ASSESSMENT OF FOLIAR OZONE INJURY ON BIOINDICATOR PLANT SPECIES AT ROCKY MOUNTAIN NATIONAL PARK

RESULTS FROM 2006 – 2009

Robert Kohut
Professor Emeritus

Boyce Thompson Institute
Cornell University
Ithaca, NY 14853

The Air Resources Division (ARD) of the US National Park Service funded these assessments of ozone injury to vegetation at Rocky Mountain National Park. The assessments were initially conducted as a field trial of the Handbook for the Assessment of Foliar Ozone Injury on Vegetation in the National Parks that was written for use in the Vital Signs Monitoring Program of the National Park Service, and conducted in subsequent years to better understand the occurrence of injury in the park.

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The author may be contacted at:

rjk9@cornell.edu

The Boyce Thompson Institute
Cornell University
Ithaca, NY 14850

INTRODUCTION

Foliar ozone injury was assessed at Rocky Mountain National Park (ROMO) from 2006 through 2009. The assessments were initiated to meet two objectives: assess the utility of the Handbook for Assessment of Foliar Ozone Injury written for the Vital Signs Monitoring Program, and determine whether ozone was injuring vegetation in the park.

The Handbook was written to support the National Park Service's Vital Signs Monitoring Program and provide both broad background and specific information necessary to design and implement a field ozone injury assessment. It provides guidance to managers and biologists who want to determine whether ozone is injuring plants in their park, and assess the extent of ozone injury in a given year and over time. It describes in detail three assessment approaches that require different commitments of resources and produce different levels of information. The Handbook describes the objectives of each approach, how field sites are located and evaluated, how field assessment plots are established, and how the assessment of foliar ozone injury is conducted. Guidance is provided on compiling foliar injury data, implementing a quality assurance program, and conducting fieldwork safely and efficiently.

The Air Resources Division (ARD) of the National Park Service funded the development of the Handbook. Subsequently, the Division wanted to determine how well it satisfied the needs of staff in designing and implementing a field assessment program. A decision was made to test the utility of the Handbook in several parks, including Rocky Mountain. The 2006 assessment effort at ROMO involved using plant distribution information to select candidate field plot sites, evaluate the adequacy of the candidate sites in the field, select sites to be used in the assessment program, conduct the field assessment of injury in August, and compile the results into a short report. At each step of the process, observations were made on how well the information in the Handbook met the needs of the park staff. The observations were subsequently used to identify the sections of the Handbook needing revision and expansion, and to produce a second edition of document. The 2nd edition of the Handbook is available on-line at the web site: http://www.nature.nps.gov/air/permits/aris/networks/docs/O3_InjuryAssessmentHandbookD1688.pdf.

Rocky Mountain National Park was selected as a site for an assessment of foliar ozone injury because of its proximity to ozone-source Front Range communities and concern about upslope movement of ozone into the park where it may produce injury on sensitive species of plants. A previous assessment of the risk of ozone injury to sensitive plants in the park indicated the risk was generally low (Appendix 1). The assessment revealed that while ozone exposures were often high, in many years low levels of soil moisture would constrain the uptake of ozone by plants and reduce or preclude the development of foliar injury. Knowing whether ozone is impacting plants is important to the long-term management of the park's resources, and to making decisions regarding the acceptability of any further changes in air quality in the park. The assessment in 2006 was performed both to evaluate the effectiveness of the Handbook and determine whether foliar ozone injury was present in the park; the assessments in subsequent years were to follow-up on the original findings.

METHODS

INDICES OF OZONE EXPOSURE

The annual ambient levels of ozone exposure at ROMO for 2006 through 2009 were assessed using several indices used in research and considered as air quality standards for ozone. Exposures were expressed as the Sum06 and W126 cumulative indices, and the maximum 3-month, cumulative W126 value (W126-3 mo) that was proposed, but not adopted, as the secondary national ambient air quality standard for ozone in 2007. While not an index, the numbers of hours at or above 60ppb, 80ppb and 100ppb (N-values) provide some insight to the frequency distribution of the hourly exposures.

Sum06 index

The Sum06 index is the 90-day maximum sum of the 0800 through 1959 hourly concentrations of ozone ≥ 60 ppb (0.60 ppm) (Heck and Cowling 1997). The index is calculated over running 90-day periods and the maximum sum can occur over any period of the year, although the chemistry of ozone generation usually results in it occurring over the summer months.

Injury thresholds for the Sum06 index in cumulative ppm-hr are:

Natural Ecosystems	8 - 12 ppm-hr	(foliar injury)
Tree Seedlings	10 - 16 ppm-hr	(1-2% reduction in growth)
Crops	15 - 20 ppm-hr	(10% reduction in 25-35% of crops)

W126 index

The W126 index is the weighted sum of the 24 one-hour ozone concentrations daily from April through October, and the number of hours of exposure to concentrations ≥ 100 ppb (0.10 ppm) during that period (Lefohn et al. 1997). The W126 index uses a sigmoidal weighting function in producing the sum: the lower concentrations are given less weight than are the higher concentrations since the higher exposures play a greater role in producing injury. The significance of the higher concentrations is also reflected in the requirement that there be a specified minimum number of hours of exposure to concentrations ≥ 100 ppb. Thus, the W126 index has two criteria that must be attained to satisfy its thresholds: a minimum sum of weighted concentrations and a minimum number of hours ≥ 100 ppb.

Injury thresholds for the W126 index in cumulative ppm-hr and numbers of hours are:

	W126	N100
Highly Sensitive Species	5.9 ppm-hr	6
Moderately Sensitive Species	23.8 ppm-hr	51
Low Sensitivity	66.6 ppm-hr	135

Another indicator of ozone exposure, designated N-value, consists of the numbers of hours of exposure each year that exceeded 60, 80 and 100 ppb. While there are no formal thresholds associated with these values, they provide insight to the distribution of exposures among these concentrations.

W126-3 mo index

Exposures were also calculated in the form of the secondary standard proposed in the OAPQS staff paper during the review of the ambient air quality standards for ozone in 2007 (US EPA OAQPS 2007). The W126-3 mo index is the maximum 3-month, cumulative 12-hour (0800-1959) W126 index. The threshold proposed for the index was 7-21 ppm-hr.

