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FOLIAR OZONE INJURY ON CUTLEAF CONEFLOWER AT ROCKY MOUNTAIN NATIONAL PARK, COLORADO

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Abstract.—Surveys for foliar ozone injury on cutleaf coneflower, spreading dogbane, and quaking aspen were conducted in Rocky Mountain National Park, Colorado, from 2006 through 2010. Foliar injury in the form of ozone stipple was found on coneflower each year. The incidence of injured plants on sites with injury ranged from 5% to 100%. The severity of injury on affected foliage was generally <4% but occurred on some leaves at a level greater than 12% in 3 years and in 1 year on 1 plant at a level >75%. No foliar ozone injury was found on spreading dogbane or quaking aspen in any year of the survey. This is the first documentation of ozone injury on vegetation in Rocky Mountain National Park. While ozone has long been a concern in the Colorado Front Range, spreading urbanization and oil and gas development are leading to increased levels of ozone in many areas in the Rocky Mountain region. Air monitoring data indicate that ozone exposures are exceeding injury thresholds in several locations and suggest that assessments of foliar ozone injury should be conducted on ozone-sensitive plant species in riparian and moist communities in those areas.

Resumen.—De 2006 a 2010 se llevaron a cabo trabajos de investigación en el Parque Nacional Rocky Mountain, estado de Colorado, para investigar el daño foliar causado por el ozono en *Rudbeckia laciniata*, *Apocynum androsaemifolium* y *Populus tremuloides*. Cada año se encontraron en *Rudbeckia laciniata* manchas ocasionadas por el ozono representativas de daño foliar. La incidencia de plantas dañadas en sitos afectados varió desde un 5% hasta un 100%. Por lo general, la severidad del daño en el follaje afectado fue menor de un 4%; sin embargo, en 3 años ocurrió a un nivel por encima del 12% en algunas hojas, y en un año a un nivel superior al 75% en una planta. No se encontró ningún daño foliar ocasionado por el ozono ni en *Apocynum androsaemifolium* ni en *Populus tremuloides* durante ninguno de los años en los que se llevó a cabo el estudio. Esta es la primera documentación existente de un daño ocasionado por el ozono sobre la vegetación del Parque Nacional Rocky Mountain. Mientras que el ozono ha sido por mucho tiempo un punto de preocupación para la cordillera frontal de Colorado (*Colorado Front Range*), la creciente urbanización y la extracción de petróleo y de gas son las causas principales que aumentan los niveles de ozono en muchas áreas de la región de las Montañas Rocosas. Los datos del monitoreo del aire indican que la exposición al ozono está excediendo los niveles críticos de daño en varias localidades; también indican que se deben llevar a cabo evaluaciones del daño foliar ocasionado por el ozono en especies de plantas susceptibles al ozono tanto en las comunidades ribereñas como en las de los humedales de esas áreas.

Tropospheric, or ground-level, ozone of anthropogenic origin is recognized as one of the most widely dispersed and phytotoxic air pollutants in the United States, and its effects on the physiology, growth, productivity, and well-being of plant species have been widely researched and documented in laboratory and field studies (EPA 1996, 2007). Ozone is not emitted by any source; it is produced by photochemical reactions in the atmosphere that are powered by sunlight and involve volatile organic compounds and oxides of nitrogen. Precursor pollutants are released in urban and industrialized sites and from energy development in rural areas. The generated ozone can be transported hundreds of miles downwind, producing deleterious impacts on agricultural crops and native plant communities along its path of movement (EPA 2001).

In Denver and other Front Range communities in Colorado, ozone is recognized as an air quality problem due to the regional release of precursor pollutants and the occurrence of abundant sunshine to power ozone's photochemical generation in the atmosphere (Colorado Department of Public Health and Environment 2009). Rocky Mountain National Park (ROMO) in Colorado is located astride the Continental Divide approximately 100 km northwest of Denver at elevations ranging from approximately 2500 m to over 4200 m. The park encompasses many air pollution–sensitive, high-elevation aquatic and terrestrial ecosystems, and it is designated a Class I air quality area, a

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status that affords the highest level of resource protection under the Clean Air Act. Periodic upslope movement of air from the Front Range metropolitan area carries ozone into the park, where monitoring by the National Park Service has found levels that approach and often exceed those recognized as being harmful to sensitive plant species. Changes in ozone concentrations with elevation in the Colorado Front Range were examined by monitoring ozone at 7 sites along an elevation gradient from Boulder, Colorado, at 1600 m to a tundra research site at 3530 m (Brodin et al. 2010). The researchers found that summer levels of ozone increased with elevation along the gradient and that ozone production and movement from urban areas appeared to be the dominant factors responsible. Even the highest elevation site showed the influence of upslope movement of ozone. Brodin et al. also noted that ozone exposure at the mid- to high-elevation sites in many instances approached and exceeded the 8-hour National Ambient Air Quality Standard of 75 ppb.

