Ozone Injury On Cutleaf Coneflower (*Rudbeckia laciniata*) And Crown-Beard (*Verbesina occidentalis*) In Great Smoky Mountains National Park

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Ozone injury on cutleaf coneflower (*Rudbeckia laciniata*) and crown-beard (*Verbesina occidentalis*) in Great Smoky Mountains National Park

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"Capsule": *Ground-level ozone causes deleterious effects to cutleaf coneflower and crown-beard in Great Smoky Mountains National Park.*

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Keywords: Ozone; Bioindicator; Visible foliar injury; Air pollution; Plant ecosystems

1. Introduction

The United States Clean Air Act, enacted in 1970 and amended in 1977, designates Class I areas as national parks and wilderness areas greater than 2400 and 2000 ha in size, respectively. Federal land managers are required to monitor air quality in these areas and assess potential impacts of air pollutants on resources within these locales (US DOI, 1982). The potential for adverse effects of air pollution in Class I areas including Great Smoky Mountains National Park (GRSM) are, therefore, of major concern to regulatory agencies, e.g., the US Environmental Protection Agency (US EPA), the National Park Service (NPS), and the general public (Shaver et al., 1994).

During the past 20 years, ozone concentrations in the USA decreased approximately 21% and 12% based on the past 1-h and the new 8-h standards (US EPA, 2001). However, not all regions of the country have experienced improvement in air quality. In the southern and north-central regions ambient ozone levels increased over the past 10 years (US EPA, 2001). Thirteen sites in 11 National Parks, including GRSM exhibited significant increases. Seasonal ozone exposures (SUM60) nearly doubled in GRSM between 1990 and 1999 (Renfro, personal communication).

Responses of terrestrial vegetation to ozone include: foliar injury, decreases in growth and productivity, changes in crop quality, and increased sensitivity to abiotic and biotic stresses (US EPA, 1996; Chappelka

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and Samuelson, 1998). In addition, ozone has been reported to affect the growth, productivity and reproductive development of several wild plant species in the USA (Duchelle and Skelly, 1981; Neufeld et al., 1992; Bergweiler and Manning, 1999) and Europe (Bergmann et al., 1995, 1996) within open-top chambers. However, research under ambient ozone conditions in the field on native, herbaceous vegetation is limited (Chappelka et al., 1997; Davison and Barnes, 1998).

Indicator plants can be used to determine the relative air quality of a specific location or region (Manning and Krupa, 1992; Manning, 1993). Indicator plants are especially useful in locations without electrical power, and have been used to evaluate ambient ozone effects to vegetation in the USA (Neufeld et al., 1992; Heagle et al., 1995; Chappelka et al., 1997) and Europe (Bytnerowicz et al., 1993; Davison and Barnes, 1998).

Long-term investigations of visible ozone injury on selected, sensitive plants within GRSM have been continuing for over a decade (Neufeld et al., 1992; Chappelka et al., 1997; 1999). Neufeld et al. (1992) identified over 30 plant species as being ozone sensitive using open-top chambers in GRSM. Visible symptoms were observed on black cherry (*Prunus serotina*), sassafras (*Sassafras albidum*), tall milkweed (*Asclepias exaltata*), and yellow-poplar (*Liriodendron tuilpifera*) during field surveys conducted in the Park from 1991 to 1993 (Chappelka et al. 1997, 1999).

As part of a long-term ecological investigation of the structure and function of plant ecosystems in the Park as affected by ambient ozone, surveys were conducted during the summers of 2000 and 2001 to determine the magnitude of visible foliar symptoms on cutleaf coneflower (*Rudbeckia laciniata*), and crown-beard (*Verbesina occidentalis*). Both species are ubiquitous in the Park, and are sensitive to ozone in open-top chamber studies (Neufeld et al., 1992). Little quantitative information is available regarding the sensitivity of these species to ambient ozone concentrations in the field. In addition, data from this study will provide information regarding variation among populations of these plants, whether due to habitat heterogeneity and/or genetic variability.