BIOINDICATOR SPECIES AND SITE SELECTION

Several plant species at ROMO are bioindicators for ozone: cutleaf coneflower (*Rudbeckia laciniata* var. *ampla*), spreading dogbane (*Apocynum androsaemifolium*), quaking aspen (*Populus tremuloides*) and Scouler's willow (*Salix scouleriana*) (US National Park Service 2003). Coneflower and dogbane were selected for emphasis in the assessment because they are herbaceous understory species that can be readily examined in large numbers, and are widely distributed in the eastern portion of the park.

Potential assessment sites for coneflower and dogbane were identified using plant community information available for the park, including the park's recently developed vegetation map and plant species GIS layers, and from information on locations of plants provided by staff based on their personal observations in the field. The potential sites were located on maps and examined for suitability in the field. Sites used in the assessment program were selected based on the number of plants present and accessibility of the site. In the first year of assessment, 2006, cutleaf coneflower was evaluated for foliar injury on 14 permanent sites and spreading dogbane on 6 permanent sites. Due to its widespread nature and secondary emphasis in the program, quaking aspen was assessed for injury on an opportunistic basis, and no permanent assessment sites were established for it. Scouler's willow was not employed in the assessment due to the difficulty in accurately identifying willows and their tendency to hybridize. Additional information on the identification and selection of assessment sites can be found in the NPS report for the initial year of the study (Flanagan and Kohut 2006).

FOLIAR INJURY ASSESSMENTS

Assessments of foliar ozone injury were conducted at two levels: surveying and scouting. The main emphasis was placed in survey assessments of cutleaf coneflower and spreading dogbane, while a scouting assessment was conducted with quaking aspen.

Survey assessments are quantitative and comprehensive in nature. A survey can be employed to provide a one-time assessment of injury, performed only when exposure and environmental conditions warrant, or conducted annually to assess the incidence of foliar injury and its trend over time. A survey uses field sites that are located using both random and non-random means, considers known locations of plant communities containing bioindicator species, and seeks to insure sites are distributed to provide spatial coverage throughout the park. It yields quantitative information on the incidence and severity of ozone injury and its spatial distribution. Assessments are conducted annually on permanent plots, but not necessarily on the same plants.

Procedures used in the survey assessments with coneflower and dogbane to select plants for evaluation and to quantify foliar ozone injury followed those presented in the Handbook for the Assessment of Foliar Ozone Injury and are summarized here. The objective was to examine at least 20 plants on each plot. When the population of plants on the plot significantly exceeded 20, the plants to be examined were identified by random selection using one of the random number tables in the Handbook. If the population of plants was less than 20, all plants on the plot were assessed. Only flowering coneflower plants were candidates for assessment, and observations made only on leaves on the flower stalk. All leaves on the stalk were examined and counted, and when foliar ozone injury was found, the number of injured leaves was determined. The severity of foliar injury was determined on all leaves if the number of leaves on the flower stalk was less than 20, or on a random selection of 20 leaves if there were more than 20 leaves on the stalk. In almost all cases there were less than 20 leaves on the stalk. The data collected allowed both the incidence (percent of plants injured on the plot) and severity (percent of leaf area affected on a plant) of injury to be calculated. On spreading dogbane, a similar process was used to select plants for assessment on each plot, and all leaves on a plant were examined for injury. The total number of leaves and number of injured leaves were determined. The severity of foliar injury on the plant was evaluated as on coneflower, except that all leaves on the plant were subject to assessment.

The severity of injury on individual leaves was estimated using the scale in the Handbook that incorporates features of the scales used by the US Forest Service in its Forest Inventory and analysis Program (US Department of Agriculture 2003) and one devised by Horsfall and Barratt (1945), but is not directly comparable to either of them. The scale is presented in Table 1.

Table 1. Assessment scale for percent of leaf area or leaves affected.

Index	Percent Affected
0	0
1	1-4
2	5-12
3	13-25
4	26-50
5	51-75
6	76-100

A scouting assessment is used to determine whether foliar ozone injury is occurring on plants in a park, and to document its presence over time. It is intended to both produce a yes/no answer with regard to the presence of foliar injury and provide information on its continued occurrence. It requires the smallest investment of time, personnel and funds, but provides limited information on the incidence, severity, and spatial distribution of injury. Annual scouting assessments provide information on the continued presence of ozone injury, and have long-term value with respect to air quality concerns. When a scouting assessment is conducted in conjunction with a survey, bioindicator plants are generally examined as they are opportunistically encountered in the field.

The scouting assessment on quaking aspen employed the procedures in the Handbook to select plants and evaluate injury. No permanent plots were established, and trees were selected and examined on an opportunistic basis. Most of the trees were sapling size so the maximum number of leaves could be readily examined. The observation made for each tree, injury or no injury, provides a measure of the incidence of ozone injury (percent of plants injured) on the evaluation site.

RESULTS

ANNUAL LEVELS OF OZONE EXPOSURE

Ambient concentrations of ozone monitored at ROMO for 2006 through 2008 were analyzed to generate annual exposure values (Table 2).

Table 2. Ozone air quality for Rocky Mountain National Park 2006-2009					
Index [1,2]	2006	2007	2008 [3]	2009	Threshold [4]
Sum 06	26	28	24	13	8-12
W126	29.6	33.2	28.9	19.9	5.9
N60	746	798	716	390	NA
N80	20	32	27	5	NA
N100	3	0	0	0	6 for W126
W126 - 3mo max	19	20	18	11	7-21

1. Sum06, W126 and W126-3mo max values are in ppm-hr
2. N-values are numbers of hours
3. Statistics for 2008 compiled using data through August 2008
4. Thresholds:
    Sum06 - natural ecosystems/foliar injury (Heck and Cowling. Environ Mgt. 1997)
    W126 – highly sensitive species (Lefohn et al. Atmos Environ. 1997)
    W126-3 mo max – (EPA. OAQPS Staff Paper EPA-452/R-07-007)

The Sum06 index exceeded the injury threshold (8-12 ppm-hr) in each of the three years. While the cumulative value for the W126 index significantly exceeded its cumulative value threshold (5.9 ppm-hr) each year, the required number of hours of exposure greater than 100 ppb (6 hr) was not attained and thus the two components of the index were not satisfied. The range for the proposed W126-3 mo index (7-21 ppm-hr) was reached each year.