Surveys of ponderosa pines (*Pinus ponderosa*) in the Colorado Front Range area, including ROMO, in the late 1980s did not find evidence of ozone injury (Graybill 1992, Peterson et al. 1993). In contrast, widespread injury to ponderosa pine had been documented in California for a number of years (Peterson et al. 1989). However, the variety of *P. ponderosa* (var. *scopulorum*) occurring in the Front Range is considered relatively insensitive to ozone compared to *P. ponderosa* var. *ponderosa*, which occurs in California (Miller et al. 1983). Other plant species were not examined in ROMO, although ambient levels of ozone were considered high enough to induce visible injury (Bohm 1992). Ozone concentrations have increased in the park in recent years (Jaffe and Ray 2007). An assessment of the risk of ozone injury to plants at ROMO conducted by the senior author (Kohut) and the levels of ozone exposure for 1995 through 2004 can be found at http://www.nature.nps.gov/air/Pubs/pdf/03Risk/ReassessmentROMO2006.pdf

Field assessments were conducted annually from 2006 through 2010 at ROMO to determine whether ambient levels of ozone were producing foliar injury on sensitive species of native plants. Knowing whether ozone is impacting plants in the park is important to managing the park's resources and making informed decisions regarding the acceptability of additional changes in the park's air quality (USDA Forest Service et al. 2010).

METHODS

The methods employed in the foliar ozone injury assessment program at ROMO were those described in the Handbook for Assessment of Foliar Ozone Injury written for the U.S. National Park Service (Kohut 2007).

Bioindicator Species

Several plant species at ROMO are bioindicators for ozone; that is, at ambient ozone concentrations, they exhibit foliar symptoms that can be recognized as ozone injury by subject matter experts. These species include cutleaf coneflower (*Rudbeckia laciniata* var. *ampla*), spreading dogbane (*Apocynum androsaemifolium*), and quaking aspen (*Populus tremuloides*) (Porter 2003). Coneflower and dogbane were selected for emphasis in the assessment because they can be readily examined in large numbers and are widely distributed in the eastern portion of the park. Quaking aspen was selected for use in a less rigorous assessment of foliar injury.

Candidate assessment sites for coneflower and dogbane were identified using plant community information available for the park and information on locations of plants based on park staff observations in the field. All candidate sites were on the eastern side of the Continental Divide since concentrations of ozone from upslope movement of air from the Front Range are highest in that area (John D. Ray, U.S. National Park Service, Air Resources Division, personal communication). Potential sites were located on maps and examined in the field for suitability. Sites used in the assessment program were selected on the basis of the number of plants present, accessibility, and distribution within the park. All assessment sites were at elevations between approximately 2400 m and 2800 m. Cutleaf coneflower was evaluated for foliar injury on 14 permanent sites (Fig. 1), and spreading dogbane was evaluated on 6 permanent sites. Due to a widespread distribution and secondary emphasis in the program, quaking aspen was assessed for injury on an opportunistic basis, and no permanent assessment sites were established for aspen.