2. Methods

2.1. Measurements

All plots are located in GRSM, which is in the southern Appalachian Mountains of western North Carolina and eastern Tennessee. Cutleaf coneflower and crown-beard were examined for visible symptoms of ozone injury during July 23–25, 2000. Cutleaf coneflower was evaluated on Clingmans Dome trail, Clingmans Dome, TN (Lat: 35°33′46″N Long: 83°29′53″W, elevation 2015 m), and crown-beard was sampled, approximately 5 km from the Twin Creeks Natural Resource Center, GRSM (Gatlinburg, TN), adjacent to the Cherokee Orchard Road Loop (Lat: 35°41′17″N Long: 83°30′04″W, elevation 572 m). Two-hundred cutleaf coneflower and 100 crown-beard were randomly examined. Plants were categorized by distance from the trail: near-trail = plants sampled $\leqslant 5$ m from the trail, and off-trail = plants > 5 m from the trail. Plants were sampled if possible, at distances > 1 m from each other. Both the cutleaf coneflower and crown-beard grew in large, dense rhizomatous stands with few accompanying species. At each location, numbers of plants, injured plants, total number of leaves per plant and ozone-injured leaves were recorded. From these data the average percentage of injured plants and leaves were calculated. In addition, injury was recorded by leaf class: L_1 = lowest leaf, L_2 next leaf, L_n = uppermost leaf on plant.

During 2001, plants at both locations were examined for ozone injury at approximately 3–5 week intervals from June to September (four sampling periods). In addition, coneflowers growing at the GRSM Purchase Knob site located near Waynesville, NC (Lat: 35°35′14″N Long: 83°04′31″W, elevation 1494 m) were sampled during the last week in July. At each location 50 plants were observed. Plants were sampled both near- and off-trail (25 plants each) using criteria developed in 2000. Numbers of uninjured and injured plants and leaves were recorded at each location, and average percentage injured plants and leaves were calculated.

2.2. Statistical analyses

Severity of ozone injury was assessed in two ways: (1) percentage of foliage injured (number of injured leaves/total number of leaves $\times$ 100) and (2) percentage of leaf area injured for leaves with foliar ozone symptoms. A modified Horsfall–Barratt rating scale (Horsfall and Barratt, 1945) was used to quantify relative severity (leaf area affected) of symptoms on injured leaves (classes = 0%, 1–6%, 7–25%, 26–50%, 51–75% and 76–100%). The midpoint of each class was used to calculate average leaf area injured per injured leaf.

Data were analyzed using chi square (Steel and Torrie, 1960) to ascertain differences in visible ozone injury between plants growing near- and off-trail for cutleaf coneflower and crown-beard. In order to determine if leaf location was important in symptom expression the Wilcoxon's signed-rank test was used (Steel and Torrie, 1960).

3. Results

3.1. Ambient ozone characterization

Cumulative ozone exposures (SUM60) from May to September 2000 and 2001 for the study sites are shown in Fig. 1. Although ozone data was not collected along Cherokee Orchard Road Loop, data from Cades Cove (elevation 564 m) are representative of this area (Jim Renfro, personal communication). Ozone exposures varied both in time and space. Exposures were greatest at Clingmans Dome and least at Cades Cove both years. Ozone levels expressed as SUM60 were greater in 2000 than 2001 at all locations (Fig. 1). There were 58 exceedances of the current US EPA 8-h standard across all monitoring sites in the Park during 2000, and only 25 in 2001, 20 of which occurred in May and June (data not shown).

Rainfall amounts differed by location and year; 78 and 93 cm for Clingmans Dome and 63 and 64 cm for Cades Cove (May–September) in 2000 and 2001, respectively. No rainfall data were available for Purchase Knob. Seasonal patterns differed also by location. During both years of the study the greatest amount of precipitation was recorded in June at Cades Cove; 21 and 15 cm in 2000 and 2001, and May, July and September at Clingmans Dome (approximately 18 cm) during 2000. Over 20 cm of precipitation was measured during June and July in 2001.

3.2. Cutleaf coneflower—2000

The average percentage of injured plants for the overall population was approximately 50%. Visible injury was significantly greater ($P < 0.001$) for plants growing near the trail than those off-trail (Table 1). Approximately 18% of the total number of leaves examined (2088 leaves) had ozone symptoms, and the percentage of injured leaves were significantly different ($P < 0.001$) when those located near the trail were compared with plants growing off-trail. Regarding the average percentage leaf area injured, approximately 10% of the leaf area exhibited visible symptoms (Table 1). The amount of leaf area injured/injured leaf was 3.5 times greater for plants sampled near the trail compared with those off-trail (Table 1).