FOLIAR OZONE INJURY

Over the three years of foliar assessment, ozone injury was observed on cutleaf coneflower each year, while no injury was observed on spreading dogbane or quaking aspen in any year.

Foliar ozone injury was found on cutleaf coneflower in each year of the assessment. Injury was observed on 9 of 14 plots assessed in 2006 and 2008, on 9 of 13 plots in 2007, and on 7 of 13 plots in 2009 (Table 3). Injury was observed on plants on six plots consistently over the four years of assessment, while plants on three plots did not show injury in any of the years. The incidence of injured plants on plots with injury ranged from 5 to 100%. The severity of injury on affected foliage was generally less than 4% and occurred at a level greater than 12% in only two years. The incidence of injury on affected foliage is presented in Table 4.

Table 3. Incidence of foliar ozone injury on cutleaf coneflower at Rocky Mountain National Park 206-2009.

			2006		2007		2008		2009	
Plot	Plot Identifier	Trailhead/Access	Injury?	% Plants	Injury?	% Plants	Injury	% Plants	Injury	% Plants
1	CLCF HQ / HDQR	Headquarters	No	0	Yes	5	Yes	13	No	0
2	RDB A FAN / ALLN	Alluvial Fan (north)	Yes	65	Yes	33	Yes	40	Yes	38
3	RUD A FAN2 / ALLS	Alluvial Fan (south)	No	0	No	0	No	0	No	0
4	HSP CF2 / HORS	Horseshoe Park	Yes	35	Yes	5	Yes	3	No	0
5	MPMCC1 / MORP	Moraine Park Museum	Yes	10	Yes	5	No	0	No	0
6	CF1 BLR / BEAR	Bear Lake Road	Yes	65	Yes	100	Yes	70	Yes	65
7	AGCC1 / ASPE	Aspenglen Campground	Yes	62	Yes	36	Yes	77	Yes	90
8	CCC3 / COWC	Cow Creek	Yes	20	No	0	No	0	Yes	35
9	CLTH4-5CC / BTHO	Cub Lake TH/Big Tmpsn. R.	No	0	No	0	No	0	No	0
10	EDV / ENDO	Endovalley Picnic Area	No	0	No	0	No	0	No	0
11	HPCC1 / HOLL	Hollowell Park	Yes	30	Yes	41	Yes	10	----	----
12	UBM2CC / UPBM	Upper Beaver Meadows	Yes	53	----	----	Yes	10	Yes	40
13	BIERCC1 / BIER	Bierstadt Lake TH	Yes	65	Yes	67	Yes	50	Yes	90
14	CCCLTH / CUBL	Cub lake TH	No	0	Yes	35	Yes	55	Yes	90

Table 4. Level of ozone injury on affected foliage. [1]				
Severity [2]	2006 [3]	2007	2008	2009
1-4	76	89	90	78
5-12	22	11	10	18
13-25	2	0	0	4
26-50	0	0	0	0
51-75	0	0	0	0
76-100	0	0	0	0

1. Expressed as percent of plants injured at that level of severity.
2. Severity is percent of leaf affected on injury scale in Table 1.
3. Percentages for 2006 are approximate due to use of a different injury scale that year.

None of the spreading dogbane examined on the six permanent plots showed any signs of ozone injury. Dogbane was assessed on the plots in 2006 and 2007, but was not assessed in 2008 since no injury had been observed in the previous two years. In 2008, however, plants were examined in a scouting type assessment, and no injury was observed on dogbane growing on both dry and more mesic sites.

Quaking aspen was examined for ozone injury on an opportunistic basis each year and no injury was observed. Of the many trees examined, ozone-like bifacial necrotic lesions were observed on a few leaves, but their limited occurrence and distribution on the trees did not satisfy the diagnostic criteria for ozone.

DIAGNOSING OZONE INJURY

Cutleaf coneflower was selected as a bioindicator for ozone at Rocky Mountain National Park because of its recognized sensitivity to ozone and relatively widespread occurrence in the park (US National Park Service 2003). Field surveys in Smokey Mountains National Park (GRSM) had identified and confirmed its sensitivity to ozone and verified its use as a bioindicator (Chappelka et al. 2003). Symptoms of ozone injury on coneflower in the east were characterized as upper leaf surface bronzing that could be readily recognized in the field.

Diagnosing ozone injury on coneflower at ROMO proved to be challenging since the injury observed there is different from that found in the Smokies. In 2006, the first year of the injury survey, the field assessment was initiated using the upper leaf surface bronzing as the search image for identifying ozone injury. On the second field plot examined, Alluvial Fan north (ALLN), plants were found with dark stipple or fleck on the upper leaf surface. The markings were interveinal, found only on the upper leaf surface, occurred with greater intensity on the older leaves, and were unrelated to any incidence or signs of insects or diseases. While these properties of the markings satisfied the diagnostic criteria for ozone injury, the markings themselves were quite different from the bronzing seen in the Smokies. In addition, some of the markings were more like fleck than stipple in that they were slightly depressed into the leaf epidermis; stipple is generally more flush with the surface of the leaf.

In addition to stipple and fleck, many leaves had small depressions of unknown origin on the surface, some of which had dark pigmented centers while others did not. A mix of markings was common on many leaves and led to the possibility that there was a progression from dimples to pigmented dimples to fleck in depressions. Observations of non-pigmented depressions over several weeks indicated they developed brown centers and are possibly the result of insect feeding or other mechanical injury. These observations also confirmed that the dimples did not develop into stipple or fleck and, consequently, are not associated with ozone injury (Jim Cheatham. US National Park Service, personal communication).