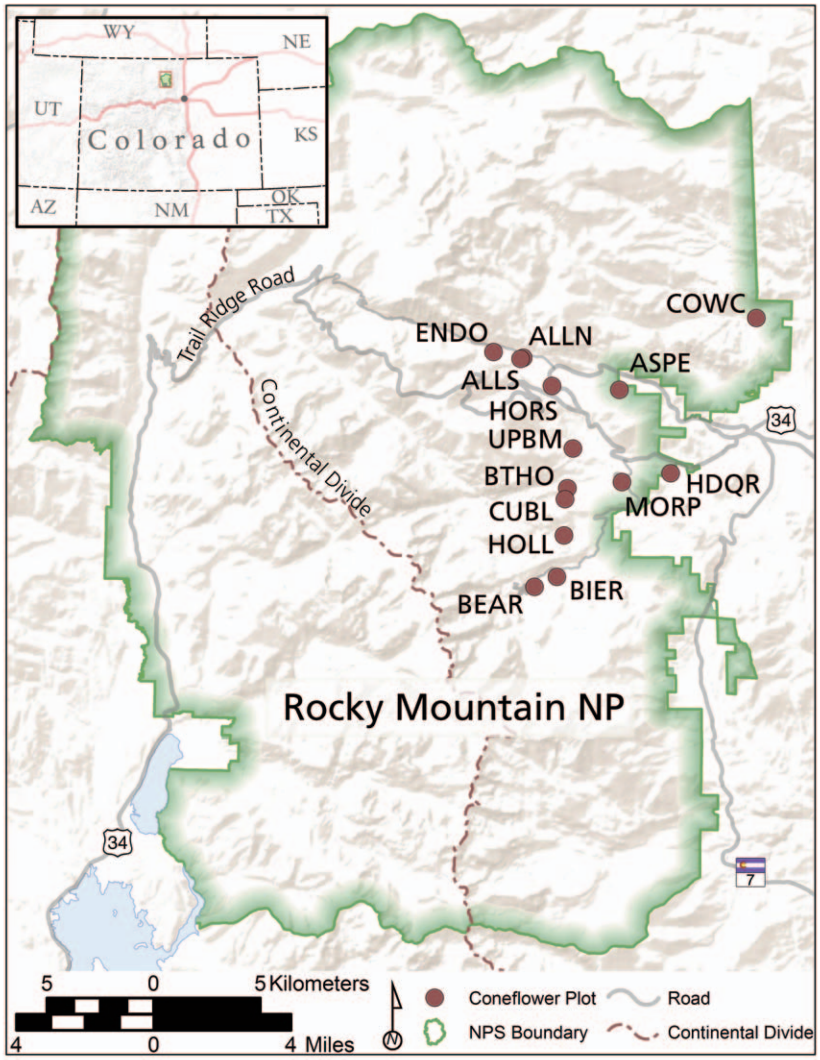

Fig. 1. Locations of permanent assessment sites for cutleaf coneflower (*Rudbeckia laciniata* var. *ampla*) at Rocky Mountain National Park.

Injury Assessment

Assessments of foliar ozone injury were conducted at 2 levels: surveying and scouting. Primary emphasis was placed in survey assessments of cutleaf coneflower and spreading dogbane, while a scouting assessment was employed with quaking aspen.

Survey assessments are quantitative and comprehensive in nature and used to assess the incidence and severity of foliar injury and its trends over time (Kohut 2007). A survey is performed annually or only when ozone exposure and environmental conditions warrant, and it uses field sites that are located with both random and nonrandom means and to ensure sites are distributed to provide spatial coverage within the park. A survey yields quantitative information on the incidence and severity of ozone injury and its spatial distribution. Though such assessments are conducted on permanent sites, the same plants are not necessarily evaluated in successive years.

On the other hand, in a scouting assessment, bioindicator plants are generally examined as they are opportunistically encountered in the field (Kohut 2007). The assessment provides a yes or no answer with regard to the presence of foliar injury in the park and its continued occurrence over time. Scouting assessments require the smallest investment of time, personnel, and funds; and because they are less quantitative than survey assessments, scouting assessments provide limited information on the incidence, severity, and spatial distribution of injury.

Survey assessments for coneflower and dogbane followed procedures in the Handbook for the Assessment of Foliar Ozone Injury (Kohut 2007) and those methods are summarized here. The objective was to examine 20 plants at each site. When the population of plants on the site significantly exceeded 20, the plants to be examined were randomly selected using one of the random number tables in the handbook. If the population of plants was <20, all plants on the site were assessed. Only flowering coneflower plants were candidates for assessment, and observations of injury were made only on leaves on the flower stalk. All leaves on the stalk were counted and examined. The severity of foliar injury was assessed on all leaves if the number of leaves on the flower stalk was <20; otherwise, 20 leaves were selected at random for assessment. In almost all cases, there were <20 leaves on the stalk. The collected data allowed calculation of several measures of injury: site incidence (percentage of plants injured on a site), plant incidence (percentage of leaves injured on a plant), and severity (percentage of leaf area affected on a plant). On spreading dogbane, a similar process was used to select plants for assessment at each site, and all leaves on a plant were examined for injury. The total number of leaves and number of injured leaves were determined for each dogbane plant. The incidence and severity of foliar injury were evaluated as they were on coneflower, except that all leaves on the plant were assessed.