The lower leaves on cutleaf coneflower exhibited the greatest amount of injury as illustrated in Fig. 2A. Over 95% of the injured leaves occurred on the lower 50% of the plant ($P < 0.0001$). The pattern of injury occurrence was similar between plants growing near- and off-trail (data not shown).

3.3. Cutleaf coneflower—2001

The total percentage of injured plants increased over time (Fig. 3A), and was greater for plants sampled near the trail than off-trail, except the last sampling period (Table 2), when all plants were injured. Similarly, 99% and 79% of the leaves sampled (near- and off-trail, respectively) during the last sampling period exhibited foliar symptoms (Table 2, Fig. 3B). The average leaf area injured/injured leaves, was 83% and 41% (Table 2) for plants near- and off-trail at the final sampling period (Fig. 3C).

Visible injury at Clingmans Dome and Purchase Knob on July 26 and 28, respectively, was compared between the two distinct populations of cutleaf coneflower. All indices tested were significantly greater ($P \leqslant 0.05$) for plants near the trail compared with off-trail for the Clingmans Dome site (Table 3). There were no differences between plants sampled near- and off-trail at the Purchase Knob site. Injury was greater for the Clingmans Dome population compared with Purchase Knob ($P < 0.003$).

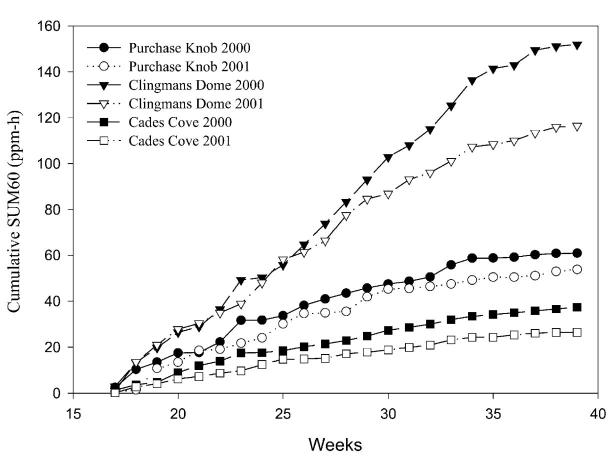

Fig. 1. Cumulative ozone exposures for three sites in Great Smoky Mountains National Park during the 2000–2001 growing seasons (May – September). Elevations = 2021, 1494 and 564 m for Clingmans Dome, Purchase Knob, and Cades Cove, GRSM, respectively. Data provided courtesy of D. Joseph, NPS-Air Resources Division.

Table 1
Visible foliar ozone injury on cutleaf coneflower at Clingmans Dome and crown-beard adjacent to the Cherokee Orchard Road Loop, by proximity to the trail, July 23–25, 2000

% Injury	Near-trail	n[a]	Off-trail	n[a]	Prob.[b]
Cutleaf coneflower					
Plants	75.0	64	36.8	136	0.0001
Leaves	28.7	819	11.3	1269	0.0001
Area injured[c]	14.5	235	4.1	143	0.0001
Crown-beard					
Plants	60.0	70	40.0	30	0.0679
Leaves	18.9	1012	9.9	444	0.0372
Area injured[c]	16.6	191	7.5	44	0.1608

[a] n = total number of plants sampled, total number of leaves sampled, and total number of injured leaves, respectively; populations approximately 100 m apart.

[b] Probabilities between populations and proximity to the trail for all indices calculated using chi square.

[c] Area injured = average % leaf area/injured leaf.