Markings on plants at the Bierstadt Lake trailhead plot (BIER) in 2006 were central to confirming that ozone injury was present and suggesting there was no relationship between dimples and stipple. Plants at this site showed ozone stipple without the presence of any pigmented or nonpigmented dimpled markings. Injury at this site was similar to classic ozone stipple that is widely recognized as a common symptom of ozone injury on many plant species (Flagler 1998; Skelly et al. 1987). Photographs of this injury were circulated to other field researchers (Art Chappelka, Auburn University, Auburn, AL; Don Davis, Penn State University, University Park, PA; Howie Neufeld, Appalachian State University, Boone, NC) and all agreed it was ozone injury. Examination by ROMO staff over several weeks of plants at other sites with a mix of foliar markings revealed that there was no relationship between or progression from dimpled markings to stipple or fleck (Jim Cheatham, U.S. National Park Service, personal communication).

Since the markings attributed to ozone at ROMO are different from those found on coneflower at GRSM, it raises the question of whether they are the result of ozone exposure. The weight of diagnostic evidence gathered in the field at ROMO indicates the injury is caused by ozone. First, the diagnostic criteria used to identify ozone injury are consistently satisfied: the stipple is interveinal, is present only on the upper surface of the leaf, and is most severe on older leaves that have had the longest exposure to ambient ozone. In addition, on one occasion where one leaf overshadowed another, the area of the leaf in the shadow was protected from injury. This protective shadow effect is a diagnostic feature for ozone etiology, but is rarely observed on cutleaf coneflower since the foliar architecture of the plants and their spacing do not afford much of an opportunity for the leaves to overlap. Second, in the summer of 2007, Dr. Howie Neufeld (Appalachian State University, Boone, NC) subjected coneflowers grown from corms obtained at ROMO in controlled exposures with ozone. Although he encountered a number of technical problems that limited the hours of exposure, the plants began to develop stipple similar to that observed at ROMO.   In addition, communication with Susan Sachs (Appalachian Highlands Science Learning Center, GRSM) indicated she has seen similar stipple injury on coneflower grown at the Learning Center. Her observation may be the result of examining the plants throughout the growing season as part of the ozone teaching programs she conducts at the Center and seeing the progressive development of injury, rather than observing the plants only late in the summer after injury has fully developed as normally occurs in an injury assessment program.

Given the mix of markings found on coneflower at some sites and the presence of typical ozone stipple on some plants, a decision was made to adopt a conservative approach to the diagnosis of ozone injury. Only those markings that are black, interveinal stipple on the leaf surface or slightly depressed into the epidermis are designated as ozone injury. These symptoms can be accurately and consistently identified. All markings that are similar in appearance to ozone injury but in deeper depressions, or markings that are colored tan, brown, white or silver are discounted. Ozone injury is visible only on the upper leaf surface with the lower surface remaining smooth, while the upper surface depressions associated with non-ozone markings are generally visible as bumps on the underside of the leaf. The conservative approach adopted for diagnosis permits ozone injury to be consistently identified without the risk of overestimating its incidence or severity.

The variety of markings found on cutleaf coneflower foliage at ROMO dictates that anyone conducting a survey have a clear understanding of the search image used to identify ozone injury, be ready to discount other markings that may be similar in appearance, and be willing to not overanalyze the markings in an effort to convince themselves that markings that should be discounted can be made to qualify as ozone injury.

Over the years of assessment, the appearance of foliar ozone injury on plants at some individual assessment sites has been very consistent. For example, injury on the Bierstadt Lake trailhead site (BIER) is consistently classic ozone stipple, while that on plants at the Bear Lake Road site (BEAR) is a complex of stipple and fleck. The consistency and nature of the foliar injury among the sites makes assessment comparatively easy at some sites and more difficult at others. The reasons for the diversity in appearance of symptoms among the sites are not known.

After careful evaluation of the markings on coneflower foliage and consideration of the difficulties encountered in identifying ozone injury and separating it from other markings, the following guidelines were adopted to allow consistent diagnosis of stipple and fleck ozone injury on coneflower.

- Stipple must be interveinal, black, found only on the upper leaf surface, and be more severe on older foliage.

- Stipple will appear to be on the leaf surface, while more fleck-like injury may be slightly depressed into the leaf epidermis. The lower leaf surface will not display any bumps under the affected areas.

- Both stipple and fleck must be black in color without any associated tan or grey edges or centers.

- All dimples on the upper leaf surface, whether uncolored or with tan, brown, white or black centers or edges will not be classified as ozone injury.

- Any surface or depressed markings that are tan or brown will not be classified as ozone injury.

- If an appropriate spatial relationship exists between adjacent leaves, a protective shadow effect may be evident on an injured leaf overshadowed by another leaf.

- Examine the entire plant with the above criteria in mind before deciding whether ozone injury is present.

- Do not “overanalyze” markings on the foliage. Attempting to split hairs on the nature of the markings, or trying to convince oneself that markings outside the prescribed search image for ozone are ozone-induced leads to confusion and inconsistency in making decisions.

- The best approach to making diagnostic decisions in the field is to employ a well-defined search image, be systematic in the assessment process, and make consistent decisions using the available information.

- When examining a leaf, first impressions are very important and a hand lens should be used to further refine that inclination.

DISCUSSION

Cutleaf coneflower was injured by ozone at ROMO, while spreading dogbane remained unaffected. The two species are found in different habitats in the park and it is believed that the soil moisture properties of the habitats influenced the responses of the plants. Cutleaf coneflower is found in riparian habitats and moist sites where the plants likely have access to adequate soil moisture throughout the growing season. This allows the plants to consistently exchange gases with the atmosphere, and take up ozone without the limitations produced by low levels of soil moisture. In contrast, spreading dogbane is generally found on exposed, open sites where soil moisture is likely a constraint to gas exchange for some or most of the growing season. Thus, the uptake of ozone by dogbane would likely be much less than that by coneflower. However, dogbane plants on more moist sites were examined opportunistically each year and were never found to have foliar ozone injury.

The Sum06 index of exposure significantly exceeded the threshold for exposure each year of assessment. The cumulative value for the W126 index also significantly exceeded the threshold, but there were few or no excursions above 100 ppb and thus the second criteria for the exposure index was not satisfied. The W126-3 mo exposure index proposed as a Secondary Ambient Air Quality Standard contains elements of both the

Sum06 and W126 indices, and was a consistent predictor of ozone injury over the four years of the assessment.

