Ambient ozone effects on gas exchange and total non-structural carbohydrate levels in cutleaf coneflower (*Rudbeckia laciniata* L.) growing in Great Smoky Mountains National Park

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ABSTRACT

Ozone-sensitive and -tolerant individuals of cutleaf coneflower (*Rudbeckia laciniata* L.) were compared for their gas exchange characteristics and total non-structural carbohydrates at Purchase Knob, a high elevation site in Great Smoky Mountains National Park, USA. Photosynthesis and stomatal conductance decreased with increased foliar stipple. Sensitive plants had lower photosynthetic rates for all leaves, except the very youngest and oldest when compared to tolerant plants. Stomatal conductance decreased with increasing leaf age, but no ozone-sensitivity differences were found. Lower leaves had less starch than upper ones, while leaves on sensitive plants had less than those on tolerant plants. These results show that ambient levels of ozone in Great Smoky Mountains National Park can adversely affect gas exchange, water use efficiency and leaf starch content in sensitive coneflower plants. Persistence of sensitive genotypes in the Park may be due to physiological recovery in low ozone years.

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1. Introduction

Great Smoky Mountains National Park (GRSM) is one 25 national parks that has experienced an increase in ozone (O_3) concentrations (Tong et al., 2006) despite a nationwide downward trend of nearly 20% over the past 30 years, based on hourly measurements (US EPA, 2000). Surveys of foliar injury have shown that nearly 6% of the flora in Great Smoky Mountains National Park, out of a total of ∼1600 species, exhibit putative O_3 symptoms in the field (Neufeld et al., 1992). After testing 30 species in open-top chambers (OTCs) for O_3 responses, 27 exhibited typical O_3 symptoms as seen in the field (Neufeld et al., 1992). Surveys by a growing number of investigators (Winner et al., 1989; Chappelka et al., 1997, 1999, 2003, 2007; Davison et al., 2003; Orendovici et al., 2003; Franzaring et al., 2000; Davis and Orendovici, 2006; Souza et al., 2006; Burkey et al.,

2006; Kline et al., 2008, 2009) have confirmed that foliar symptoms are common among wild plant species exposed to ambient O_3 concentrations in the eastern United States.

One of the most O_3-sensitive herbaceous species in GRSM is cutleaf coneflower, *Rudbeckia laciniata* (L.) (Neufeld et al., 1992; Chappelka et al., 2003; Davison et al., 2003; Finkelstein et al., 2004; Burkey et al., 2006). This perennial is typically found in moist, high elevational habitats, although it also grows at lower elevations if close to a source of water (Radford et al., 1968). There are five recognized varieties, and the species is widely distributed among 45 of the conterminous states (absent from CA, NV and OR; USDA Plant Database, 2004). Two of the varieties occur in GRSM: *R. laciniata* (L.) var. *laciniata* and *R. laciniata* (L.) var. *digitata* (Mill.) Fiori, a somewhat smaller plant than var. *laciniata* and commonly found at Purchase Knob (PK) on the North Carolina side of the Park (Cox and Urbatsch, 1990; Weakley, 2011).

Foliar injury does not usually occur until early July, but has been observed on basal rosette and lower flowering stem leaves as early as mid-May after acute O_3 episodes (Neufeld, pers. obs.). Over a season,

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injury tends to be greater on the lower flowering stem leaves (Chappelka et al., 2003; Roberts, 2007), mainly because these are older and have been exposed to more O_3. Neufeld et al. (1992) categorized this plant as "highly sensitive" after fumigating individuals in OTCs. Szantoi et al. (2009), working in Alabama, found similar results in an OTC experiment investigating the response of cutleaf coneflower to O_3 and ethylenediurea. However, Kline et al. (2009), in another OTC experiment, found cutleaf coneflower to be relatively tolerant to O_3 exposure, and attributed that to obtaining their material from a population native to a high O_3 area in Pennsylvania which may have contained only O_3-tolerant individuals.