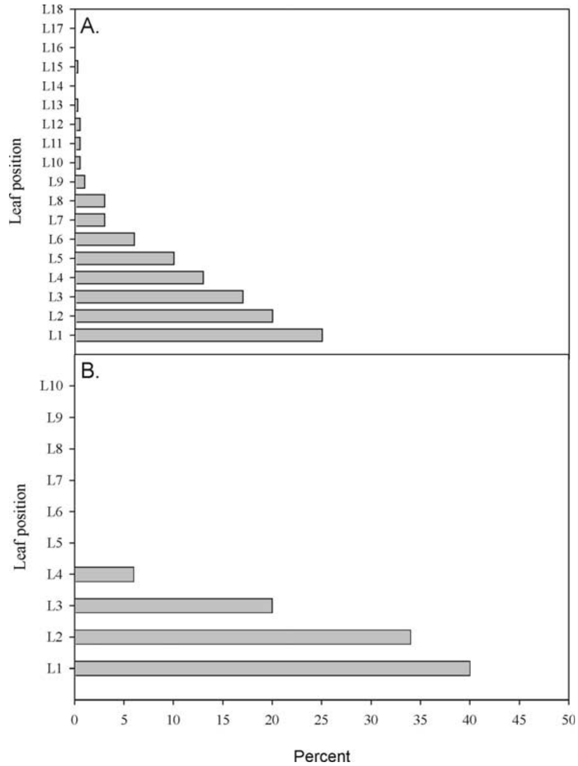

Fig. 2. Ozone injury distribution (% injured leaves) by leaf number recorded July 23–25, 2000; L1 = lowest leaf and Ln = upper leaf: (A) cutleaf coneflower at Clingmans Dome, $n = 378$, and (B) crown-beard growing at Cherokee Orchard Road Loop, $n = 235$.

3.4. Crown-beard—2000

Approximately 50% of all plants sampled were injured. However, injury was 1.5 times greater for plants growing near the trail than those off-trail (Table 1). Similarly, 16% of the overall population (1456 total leaves examined) had visible symptoms, and the percentage of injured leaves was greater near the trail (Table 1). Fifteen percent of the leaf area showed visible symptoms (Table 1). The difference in amount of leaf area injured was non-significant for plants growing near the trail compared with those off-trail. Percent injury was greater for the lower leaves (Fig. 2B). In fact, injury was only found on the first four leaf-sets. Incidence of injury was greater for plants located on the trail, but the pattern of injury was similar near- and off-trail (data not shown).

3.5. Crown-beard—2001

The percentage of injured plants increased over time (Fig. 4A) from 0 to 56% between June 13 and July 24 with no differences observed between plants near- and off-trail during any sampling period (Table 2). Approximately 17% of the leaves sampled were injured (Fig. 4B). The average percentage of leaf area injured/injured leaves generally increased over time (Table 2) and differed between plants near- and off-trail during the last two sampling periods (Fig. 4C). Although the percentage of leaf area injured was greater for plants growing off-trail for the July 5 sampling period, these results were not different due to the low number of injured leaves; 18 and six, respectively, for near- and off-trail.

4. Discussion

During the July data collection in 2000, approximately 50% of the cutleaf coneflower and crown-beard sampled exhibited visible foliar symptoms of ozone injury. These results are similar to those observed for other plant species previously sampled at various locations throughout GRSM (Chappelka et al., 1997). Incidence of injury (% plants and leaves injured) was greatest for plants growing near the trail for both cutleaf coneflower and crown-beard. Regarding the

Table 2
Probability values (chi square) for foliar ozone symptoms near-trail vs. off-trail for cutleaf coneflower in 2001 at Clingmans Dome, and crown-beard adjacent to the Cherokee Orchard Road Loop

% Injury	Probability values			
	June 13[a]	July 5	July 26	September 2[b]
Cutleaf coneflower				
Plants	< 0.0001	< 0.0001	< 0.0001	–
Leaves	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Area injured[c]	–	0.0439	0.0545	< 0.0001
Crown-beard				
Plants	–	0.071	0.949	0.264
Leaves	–	0.064	0.076	0.115
Area injured[c]	–	0.484	0.006	0.001

[a] No values reported for plant injury of cutleaf coneflower due to 0% injury for plants off-trail. No values reported for June 13 for crown-beard due to 0% injury for all plants observed.
[b] No values reported for plant injury of cutleaf coneflower since 100% of the plants were injured.
[c] Area injured = average % leaf area/injured leaf.