Quaking aspen occurred on a variety of sites and were examined annually. Regardless of the moisture level at the site, no ozone injury was observed on any of the trees. In 2008, bifacial lesions commonly associated with ozone injury were observed at one site with higher soil moisture, however the pattern and distribution of the markings did not satisfy the diagnostic criteria for ozone injury.

The black stipple ozone injury on coneflower at ROMO is significantly different from the upper surface bronzing observed on plants at GRSM. The reason for the difference is not readily apparent, but could be related to the genetic properties of the coneflower varieties, the properties of the ozone exposure regimes, or the nature of the environment in which the exposures take place.

Attention should be directed to conducting simple studies that will help confirm the role of ozone in producing the stipple found on coneflower at ROMO. Experimental approaches that could be employed are reciprocal transplant and controlled exposure studies. Both approaches would use coneflowers from ROMO and GRSM. In the reciprocal transplant study, root sections would be collected in both parks and used to propagate plants that would be grown for two years both in the home and reciprocal park. Plants would be grown outdoors in pots with individual screen enclosures to prevent gene escape by cross-pollination with native plants, and exposed to ambient levels of ozone. The plants would be in the vegetative rosette stage during the first year, and produce flower stalks in the reproductive stage in the second year. In both years, each plant would be examined weekly for the presence of ozone injury with assessments made of its nature, location and severity. All plants would be destroyed at the end of the study. A reciprocal transplant study could provide valuable insight to the timing, development and nature of ozone injury on the two provenances of coneflower. While the development of stipple on ROMO plants at GRSM would provide strong support for the ozone-induced etiology, the lack of stipple would not reject that etiology since other environmental variables unique to ROMO may condition the response of the plants there to ozone.

The best approach to assess the development of foliar ozone injury on ROMO coneflowers is to use them in controlled ozone exposures. Plants from both ROMO and GRSM would be grown in either closed or open-top chambers in which controlled levels of ozone are maintained throughout the growing season. As in the reciprocal transplant study, plants would be exposed for two years and examined weekly to allow the nature and development of foliar injury can be assessed and compared. The advantage of this approach is that the levels of ozone exposure are controlled and can be adjusted to assure the development of foliar injury. The main disadvantage is that the facilities, equipment and manpower required are found only in a few locations and are costly to employ. As with the reciprocal transplant study, unless the controlled exposures are conducted at ROMO or in a similar environment, the lack of stipple on the ROMO coneflower would not be adequate to reject ozone as the causal agent since other site-specific environmental variables at ROMO may condition the response of the plants to ozone. Since no university or research institute in the Rocky Mountains has the facilities and equipment to perform such a study, it is unlikely it could be conducted under environmental conditions representative of the region.

The presence of ozone injury on sensitive plants at ROMO raises the question of whether ozone may be affecting plants elsewhere in the Rocky Mountain region. The recent increase in oil and gas drilling in the Colorado and Wyoming has resulted in elevated levels of ambient ozone in remote areas in these states that previously had low background levels of the pollutant. When these increases occur in areas where there are riparian, wet or mesic plant communities containing ozone-sensitive species, there is an increased probability that foliar ozone injury can occur.

The U.S. Forest Service's Forest Inventory and Analysis Program (FIA) has previously looked for ozone injury on trees and ground plants during their health monitoring assessments in the Rocky Mountain Region. Those assessments did not detect any ozone injury after several years of investigation, and at present the FIA is no longer conducting ozone assessments in the Rocky Mountain region (Gretchen Smith, personal communication). In light of the injury found at ROMO and the increased levels of ozone occurring in remote regions, a strong case can be made for initiating a program that would concentrate on assessing plants in riparian and moist communities in the Rocky Mountain region for foliar ozone injury.

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APPENDIX 1

ASSESSMENT OF THE RISK OF FOLIAR INJURY FROM OZONE ON VEGETATION IN ROCKY MOUNTAIN NATIONAL PARK FOR 1995 THROUGH 2004

Objective

This assessment employs a biologically based method to evaluate the risk of foliar injury from ozone at Rocky Mountain National Park. The assessment allows resource managers at the park to better understand the risk of ozone injury to vegetation within the park and permits them to make a better informed decision regarding the need to monitor the impacts of ozone on plants.

The assessment of risk uses ozone exposure and soil moisture data for 1995 through 2004. Together the assessments provide insight to the changes in ozone exposure over a 10-year period, and a comprehensive examination of the risk of injury to plants from ozone and how it may have changed over time.

This introduction provides an overview of the risk assessment process and the data used.

Risk Assessment Methodology

The risk assessment is based on a Triad model that holds that the response of a plant to ozone is the result of the interaction of the plant, the level of exposure and the exposure environment. While interactions among the three variables determine the response, the state of any one of them can serve to accentuate or preclude the production of foliar injury. The response is greatest when all three variables and their interactions are optimized relative to the conditions that foster injury. The optimized states are: the species of plants are highly sensitive to ozone, the exposure levels of ozone significantly exceed the thresholds for foliar injury, and the environmental conditions foster gas exchange and the uptake of ozone by plants.

To conduct a risk assessment for a specific site, information was obtained on the ozone-sensitive plant species found there, the levels of ozone exposure that occur over a number of years, and, since soil moisture is a critical variable controlling gas exchange, the levels of soil moisture that exist during the periods of ozone exposure. The information was evaluated to determine the degree to which the levels of ozone exposure and soil moisture conditions integrate to create an environment that leads to the production of foliar injury on sensitive species at the site.

Ozone-Sensitive Plant Species

In 2003 a workshop was convened by the National Park Service to review the ozone research literature and apply the field experience of the attendees to develop a comprehensive list of ozone-sensitive plant species for the eastern and western United States. Because of the emphasis of previous field studies and research, information on the ozone-sensitivity of tropical, arctic and rare species is limited. The workshop identified both sensitive and bioindicator species for ozone, and published its determinations in a National Park Service Report (U.S. National Park Service 2003). An ozone bioindicator species is one whose high level of sensitivity and characteristic pattern of foliar injury allow it to be confidently used to ascertain the occurrence of injurious levels of ozone exposure in the field. With regard to the Triad model, a bioindicator species integrates the effects of exposure and environment while optimizing plant sensitivity. A bioindicator serves as an early-warning agent for the plant community with respect to the potential impacts of ozone. Ozone-sensitive and bioindicator plant species at each site were identified by comparing the site's floral list from NPSpecies with the list of sensitive species developed at the workshop.