In the scouting assessment of quaking aspen, no permanent sites were established, and trees were selected and examined on an opportunistic basis. Most of the trees selected were sapling size so that a large number of leaves could be readily examined. The observation made for each tree, injury or no injury, provided a measure of the incidence of ozone injury (percentage of plants injured) at the evaluation site.

Foliar injury commonly produced by ozone includes stipple, fleck, and bifacial necrosis (Skelly et al. 1987, Flagler 1998, Innes et al. 2001). Stipple is the most common form of ozone injury and is the form produced on coneflower and dogbane. It is characterized by the appearance of interveinal, dot-like areas of tan, red, brown, purple, or black pigmentation on the upper surface of the leaf. Stipple can be either distributed over the surface of the leaf or concentrated in certain areas, and it may range from widely scattered dots to those whose density provides uniform coloration to part or all of the leaf surface. Coloration, density, and distribution are functions of plant species and the duration and nature of exposure.

Fleck is characterized by small, discrete areas of dead tissue visible only on the upper surface of the leaf. Fleck is produced by the death of cells in the palisade mesophyll of the leaf. The lesions may be irregular in shape and range in color from tan to black. Bifacial necrosis is a more severe form of injury resulting from cell death in both the palisade and spongy mesophyll and epidermal tissues of the leaf. Injury appears on both sides of the leaf, and dead tissue may take on a papery texture. Coloration in bifacial necrosis can range from light tan to black. Fleck and bifacial necrosis are the forms of foliar ozone injury produced on quaking aspen.

For coneflower and dogbane, the severity of injury on individual leaves was assessed using the scale in the handbook (Kohut 2007), which incorporates features of the scales used by the USDA Forest Service in its Forest Inventory and Analysis Program (USDA Forest Service 2010) and one devised by Horsfall and Barratt (1945), but the handbook scale is not directly comparable to either of the others. The injury indices and respective percent leaf area or leaves affected are index 0, no injury; 1, 1%–4%; 2, 5%–12%; 3, 13%–25%; 4, 26%–50%; 5, 51%–75%; and 6, 76%–100%.

Ozone Exposure Indices

The National Park Service monitors ambient ozone at ROMO's Longs Peak meteorology site. The annual levels of ozone exposure at ROMO for 2006–2010 were characterized using several indices, which were employed in plant-effects research and considered for use as air quality standards for ozone. Exposures were expressed as the Sum06, W126, and W126–3mo cumulative indices and as the numbers of hours at or above 60 ppb, 80 ppb, and

100 ppb (N values). In January 2010, the EPA proposed using the W126–3 mo as the index for a revised secondary ozone National Ambient Air Quality Standard to protect vegetation (EPA 2010).

Sum06.—The Sum06 index is the 90-day maximum sum of the 08:00–19:59 hourly concentrations of ozone ≥60 ppb (0.06 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 seasonal pattern of ozone generation usually results in it occurring over the summer months.

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

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.—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 and Runeckles 1987, Lefohn et al. 1988, 1997). The W126 index uses a sigmoidal weighting function in producing the sum: the lower concentrations are given less weight than the higher concentrations, because 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 2 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 the following:

	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

W126–3 mo.—Exposures were also calculated in the form of the secondary standard proposed in EPA's 2010 Federal Register Notice on the ambient air quality standards for ozone (EPA 2010). The W126-3 mo index is the running maximum 3-month, cumulative 12-hour (08:00–19:59) W126 weighted value.

Injury thresholds for the W126–3 mo index in cumulative ppm-hr are the following:

Foliar injury in natural ecosystems	5–9 ppm-hr (foliar injury)
Growth reductions	
Tree seedlings in natural ecosystems	7–13 ppm-hr
Tree seedlings/saplings in plantations	9–14 ppm-hr

The EPA proposed a W126–3 mo standard to protect vegetation in the range of 7–15 ppm-hr.

