol produced per plant increased significantly as a function of CO_2 (Table 1).

How do the responses observed here apply to the potential spread of poison ivy and urushiol in a future, higher CO_2 world? Clearly, the growth response of poison ivy to CO_2 will be dependent on other abiotic resources, particularly light, nutrients, and water availability. Light, for example, is recognized as a limitation for the development of understory vines, and recent reports suggest that elevated CO_2 could benefit the response of deep-shaded tropical vines (Granados and Korner 2002) as well as provide additional carbon during the spring when temperate vines may be carbon limited (Zotz et al. 2006). If vine growth is, in effect, limited by nutrients or water, then production of secondary compounds, such as urushiol, may represent an alternative carbon sink, and higher concentrations of such compounds could result as CO_2 rises (see Bryant et al. 1983). Conversely, the growth response to CO_2 under optimal conditions may not include an increase in concentration but, instead, greater productivity of urushiol per plant (i.e., larger plants), consistent with the current study. However, both scenarios would be associated with a greater potential for contact dermatitis either through increased concentration or by greater likelihood of human contact.

Overall, the growth response of poison ivy to CO_2 reported here is consistent with that shown previously in situ (Mohan et al. 2006). Moreover, these data confirm the sensitivity of poison ivy growth to recent and small changes in atmospheric CO_2 and suggest that future increases in CO_2 are likely to stimulate the growth of poison ivy and production of urushiol, even if herbivory increases. Recent in situ observations have, in fact, indicated that vines are likely to benefit, relative to trees, as atmospheric CO_2 increases (e.g., Belote et al. 2004; Phillips et al. 2002). It has been thought that as CO_2 increases, vines can allocate more photosynthate to additional leaf tissue because of low allocation to support structures (Sasek and Strain 1990; Tsugawa et al. 1980); greater light capture, in turn, would prove over time to confer greater competitive advantage. The current data for poison ivy are consistent with these observations but suggest further that the rate of rhizome elongation, which is proportional to the rate of asexual spread, may also increase significantly in response to CO_2 .

In summary, the potential consequences of a warmer planet with respect to pathogens, air and water quality, and respiratory disease are well recognized by the health-care community (e.g., Patz and Kovatz 2002). Less recognized (or evaluated) are the direct or indirect consequences of increasing CO_2 on plant biology and public health. Although the response of poison ivy to global change cannot be elucidated by examining a single parameter, understanding and quantifying the impact of recent and projected changes in CO_2 as a dynamic, unrecognized aspect of poison ivy growth and urushiol production deserves particular attention. Given the importance of contact dermatitis as a public health issue, it is hoped that the efforts described here and previously (Mohan et al. 2006) will encourage further collaborative work between medical and plant scientists in the interaction between plant-induced contact dermatitis and human-induced climatic change, for example, the mapping of poison ivy within urban locations, (i.e., higher CO_2 environments; see Ziska et al. 2003a) and the sharing of such maps with health-care providers.

Sources of Materials

- ¹ Growth chambers, EGC Corporation, Chagrin Falls, OH.
- ² Infrared gas analyzer, model WMA-2, PP Systems, Haverhill, MA.
- ³ Centrifuge, Avanti J-20 XP, Beckman-Coulter, Fullerton, CA.
- ⁴ Gas chromatograph, model 6890A, Hewlett Packard, Palo Alto, CA.
- ⁵ Chemstation software, Hewlett Packard, Palo Alto, CA.
- ⁶ SPB-50 column, Supelco, Bellefonte PA.
- ⁷ Statview, SAS, Cary, NC.

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