Levels of Ozone Exposure

Ozone exposure data were obtained from on-site monitoring. While ozone monitoring provides the most accurate assessment of ozone exposure, data from a single monitor may not accurately represent exposures throughout a large park, or a park with significant elevation differences.

The Air Resources Division of the National Park Service analyzed ozone air monitoring data from the park to produce annual indices of exposure. Since the ozone research community has not completely accepted one index of exposure as fully characterizing the threshold for foliar injury to vegetation, the assessment employed three indices to assure a comprehensive approach was taken in the assessment.

One index is the Sum06 and its attendant thresholds for injury (Heck and Cowling 1997). This index is comprised of the 90-day maximum sum of the 0800 through 1959 hourly concentrations of ozone $\geq$ 60 ppb (0.60 ppm). The index is calculated over running 90-day periods and the maximum sum can occur over any period of the year, although the chemistry of ozone generation usually results in it occurring over the summer months. For risk assessment purposes, it is also necessary to know the three-month period over which each year's maximum index occurs.

Another index is the W126 and its associated thresholds (Lefohn et al. 1997). The W126 index is the weighted sum of the 24 one-hour ozone concentrations daily from April through October, and the number of hours of exposure to concentrations $\geq$ 100 ppb (0.10 ppm) during that period. The W126 index uses a sigmoidal weighting function in producing the sum: the lower concentrations are given less weight than are the higher concentrations since the higher exposures play a greater role in producing injury. The significance of the higher concentrations is also reflected in the requirement that there be a specified minimum number of hours of exposure to concentrations $\geq$ 100 ppb. Thus, the W126 index has two criteria that must be realized to satisfy its thresholds: a minimum sum of weighted concentrations and a minimum number of hours $\geq$ 100 ppb.

The last indicator of ozone exposure, designated N-value, consists of the numbers of hours of exposure each year that exceeded 60, 80 and 100 ppb. While there are no formal thresholds associated with these values, they provide insight to the distribution of exposures among these concentrations, and to the numbers of hours at and above 80 and 100 ppb, levels of exposure that are associated with the production of foliar injury.

Soil Moisture Status

Although gas exchange in plants is influenced by many environmental variables, soil moisture status is a critical factor since stomatal closure during periods of low soil moisture can severely limit gas exchange. Since site-specific soil moisture data are not available for the sites, the USDA's Palmer Z Index was selected to represent soil moisture conditions. The Palmer Z Index is a measure of the short-term departure of soil moisture from the long-term mean for the area. Consequently, the index automatically takes into account the diversity in precipitation among the parks, and emphasizes the difference that exists between the monthly soil moisture norm for the site and its actual state. The index is calculated monthly for up to ten regions in each of the 48 contiguous states, and measures drought on a scale from 0.0 to –4.0, a range representing normal to severe conditions. The regions are considered to be relatively homogeneous by USDA, but contain a diversity of soil, elevation and site variables that influence the soil moisture conditions at any specific location. The Palmer Z Index is not site specific and may not fully represent the soil moisture conditions at a park during a specific month.

The objective of this aspect of the risk assessment was to determine whether there is a consistent relationship between the level of ozone exposure and soil moisture status for the site by using the five years of data available. Atmospheric conditions that foster the production of ozone, such as clear sky, high UV levels and higher temperatures, are ones associated with the presence of few clouds and reduced precipitation. Consequently, years with high levels of atmospheric ozone may also experience low levels of soil moisture. This inverse relationship can constrain the uptake of ozone by plants in years with high levels of ozone and significantly reduce the likelihood that foliar injury will be produced. Knowing whether this relationship exists at a site is essential in determining whether certain levels of ozone exposure pose a risk to vegetation.

Palmer Z data were obtained from the USDA web site and tabulated for the three-month period over which the Sum06 exposure indices were compiled, and for the May to October period associated with the W126 exposure indices. Visual analysis of the exposure and soil moisture data was undertaken to determine whether there was an association between the levels of ozone exposure and soil moisture at each site.

Site-Specific Assessment

After information on the presence of sensitive species, levels of ozone exposure and relationships between exposure and soil moisture was compiled, it was synthesized into an assessment of risk of foliar injury for the park. Risk was classified as high, medium or low.

The Sum06 and W126 exposure indices were examined to determine whether they exceeded their respective thresholds for injury, and the frequency with which the thresholds were exceeded over the five-year assessment period. The N-value data were examined to assess the distribution of exposures in a given year, and the consistency of exposure over the five years.

Evaluation of the relationship between ozone exposure and soil moisture might indicate they are inversely related, or they are not related and months of drought occur independent of the level of ozone exposure. When exposure and drought are inversely related, the uptake of ozone is constrained by drought stress in the highest exposure years. In this instance, the risk of foliar ozone injury is likely greatest in years with lower levels of exposure that still exceed the injury thresholds and with soil moisture conditions that are more favorable for the uptake of ozone. In these cases, the greatest risk of foliar injury does not necessarily occur in the year with the highest level of ozone exposure. When exposure and soil moisture are not related, the risk of foliar injury in a given year is a function of the random co-occurrence of high exposure and favorable moisture conditions.

The risk of foliar ozone injury at the park was determined by analyzing the plant, exposure and moisture data. The process was not quantitative, but based upon three primary evaluations: the extent and consistency by which the ozone injury thresholds were exceeded by the Sum06 and W126 exposure indices, the nature of the relationship between exposure and soil moisture, and the extent to which soil moisture conditions constrained the uptake of ozone in high exposure years. The evaluation of these factors and the assessment of their interactions with ozone-sensitive plant species is consistent with the Triad model of risk assessment, and comprises the framework for determining whether the risk of foliar ozone injury was high, moderate or low. The accuracy of the park's risk assessment is dependent upon the quality of the plant list, the accuracy of the ozone exposure data and the degree to which the regional soil moisture data represent conditions at the site.