N-values.—N-values—the number of hours of exposure each year that equaled or exceeded 60, 80 and 100 ppb—were also determined. While not associated with the values by themselves, injury thresholds provide insight into the frequency of high ozone exposures, and the N100 value is used in conjunction with the W126 index (Lefohn and Runeckles 1987, Lefohn et al. 1988).

RESULTS

In annual assessments from 2006 through 2010, foliar 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 on 9 of 14 assessment sites in 2006 and 2008, 9 of 13 sites in 2007, 7 of 13 sites in 2009, and 10 of 13 sites in 2010 (Table 1). Plants on 5 sites were injured in all 5 years of assessment, plants on 4 sites were injured in 4 years of assessment, and the presence of injury on the remaining sites varied among years. The incidence of injured plants on sites with injury ranged from 5% to 100%. While the severity of injury on affected foliage was generally <4%, in 3 years injury occurred on some plants at a level greater than 12%, and in one year a few plants on one site had >75% injury. Table 2 contains summary data on the severity of injury on affected foliage.

Injury on coneflower at ROMO was primarily stipple, characterized as discrete, black, interveinal dots of pigmentation (Fig. 2). Injury was found only on the upper leaf surface and occurred with greater intensity on the older leaves. Also, injury was unrelated to any incidence or signs of insects or disease. These properties of the markings satisfy the diagnostic criteria for stipple ozone injury (Skelly et al.

TABLE 1. Incidence of foliar ozone injury on cutleaf coneflower at Rocky Mountain National Park 2006–2010 presented as whether or not injury was present and the percentage of plants affected on the site.

			2006		2007		2008		2009		2010	
Site	Site identifier	Site location	Injury?	% Plants	Injury?	% Plants	Injury?	% Plants	Injury?	% Plants	Injury?	% Plants
1	HDQTR	Headquarters	No	0	Yes	5	Yes	13	No	0	Yes	40
2	ALLN	Alluvial Fan (north)	Yes	65	Yes	33	Yes	40	Yes	38	Yes	45
3	ALLS	Alluvial Fan (south)	No	0	No	0	No	0	No	0	Yes	10
4	HORS	Horseshoe Park	Yes	35	Yes	5	Yes	3	No	0	Yes	5
5	MORP	Moraine Park Museum	Yes	10	Yes	5	No	0	No	0	No	0
6	BEAR	Bear Lake Road	Yes	65	Yes	100	Yes	70	Yes	65	No	0
7	ASPE	Aspenglen Campground	Yes	62	Yes	36	Yes	77	Yes	90	Yes	75
8	COWC	Cow Creek	Yes	20	No	0	No	0	Yes	35	Yes	10
9	BTHO	Big Thompson River	No	0	No	0	No	0	No	0	Yes	20
10	ENDO	Endovalley Picnic Area	No	0	No	0	No	0	No	0	—	—
11	HOLL	Hollowell Park	Yes	30	Yes	41	Yes	10	—	—	No	0
12	UPBM	Upper Beaver Meadows	Yes	53	—	—	Yes	10	Yes	40	Yes	50
13	BIER	Bierstadt Lake Trailhead	Yes	65	Yes	67	Yes	50	Yes	90	Yes	50
14	CUBL	Cub Lake Trailhead	No	0	Yes	35	Yes	55	Yes	90	Yes	90

TABLE 2. Severity of ozone injury on affected cutleaf coneflower foliage[a].

Severity[b]	2006[c]	2007	2008	2009	2010
1–4	76	89	90	78	81
5–12	22	11	10	18	16
13–25	2	0	0	4	2
26–50	0	0	0	0	0
51–75	0	0	0	0	0
76–100	0	0	0	0	1

[a]Expressed as the percentage of leaves injured at that level of severity.
[b]Severity is the percentage of leaf affected as graded on injury scale.
[c]Percentages for 2006 are approximate due to use of a slightly different injury scale in that year.

1987, Kohut 2007). However, the presence of black stipple was quite different from the bronzing (coalesced rust-brown pigmented stipples) produced on coneflower at Great Smoky Mountains National Park (GRSM), North Carolina and Tennessee (Neufeld et al. 1992, Grulke et al. 2007). In addition, some of the markings on coneflower at ROMO were more like fleck in that they were slightly larger, more irregular in shape, and slightly depressed into the leaf epidermis; stipple is generally more uniformly shaped and flush with the leaf surface.