Extensive genetic variability in O_3 sensitivity occurs in several wildflower species in GRSM, including the cutleaf coneflower, tall milkweed (Asclepias exaltata (L.)) and crownbeard (Verbesina occidentalis (L.)) (Chappelka et al., 1997, 2003; Davison et al., 2003; Souza, 2003; Souza et al., 2006; Burkey et al., 2006; Chappelka et al., 2007). Similar variation in sensitivity has also been reported for wildflowers in Europe (Davison and Barnes, 1998; Bassin et al., 2004). In GRSM, sensitive plants develop injury both sooner, and to a greater extent, than tolerant plants growing <1 m away (Grulke et al., 2007).

Ozone causes decreases in photosynthesis (A_n) and stomatal conductance (g_s) in a wide variety of native plant species (Reich, 1983; Grantz et al., 2003; Fiscus et al., 2005) including cutleaf coneflower (Grulke et al., 2007). This reduction in A_n may result from impaired photosynthetic electron transport, reduced amounts or activity of Rubisco, and/or lower rates of diffusion of CO_2 due to low g_s (Fiscus et al., 2005). Reductions in A_n may lead to a lower quantity of photosynthates in leaves (Darrall, 1989). Ozone also indirectly or directly inhibits photosynthate translocation (Grantz and Farrar, 1999, 2000; Fiscus et al., 2005), one consequence of which would be to reduce the root:shoot ratio (Hogsett et al., 1985; Grantz and Yang, 1996, 2000). Finally, O_3-induced leaf senescence, which shortens the timespan for CO_2 uptake, lowers the seasonal carbon assimilation potential of plants (Grantz and Yang, 2000; Grantz et al., 2003).

In this paper we report the results of gas exchange studies as affected by O_3-induced foliar injury over a range of leaf age/positions for cutleaf coneflower plants growing in a shaded forest understory. Our main objectives were to determine (1) leaf age-specific gas exchange responses in sensitive and tolerant individuals of cutleaf coneflower plants exposed to ambient levels of O_3, and (2) whether, as a consequence, there were any differences in the non-structural carbohydrate concentrations in leaves and rhizomes between plants of the two sensitivity classes. An earlier study by Grulke et al. (2007) on coneflowers growing in full sun, did report lower g_s for all leaf age/positions of sensitive compared to tolerant plants, but did not analyze A_n on these same leaves.

Our specific hypotheses were (1) comparably aged young leaves of sensitive plants (i.e., prior to injury) would have higher rates of A_n as a result of compensatory responses (Pell et al., 1994); (2) age/position related declines in gas exchange would be greater for sensitive plants (Pell et al., 1992), and (3) sensitive plants would have lower amounts of rhizomal TNC due to these O_3-induced reductions in A_n and/or translocation. For leaves, TNC amounts could either be transiently increased (Zheng et al., 2000) or decreased (Miller et al., 1995; Lux et al., 1997; Grantz and Yang, 2000; Braun et al., 2004) depending on the relative impacts of O_3 on A_n versus translocation.

2. Materials and methods

2.1. Site characteristics, O_3 indices and O_3-induced leaf injury rating

Our field site was located in GRSM at the Appalachian High Elevation Learning Center at Purchase Knob (PK), NC (Lat: 35°35′14″N, Long: 83°04′31″W, elevation 1494 m). This site supported large stands of coneflowers (R. laciniata var. digitata).

Temperature and precipitation data were obtained from the National Climatic Data Center in Asheville, NC for the Cataloochee Valley, GRSM, which was the nearest weather station to PK.

Ozone concentrations were measured by an electronic monitor (Model 49C, Thermo Environmental Instruments, Franklin, MA) in 2002 and 2003 by the North Carolina Division of Air Quality. Data reported are from May 1st to August 31st. Ogawa-type passive O_3 sensors were also used at Purchase Knob (Ogawa & Co. USA Inc., Pompano Beach, FL) and analyzed by the National Park Service in 2002 and 2003. Passive sensors monitor the total O_3 exposure (SUM00; sum of all hourly mean concentrations over the season) for the time period they are in the field and analysis is based on the colorimetric oxidation of sodium nitrite to sodium nitrate (Ray, 2001).