A risk rating of high suggests a high probability of foliar injury in most years, while a rating of low suggests a low probability of injury in any year. A rating of moderate is assigned when analysis indicates injury is likely to occur at some point in the ten-year assessment period, but the chance of injury occurring consistently is low. In other words, foliar injury will probably occur at a park rated moderate, but it is not anticipated it will occur regularly or frequently. Parks rated moderate are likely to experience a wide temporal variation in the occurrence of injury, and over a period of time may experience injury for one or more years while also experiencing several years without injury.

Literature Cited

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ROCKY MOUNTAIN NATIONAL PARK
(ROMO)

Plant Species Sensitive to Ozone

Latin Name	*Common Name*	*Family*
Amelanchier alnifolia	Saskatoon serviceberry	Rosaceae
Apocynum androsaemifolium	Spreading dogbane	Apocynaceae
Populus tremuloides	Quaking aspen	Salicaceae
Rudbeckia laciniata	Cut-leaf coneflower	Asteraceae
Salix scouleriana	Scouler's willow	Saliaceae

Representative Ozone Injury Thresholds

<u>Sum06</u> -- The running 90-day maximum sum of the 0800-2000 hourly ozone concentrations of ozone equal to or greater than 0.06 ppm. Index is in cumulative ppm-hr.

Natural Ecosystems	8 - 12 ppm-hr	(foliar injury)
Tree Seedlings	10 - 16 ppm-hr	(1-2% reduction in growth)
Crops	15 - 20 ppm-hr	(10% reduction in 25-35% of crops)

<u>W126</u> -- A cumulative index of exposure that uses a sigmoidal weighting function to give added significance to higher concentrations of ozone while retaining and giving less weight to mid and lower concentrations. The number of hours over 100 ppb (N100) is also considered in assessing the possible impact of the exposure. The W126 index is in cumulative ppm-hr.

	W126	N100
Highly Sensitive Species	5.9 ppm-hr	6
Moderately Sensitive Species	23.8 ppm-hr	51
Low Sensitivity	66.6 ppm-hr	135

Ozone Exposure Data

Ambient concentrations of ozone monitored on-site were analyzed to generate annual exposure values. The exposure values include the Sum06 and W126 exposure indices in ppm-hr and the annual number of hours above 60, 80 and 100 ppb (N60, N80 and N100, respectively).

Ozone air quality data for ROMO					
	1995	1996	1997	1998	1999
Sum06	15	24	10	28	13
W126	29.1	37.0	28.7	47.0	6.9
N60	331	566	388	843	431
N80	21	21	8	36	15
N100	0	0	0	2	1

Ozone air quality data for ROMO					
	2000	2001	2002	2003	2004
Sum06	38	9	49	43	22
W126	50.9	22.8	71.4	67.4	43.4
N60	931	306	1394	1332	757
N80	52	7	133	101	9
N100	2	0	4	4	0

Soil Moisture Status

The uptake of ambient ozone by a plant is highly dependent upon the environmental conditions under which the exposure takes place, and the level of soil moisture is an important environmental variable controlling the process. Understanding the soil moisture status can provide insight to how effective an exposure may be in leading to foliar injury. The Palmer Z Index was selected to indicate soil moisture status since it represents the short-term departure of soil moisture from the average for each month for the site. The objectives of the assessment were to examine the relationship between high annual levels of ozone and soil moisture status, and to consider the impact reduced soil moisture status would have on the effectiveness of exposure.

The Palmer Z Index is calculated for up to 10 regions within a state and therefore is not a site-specific index. Without site-specific data, ozone/soil moisture relationships can only be estimated. Site-specific criteria such as aspect, elevation, and soil type can alter soil moisture conditions such that they depart from those determined for the region. However, in lieu of site-specific data, the Palmer Z Index is the best estimate of short-term soil moisture status and its change throughout the growing season.

Palmer Z data were compiled for the site for both the three months used to calculate the Sum06 index and for the April through October period for the W126 index for 1995 through 1999. The Palmer Z index ranges from approximately +4.0 (extreme wetness) to –4.0 (extreme drought) with ±0.9 representing normal soil moisture.

Rocky Mountain National Park sits astride two of the state divisions used by the National Weather Service Prediction Center in its calculation of Palmer Z data. With respect to the park, Zone 2 covers the area from the Continental Divide west and Zone 4 covers the area east of the Continental Divide. Most of the park resides in Zone 2. For the period 1995 through 1999, soil moisture conditions in the two zones were similar, and data from Zone 2 were used to characterize soil moisture conditions in the park. For the period 2000 through 2004, soil moisture indices in the zones were somewhat different, and data for both zones are presented below. Since ozone exposures in the park are likely the result of upslope movement from the Denver Metropolitan area, data from Zone 4 are emphasized in the assessment.

Soil moisture status for the Sum06 index period.

Palmer Z Index data for 3-month Sum06 period at ROMO					
	1995	1996	1997	1998	1999
Month 1	5.31	0.86	3.50	-0.85	1.58
Month 2	1.79	1.08	-0.65	0.67	1.70
Month 3	0.89	0.32	3.47	5.84	3.00

Soil moisture status for the April through October period for the W126 index.

Palmer Z Index data for the 7-month W126 period at ROMO					
	1995	1996	1997	1998	1999
April	3.79	-0.69	3.50	2.92	7.48
May	7.46	1.38	-0.65	-0.85	1.58
June	5.31	0.86	3.47	0.67	1.70
July	1.79	1.08	3.35	5.84	3.00
August	0.89	0.32	6.34	1.81	5.30
September	3.10	3.91	2.47	-0.80	2.21
October	-0.26	-0.55	3.49	2.04	-0.11

WEST OF THE CONTINENTAL DIVIDE: 2000 - 2004

Soil moisture status for the Sum06 index period.

Palmer Z Index data for 3-month Sum06 period at ROMO					
	2000	2001	2002	2003	2004
Month 1	-1.86	-1.68	-5.65	-1.14	1.54
Month 2	-3.08	0.48	-4.80	-3.43	-2.54
Month 3	-0.45	-2.11	-2.46	-0.88	-1.27

Soil moisture status for the April through October period for the W126 index.