None of the spreading dogbane examined on 6 permanent sites showed any signs of ozone injury. The dogbane sites were formally assessed in 2006 and 2007 but not in subsequent years, since no injury had been observed in the initial 2 years. Each year, however, dogbane was examined in a scouting assessment as the park was traversed on foot, and no injury was found then either.

Quaking aspen was examined for ozone injury in a scouting assessment 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 pattern of distribution on the tree did not satisfy the diagnostic criteria for ozone.

Ambient concentrations of ozone monitored at ROMO for 2006–2010 were analyzed to generate annual exposure values (Table 3). The Sum06 index exceeded the injury threshold (8–12 ppm-hr) in each of the 5 years. While the W126 index exceeded its cumulative value threshold (5.9 ppm-hr) each year, the required number of hours of exposure >100 ppb (6 hours) was not attained in any year; thus, the 2 components of the index were not satisfied. The threshold range of the W126–3 mo index for foliar injury (7–15 ppm-hr) was reached each year.

Fig. 2. Ozone stipple on cutleaf coneflower (*Rudbeckia laciniata* var. *ampla*) at Rocky Mountain National Park.

TABLE 3. Ozone air quality for Rocky Mountain National Park 2006–2010[a].

Index[b,c]	2006	2007	2008	2009	2010	Threshold[d]
Sum06	26	28	24	13	27	8–12
W126	29.6	33.2	28.9	19.9	18.9	5.9
W126–3 mo	19	20	18	11	19	7–15
N60	746	798	716	390	900	NA
N80	20	32	27	5	37	NA
N100	3	0	0	0	0	6 for W126

[a]Air quality data provided by U.S. National Park Service.
[b]Sum06, W126 and W126–3 mo values are in ppm-hr.
[c]N values are numbers of hours.
[d]Thresholds: Sum06—natural ecosystems/foliar injury (Heck and Cowling 1997); W126—highly sensitive species (Lefohn et al. 1997); W126–3 mo—foliar injury in natural ecosystems (EPA 2010).

TABLE 4. Ozone air quality for Jonah gas field, Wyoming, 2005–2007[a].

Index[b]	2005	2006	2007	Threshold[c]
Sum06	15	19	16	8–12
W126–3 mo	13	14	12	7–15

[a]Air quality data from Wyoming Department of Environmental Quality (2010).
[b]Sum06 and W126–3 mo values are in ppm-hr.
[c]Thresholds: Sum06—natural ecosystems/foliar injury (Heck and Cowling 1997); W126–3 mo—foliar injury in natural ecosystems (EPA 2010).

DISCUSSION

This is the first documentation of ozone injury on vegetation at ROMO and the first report of ozone injury on native vegetation in the Rocky Mountain region. The presence of ozone injury on plants at ROMO raises the question of whether ozone may be affecting sensitive plants elsewhere in the region. While ozone has long been a concern in the Colorado Front Range, spreading urbanization and oil and gas development are leading to increased levels of ozone in many areas in the Rocky Mountain region. The recent increase in oil and gas drilling in Wyoming has resulted in elevated concentrations of ambient ozone in remote areas that previously had low background levels of the pollutant (Wyoming Department of Environmental Quality 2010). Ozone air monitoring

TABLE 5. Ozone air quality for monitoring sites at selected national parks for 2005–2009[a].

Index[b,c]	2005	2006	2007	2008	2009	Threshold[d]
GRAND CANYON – THE ABYSS						
W126	60.6	71.9	68.1	56.8	41.9	5.9
W126–3 mo	20	19	20	19	15	7–15
N60	824	1248	1130	732	353	NA
N80	27	2	2	0	0	NA
N100	0	0	0	0	0	6 for W126
MESA VERDE – RESOURCE MANAGEMENT AREA						
W126	66.2	67.7	56.5	40.6	54.6	5.9
W126–3 mo	17	19	21	18	15	7–15
N60	1041	1167	825	469	696	NA
N80	11	12	0	0	1	NA
N100	0	0	0	0	0	6 for W126
CANYONLANDS – ISLAND IN THE SKY						
W126	46.3	57.1	56.8	58.4	51.6	5.9
W126–3 mo	18	16	17	17	15	7–15
N60	592	806	877	772	484	NA
N80	1	0	1	2	0	NA
N100	0	0	0	0	0	6 for W126
ZION – DAALTON'S WASH						
W126	50.2	50.4	45.3	52.9	37.1	5.9
W126–3 mo	—	23	22	20	17	7–15
N60	802	855	788	882	421	NA
N80	48	8	4	4	2	NA
N100	12	0	0	0	0	6 for W126