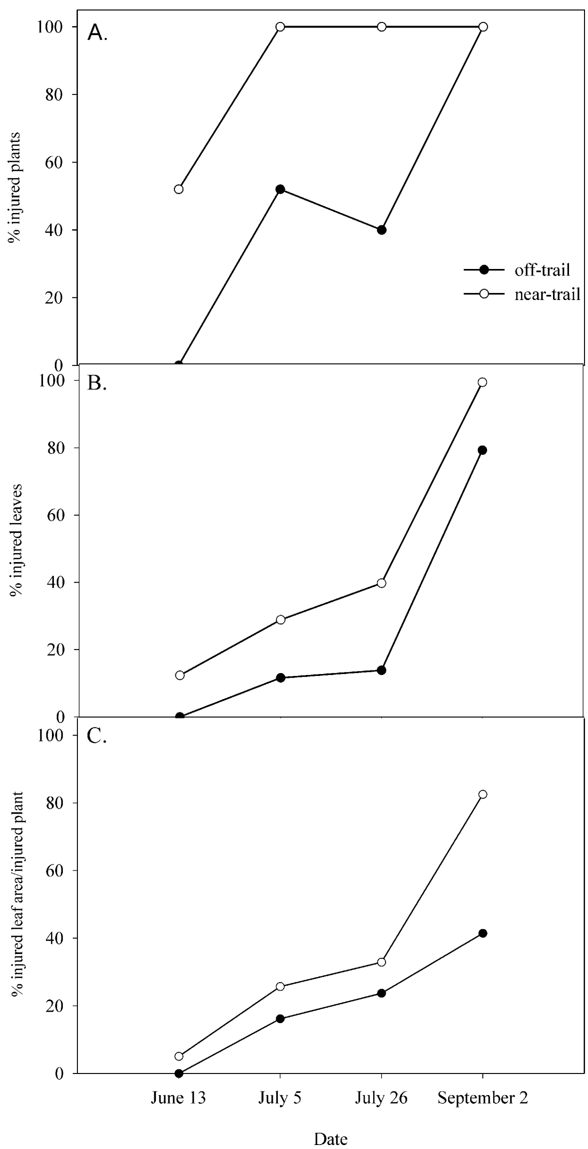

Fig. 3. Seasonal progression of visible ozone injury of cutleaf coneflower growing on Clingmans Dome, GRSM during 2001: (A) percent injured plants, (B) percent injured leaves, and (C) percent injured leaf area/injured leaf.

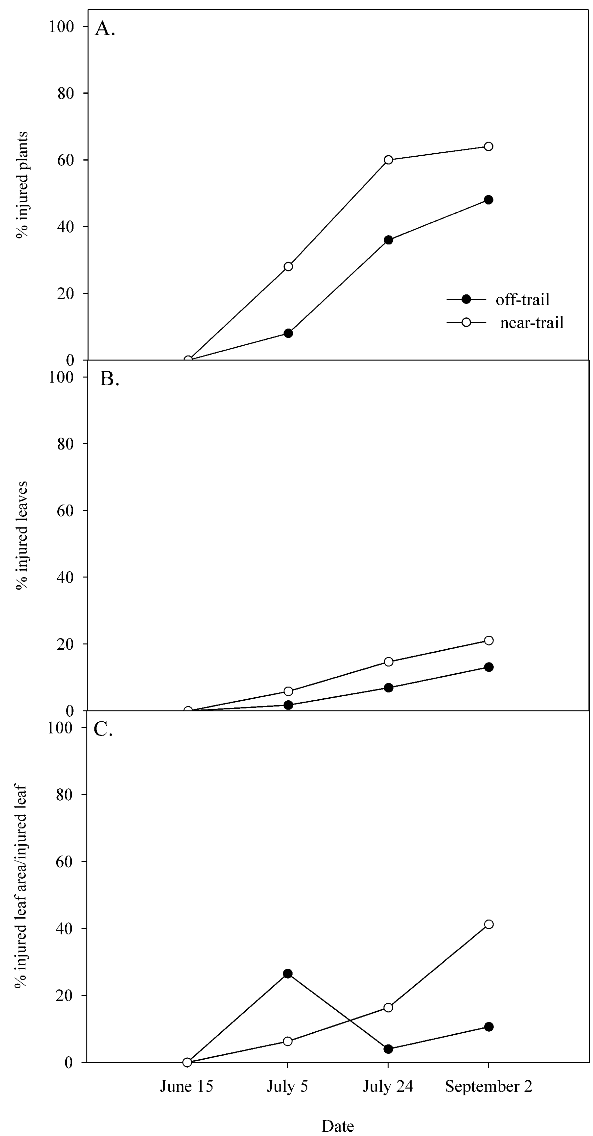

Fig. 4. Seasonal progression of visible ozone injury of crown-beard growing at Cherokee Orchard Road Loop, GRSM during 2001: (A) percent injured plants, (B) percent injured leaves, and (C) percent injured leaf area/injured leaf.