Passive sensors were placed on poles at two heights (2 m and 1 m) in two locations: (1) inside the forest where gas exchange measurements were conducted (see below) and (3) co-located atop the active monitor shed ~30 m from the forest at ~4 m in height. Sensors were analyzed biweekly from May 14th thru August 23rd in 2002 and June 24th thru August 27th in 2003.

Several O_3 exposure indices were calculated from these data, including the SUM00 (passive sensors and electronic monitor); SUM60 (the sum of all mean hourly concentrations ≥60 ppb; electronic monitor); and N80 (number of hours with mean concentrations ≥80 ppb; electronic monitor). Indices from the electronic monitor were adjusted for missing values according to the protocol of Lee et al. (1991). Electronic monitor measurements began May 1st and ended September 30th although exposures are only reported through August 31st, which corresponds to the main physiologically active period for these plants.

Ozone injury was assessed for leaves on the main flowering stem of plants used for gas exchange measurements. Injury in this species results in numerous, small, dark purple to brownish spots or stipples, on the adaxial surface of the leaves (Chappelka et al., 2003). We used a customized injury rating scale to categorize the percent leaf area with stipple: 1 = 0%; 2 = 1–6%; 3 = 7–25%; 4 = 26–50%; 5 = 51–75%; 6 = >75%.

2.2. Gas exchange

Photosynthesis (A_n) and stomatal conductance (g_s), were measured over a 10 day period beginning August 1st in 2002. Five plants classified as sensitive (3 or greater rating on foliar injury scale for any leaf on the flowering stem) and 5 tolerant plants (<3 maximum rating on foliar injury scale) were randomly selected for measurement. Plants were located along a trail in a forest stand composed primarily of yellow buckeye (Aesculus flava Solander) with an overstory leaf area index of ~2.5 m^2/m^2 as estimated using a plant canopy analyzer (model 2000, Li-Cor, Inc., Lincoln, NE). Sensitive and tolerant plants were within 1–2 m of each other.

Gas exchange was measured on all leaves (max = 9) on the main flowering stem beginning with the bottom (oldest) leaf, between 0900 and 1400 h. Preliminary measurements showed no appreciable declines in maximum A_n or g_s during this time interval (data not shown). Measurements alternated between sensitive and tolerant plants, and were conducted using a Li-Cor 6200 photosynthesis system equipped with the 1 L chamber (Li-Cor, Inc., Lincoln, NE). Preliminary experiments established that A_n saturated at a photosynthetic photon flux density (PPFD) of ~700 µmol m^{-2} s^{-1} so all subsequent measurements were made at a PPFD of 650–700 µmol m^{-2} s^{-1} using a 20 W halogen bulb. A heat-absorbing glass plate was placed between the light source and chamber, resulting in less than a 2 °C rise in leaf temperatures during measurements. Other environmental conditions were not controlled, but remained similar during measurements of replicate leaves. Leaf temperatures in the cuvette ranged from 23 to 27 °C, relative humidity varied between 50 and 70%, and external carbon dioxide concentrations ranged between 380 and 400 ppm. Leaves were allowed to acclimate for 5 min before any measurements were recorded. If repeated measurements of A_n or g_s differed by more than 10%, then leaves were given more time to equilibrate.

2.3. Total non-structural carbohydrates

Total non-structural carbohydrates (TNC) were determined at the end of the growing season in 2003 (mid-September, but prior to initiation of fall leaf senescence) for 8 of the plants used for gas exchange measurements the previous year at PK (4 sensitive, 4 tolerant). Each leaf on the flowering stem was scored for injury, bagged separately along with a sample from the rhizome, and then immediately stored in liquid nitrogen, before being freeze-dried later. Starch and soluble sugars were determined enzymatically by the UV method (R-Biopharm, Inc., Marshall, MI, USA), and were extracted from 25 mg samples using a solution of dimethylsulfoxide/HCl (see Booker, 2000 for details). Following neutralization and filtration, starch and soluble sugars were assayed according to kit instructions using amyloglucosidase to hydrolyze starch into glucose. Results are reported in units of mg glucose equivalents per g dry weight.