[a]Air quality data provided by U.S. National Park Service (John Ray, personal communication, 2010).
[b]W126 and W126–3 mo values are in ppm-hr.
[c]N values are numbers of hours.
[d]Thresholds: W126—highly sensitive species (Lefohn et al. 1997); W126–3 mo—foliar injury in natural ecosystems (EPA 2010).

data from the Jonah gas field in Wyoming for 2005, 2006, and 2007 were compiled into the Sum06 and W126–3 mo indices by the Wyoming DEQ (Table 4). Each year, the ambient levels of ozone exposure exceeded the injury thresholds for sensitive plant species for each index. Analysis by the National Park Service of ozone air monitoring data from Grand Canyon, Mesa Verde, Canyonlands, and Zion National Parks indicate that ozone exposures, as represented by the proposed W126–3 mo ozone exposure index, exceeded the injury threshold annually from 2005 through 2009 (Table 5).

The U.S. Forest Service's Forest Inventory and Analysis Program (FIA) assessed trees and ground plants for ozone injury during their health-monitoring assessments in the Rocky Mountain region. However, no foliar ozone injury was observed after several years of assessment (EPA 2007), and the FIA no longer conducts ozone injury surveys in the region. In light of the injury found at ROMO and the elevated levels of ozone occurring in areas where it had previously been at background levels, a strong case can be made for initiating a comprehensive program to assess foliar ozone injury on ozone-sensitive plant species in riparian and moist communities in the Rocky Mountain region.

Cutleaf coneflower was selected as a bioindicator at ROMO because of its recognized sensitivity to ozone and its relatively widespread occurrence in the park (Neufeld et al. 1992, Porter 2003). Controlled exposure studies and field surveys at GRSM identified and confirmed the coneflower's sensitivity to ozone and verified its use as a bioindicator (Neufeld et al. 1992, Chappelka et al. 2003). Symptoms of ozone injury on coneflower in the eastern United States, including GRSM, are characterized as rust or purple-brown bronzing restricted to the upper leaf surface. These symptoms can be readily recognized in the field (Neufeld et al. 1992, Grulke et al. 2007).

Thus, it was anticipated that ozone injury to coneflower at ROMO would appear as bronzing of the upper leaf surface. However, the black, scattered stipple found at ROMO is significantly different from the bronzing found at GRSM. On the second field site, Alluvial Fan North, examined at ROMO in 2006, plants were found with a combination of dark stipple and fleck on the upper leaf surface. Later on that sampling day, foliar injury observed on plants at the Bierstadt Lake Trail site was the classic black stipple widely recognized as a common form of ozone injury on many plant species (Skelly et al. 1987, Flagler 1998). Photographs of this injury were circulated to other researchers familiar with ozone injury in the field (Art Chappelka, Auburn University, Auburn, AL; Don Davis, Penn State University, University Park, PA; Howard Neufeld, Appalachian State University, Boone, NC), and all agreed it was ozone injury.

While the injury attributed to ozone at ROMO is different from that found on coneflower in the eastern United States, several lines of evidence confirm that the markings are the result of exposure to ozone. First, the diagnostic criteria used to identify ozone injury are consistently satisfied: the stipple is interveinal, present only on the upper leaf surface, and most severe on older leaves that have had the longest exposure to ambient ozone. On one occasion where a coneflower 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 opportunity for leaves to overlap. Second, in the summer of 2007, Dr. Howard Neufeld (Appalachian State University, Boone, NC) subjected coneflowers grown from root sections obtained at ROMO to controlled ozone exposures. Although technical problems limited the hours of exposure, the plants began to develop black stipple similar to that observed at ROMO. In addition, Susan Sachs (Appalachian Highlands Science Learning Center, GRSM, personal communication) indicated that she has seen similar stipple injury on coneflower grown at the Learning Center. Her observation of stipple may result from her examination of plants throughout the growing season as part of the ozone education program she conducts at the center, whereas field injury assessment programs generally record observations only late in the summer.