Table 3
Visible foliar ozone injury on cutleaf coneflower at Clingmans Dome and Purchase Knob, GRSM by proximity to the trail, July 26–28, 2001

% Injury	Clingmans Dome					Purchase Knob				
	Near	n[a]	Off	n[a]	Prob.[b]	Near	n[a]	Off	n[a]	Prob.[b]
Plants	100.0	25.0	40.0	25	0.0001	52.0	25	28.0	25	0.0864
Leaves	39.7	219	13.8	239	0.0001	12.4	242	7.9	242	0.1638
Area injured[c]	32.9	87	23.7	33	0.0522	15.3	30	15.8	19	0.6578

[a] n = total number of plants sampled, total number of leaves sampled, and total number of injured leaves, respectively; populations approximately 100 m apart.

[b] Probabilities between on and off-trail for all indices tested using chi square.

[c] Area injured = average % leaf area/injured leaf.

percentage of leaf area injured, cutleaf coneflower, but not crown-beard, exhibited differences between plants growing near- and off-trail. Differences in the amount of injury recorded were observed between two dissimilar populations of cutleaf coneflower. The differences observed are probably related to environmental factors, although genetic differences cannot be ruled out (Davison et al., this volume). As expected (typical of ozone injury), symptoms were greatest on the lower leaves (Chappelka and Chevone, 1992). Duration of ozone exposure is greater for the lower leaves, but there may also be changes in sensitivity with age.

Incidence of ozone injury increased for both species over time during 2001. The percentage of injured plants and leaves was greater for cutleaf coneflower than crown-beard throughout the growing season. Neufeld et al. (1992) reported that both species were extremely sensitive to ozone in open-top chamber studies at GRSM. The observed differences in sensitivity among these species could result from a couple of factors. Ozone exposures were greater at the higher elevations where cutleaf coneflower grew (refer to Fig. 1). Elevational gradients of ozone exposures and foliar injuries are reported for several other plant species (Winner et al., 1989; Chappelka et al., 1999), with the greatest exposures and injuries reported at the higher elevations.

Another explanation could be genetic differences among the species regarding sensitivity. Chappelka et al. (1997) reported in a 1-year study in GRSM that 75% of the tall milkweed (*Asclepias exaltata*) sampled in a Park-wide survey (approximately 1500 plants) were injured. However, no discernible relationships among ozone symptoms, slope, aspect, or elevation could be found. Common garden experiments with both species located at several elevations throughout GRSM are being conducted to test the validity of this hypothesis.

Ozone injury was greatest at the edge of the stand (near the trail) for cutleaf coneflower, but not crown-beard. Injury was not different for crown-beard growing near the trail compared with off-trail. The severity of injury, however, was greater for crown-beard growing at the edge of the stand (near the trail). The reasons for these differences (or lack thereof) are unknown, but may be related to genetic differentiation between individuals, or environmental factors within stands (Davison et al., this volume). They determined that the genetic makeup of the population sampled at Clingmans Dome was fairly homogeneous.

The data of Davison et al. (this volume) suggest that environmental factors, primarily light levels, may be more important than genetic variability within a species for the differences observed between plants growing at the edge of stands vs. those growing within the interior. There were no differences in injury at the Purchase Knob between plants growing near the trail with those off-trail. The population at the Purchase Knob is not as dense as the one at Clingmans Dome, indicating that light levels and ozone concentrations are different within the stand and possibly the plant canopy (Davison et al., this volume). However, more genetic variability was observed for cutleaf coneflower growing at Purchase Knob.

The greatest number of injured leaves occurred on the lower portions of both cutleaf coneflower and crown-beard. This is a common phenomenon, and is reported for many different plant species (Chappelka and Chevone, 1992; Neufeld et al., 1992). It is interesting to note that leaves on cutleaf coneflower tended to turn chlorotic and persist on the plant, whereas those on crown-beard tended to senesce and drop off. The reasons for these differences are unknown.

5. Conclusions

Cutleaf coneflower had more foliar symptoms to ambient concentrations of ozone than crown-beard throughout this 2-year study (2000–2001). Incidence and severity of injury was greater for cutleaf coneflower growing at the edge (near-trail) of the Clingmans Dome stand, and this response is probably environmentally (light) controlled. The same relationship was not observed with a more heterogeneous population located at Purchase Knob. A significant relationship regarding differences in sensitivity within the population at Cherokee Orchard Road Loop was not observed for crown-beard. Ozone injury was greatest on the lower leaves for both species. Dissimilarities in injury occurred between two different populations of cutleaf coneflower, and may be due to differences in elevation, microclimate, and/or genetic variability. This is the first report of foliar injury on these plant species exposed to ambient ozone concentrations in the Park.

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