Palmer Z Index data for the 7-month W126 period at ROMO					
	2000	2001	2002	2003	2004
April	-2.64	-1.46	-4.20	-1.77	1.54
May	-1.71	-1.12	-4.29	-0.60	-2.54
June	-1.86	-2.64	-5.65	-1.14	-1.27
July	-3.08	-1.68	-4.80	-3.43	-1.38
August	-0.45	0.48	-2.46	-0.88	-2.00
September	-0.60	-2.11	2.10	0.86	3.58
October	-0.23	-1.72	0.78	-3.01	0.59

EAST OF THE CONTINENTAL DIVIDE: 2000 - 2004

Soil moisture status for the Sum06 index period**.**

Palmer Z Index data for 3-month Sum06 period at ROMO					
	2000	2001	2002	2003	2004
Month 1	-2.28	0.38	-3.81	1.87	1.72
Month 2	-1.92	0.27	-4.45	-1.51	-2.81
Month 3	0.12	-0.13	-1.89	1.33	2.00

Soil moisture status for the April through October period for the W126 index.

Palmer Z Index data for the 7-month W126 period at ROMO					
	2000	2001	2002	2003	2004
April	-1.08	0.03	-4.03	0.44	1.72
May	-2.35	1.25	-2.72	-0.91	-2.81
June	-2.28	-1.63	-3.81	1.87	2.00
July	-1.92	0.38	-4.45	-1.51	2.22
August	0.12	0.27	-1.89	1.33	3.20
September	0.89	-0.13	0.38	-0.81	1.65
October	-0.77	-1.50	1.32	-2.22	1.05

Risk Analysis

- There are a few ozone-sensitive species at the site, some of which are bioindicators for ozone.

1995-1999

- The Sum06 index exceeds the threshold for injury to vegetation. While the W126 accumulative value is above the threshold, the N100 count is below the required number and thus the criteria for injury are not satisfied.

- The N-values for the site show concentrations frequently exceeded 60 ppb and exceeded 80 ppb for a few hours each year. No year had more than two hours in which the concentration exceeded 100 ppb, and three years had no hours at this level. These levels of exposure are not likely to injure vegetation.

- During the five-year assessment period, soil moisture levels were normal to high and favored the uptake of ozone. Since there were no months of drought, it is not possible to determine whether a relationship exists between the level of soil moisture and either the 90-day cumulative Sum06 or the seasonal W126 index of exposure.

2000-2004

- The Sum06 index exceeds the threshold for injury to vegetation. In some years the threshold is exceeded by a considerable margin. While the W126 accumulative value is above the threshold, the N100 count is below the required number and thus the criteria for injury are not satisfied.

- The N-values for the site show concentrations frequently exceeded 60 ppb and often exceeded 80 ppb in some years. No year had more than four hours in which the concentration exceeded 100 ppb, and there were some years in which 100 ppb was not reached. These levels of exposure may possibly injure vegetation.

- West of the Continental Divide, soil moisture levels during the 90-day Sum06 and the seasonal W126 accumulation periods appear to be unrelated to the levels of ozone exposure. However, the numbers of months and levels of drought experienced each year would significantly reduce the uptake of ozone by plants. For the Sum06 index, all years experienced at least two months of mild to severe drought during the 90-day period, while for the W126 index there were four to six months of mild to severe drought during the seven-month accumulation period each year. East of the Continental Divide, soil moisture levels during the 90-day Sum06 and the seasonal W126 accumulation periods appear to be inversely related to ozone concentrations: when ozone is high, soil moisture is low. This relationship reduces the uptake of ozone and the effectiveness of the exposure in producing foliar injury. There are, however, some inconsistencies in the relationship for both indices. The years with the three highest Sum06 ozone exposure values, 2002, 2003 and 2000 had respectively, three months of mild to severe drought, one month of mild drought, and two months of mild and moderate drought. The year with the second lowest level of exposure, 2004, experienced one month of moderate drought, while the year with the lowest exposure, 2001, had no drought. There was also an inverse relationship between W126 index of exposure and incidence of drought, with some inconsistency. The year with the highest exposure, 2002, had five months of mild to severe drought, while the second highest year, 2003, had two months of mild and moderate drought. The two years with next lower levels of exposure, 2000 and 2004, had four months of mild and moderate drought, and one month of moderate drought, respectively. The year with the lowest exposure, 2001, had two months of mild drought. Generally, the levels of drought experienced in the higher exposure years would reduce the uptake of ozone by plants and reduce the likelihood of foliar injury.

Levels of ozone exposure increased significantly over the 10-year assessment period at Rocky Mountain National Park, however the risk of foliar ozone injury is generally low. The threshold for injury is consistently satisfied for the Sum06 index. The cumulative value for the W126 index also consistently exceeds the threshold, however the associated criterion for the number of hours greater than 100ppb is not satisfied in any year. The N-values indicate that exposure to concentrations of ozone greater than 60 and 80 ppb increased significantly between the two assessment periods, and although exposures over 100 ppb are rare, they too increased. During the first 5-year assessment period, soil moisture levels were favorable for the uptake of ozone, but ambient levels of exposure were lower and foliar injury was not likely to be produced. Levels of exposure increased significantly in the second five-year assessment period, however the incidence of mild to severe drought also increased. The occurrence of low levels of soil moisture during periods of high ozone exposure greatly reduces the potential for foliar injury since the uptake of ozone by the plant is reduced. Extended meteorological conditions that foster the production of atmospheric ozone, such as intense sunlight and high temperatures, are also associated with reduced precipitation and greater evaporation of soil moisture all of which reduce gas exchange by plants. If there is a year in which this relationship becomes uncoupled and high levels of exposure occur under conditions of normal soil moisture or mild drought, the risk of foliar injury will increase.

It is worth noting that Zones 2 and 4 are large and contain considerable diversity in elevation and ecology. It is not possible to determine whether the soil moisture levels calculated for the zones are necessarily representative of those in Rocky Mountain National Park. If during periods of elevated ozone exposure the soil moisture levels in the park are higher than those calculated for the zone, the risk of ozone injury could be greater in a given year.

If the level of risk increases in the future, a program to assess the incidence of foliar ozone injury on plants at the site could use one or more of the following bioindicator species: spreading dogbane, quaking aspen, cut-leaf coneflower, and Scouler's willow.