While cutleaf coneflower was consistently injured by ozone at ROMO, spreading dogbane remained unaffected. The 2 species are found in different habitats in the park, and it is believed that soil moisture properties of their habitats may have influenced their responses. At ROMO, cutleaf coneflower is found in riparian habitats and moist sites, where plants likely have access to adequate soil moisture throughout the growing season. This environment allows plants to consistently exchange gases with the atmosphere and take up ozone without the limitations on uptake produced by stomatal closure in response to low levels of soil moisture (Tingey and Hogsett 1985, Dobson et al. 1990). In contrast, spreading dogbane is generally found on exposed, open sites where soil moisture is likely low and where moisture stress serves as a constraint to gas exchange for some or most of the growing season. Thus, the uptake of ozone by dogbane would likely be less than that by coneflower. However, some dogbane plants examined opportunistically each year were on moist sites and were never found to have foliar ozone injury.

Showman (1991) documented the importance of soil moisture in regulating the response of bioindicator species to ozone in the field. In 1988 and 1989, he assessed foliar ozone injury on up to 7 bioindicator species on 13 permanent sites in Indiana and on 11 sites in Ohio. In 1988, ambient levels of ozone at nearby monitoring sites showed up to 63 hours of concentrations above 120 ppb while precipitation in the area was 5.6 inches below average. In 1989, ozone levels at the same monitoring sites exceeded 120 ppb for only 2 hours while precipitation was 8.7 inches above normal. In 1988, the year of higher ozone exposure and drier conditions, ozone injury was observed only on common milkweed (*Asclepias syriaca*) on 1 site in Indiana and 2 sites in Ohio, while all other bioindicator species remained uninjured. In contrast, in 1989, the year of lower ozone exposure and wetter conditions, injury was observed on common milkweed on 11 of 13 sites in Indiana and on all 11 sites in Ohio. In addition, injury was also found on blackberry (*Rubus allegheniensis*), sweet gum (*Liquidambar styraciflua*), tulip poplar (*Liriodendron tulipifera*), and wild grape (*Vitis vinifera*) in Indiana, and on white ash (*Fraxinus americana*), wild grape, and blackberry in Ohio. These observations illustrate the importance of soil moisture in regulating the potential effects of ambient levels of ozone by influencing stomatal opening, the exchange of gases with the atmosphere, and the concomitant uptake of ozone.

Quaking aspen at ROMO occurred on a variety of sites, and regardless of the soil moisture level at the sites, no ozone injury was observed on any of the trees. In 2008, bifacial lesions were observed on aspen 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 ozone exposure indices for 2006–2010 indicate that ambient ozone at ROMO reached levels that are harmful to very sensitive species of plants. The Sum06 index of exposure significantly exceeded the threshold for injury in each year of the assessment. The cumulative value for the W126 index also significantly exceeded the threshold, but there were few or no excursions >100 ppb each year; thus, the second criterion of the exposure index was not satisfied. The W126–3 mo exposure index thresholds for foliar injury were exceeded each year. The W126–3 mo index, proposed by the EPA as the form of the secondary National Ambient Air Quality Standard, was a consistent predictor of ozone injury over the 5 years of assessment.

It is not apparent why ozone injury on coneflower variety *ampla* occurred as black stipple at ROMO rather than the bronzing observed on varieties *laciniata* and *digitata* at GRSM. The difference could be related to the genetic or physiological properties of the western and eastern coneflower varieties, the characteristics of the ozone exposure regimes, or the nature of the environments in which the plants grow and exposures occur. In a study to assess the differences between ozone-sensitive and -insensitive lines of coneflower in GRSM, Grulke et al. (2007) found that no single stomatal or physiological property was adequate to explain the difference and concluded that physiological attributes that vary independently within an individual plant can collectively confer sensitivity or insensitivity to ozone. Given these findings and the differences in ozone exposure regimes and environments at ROMO and GRSM, it is likely that the explanation for the different response to ozone of the plants at the 2 parks will also be complex. To explain the differences in injury symptoms, effort should be directed at conducting controlled exposure or reciprocal transplant studies supported by physical and physiological assessments using coneflower varieties from both ROMO and GRSM.

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