2.4. Statistical analyses

A variety of statistical methods were applied to the data to analyze for potential differences in gas exchange as a function of leaf age/position or injury. To assess the relationship between foliar injury and gas exchange, linear regression was used initially. However, since leaf age/position and injury were confounded, we separated

the plants by sensitivity class. Because leaf age/position is essentially a discrete variable (there cannot be a leaf 2.5 for example) we decided to use a categorical analysis (two-way univariate ANOVA) with sensitivity and leaf position as the main effects to analyze these results. All analyses were conducted using SAS (SAS Ver. 9.1, SAS Institute, Cary, NC).

Total non-structural carbohydrates in the leaves of sensitive plants at PK were grouped into upper (<category 3 injury rating) and lower (≥3 category injury rating) leaves, while those on tolerant plants were grouped by leaf positions comparable to the two injury categories on the sensitive plants. Concentrations of TNCs in each group were compared using a two-sample two-way *t*-test.

Data for all tests were normally distributed with homogeneous variances, so no transformations were necessary. For all statistical analyses, differences were considered significant if $p < 0.05$.

3. Results

3.1. Ozone exposures

Year 2002 was hotter and drier in June, July and September than 2003, while May and August were cooler (Table 1). Precipitation was greater in 2003 compared with 2002 for all months, especially May and June (Table 1).

Coincident with these weather differences, SUM00 O_3 exposures were greater in 2002 than 2003 (Table 2), while differences in the SUM60s and N80s were even larger. The SUM60 was 2.3× higher and the N80 9.8× higher in 2002 versus 2003. The greater SUM60s and N80s indicate that elevated O_3 concentrations were more frequent in 2002 than in 2003.

Ozone in the forested site at PK was measured by passive sensors at both 1 and 2 m above the ground (which covers the range in height of the coneflowers), and the results presented are the mean SUM00 estimates of these two heights (Table 2). For comparable sampling periods with the electronic monitor (May 14 thru August 23 for 2002; June 24 thru August 27 for 2003), the co-located passive sensors underestimated the SUM00 values of the electronic monitor by 7.3% in 2002 and 8.1% in 2003. Using these correction factors, and then adjusting to match the season length for the electronic monitor (May 1 thru August 31), the SUM00 (as measured by the passive sensors located in the forest) were estimated to be 136.6 ppm*hr and 120.3 ppm*hr in 2002 and 2003, respectively (Table 2). These values constitute ∼16% reductions in the SUM00s compared with the electronic monitor located 30 m away in an open field.

3.2. Gas exchange

When A_n and g_s were plotted as functions of leaf injury without regard to plant O_3 sensitivity, both declined linearly as leaf injury increased (Fig. 1). The wide scatter in rates at leaf injury rating 1 was due mainly to variability in rates among tolerant plants. Because these leaves had no foliar injury, this variability probably resulted from a leaf age influence. In sensitive plants, leaf injury and

Table 1
Temperature and precipitation totals for Purchase Knob in 2002 and 2003. ΔTemp and ΔPrecip = 2002 values−2003 values. Data obtained from nearest weather station in GRSM at Cataloochee Valley.

Month	Mean temperature (°C)			Precipitation (mm)		
	2002	2003	ΔTemp	2002	2003	ΔPrecip
May	15.3	18.3	−3.0	121	179	−58
June	19.3	19.0	0.3	41	109	−68
July	21.8	21.6	0.2	163	166	−3
August	22.0	22.6	−0.6	64	71	−7
September	19.5	16.7	2.8	*	*	*
Total[a]				106	110	−4

*September data not available.
[a] Precipitation values weighted by percent missing hours (e.g., if 5% of data missing, monthly totals multiplied by 1.05).

Table 2
SUM00, SUM06 (ppm*h) and N80 ozone indices[a] (h) from May 1 to August 31 in 2002 and 2003 using an electronic monitor and passive sensors at Purchase Knob.

Electronic monitor	Ozone indices		
	SUM00[b]	SUM60	N80
Year			
2002	162.6	96.4	273
2003	144.1	42.1	28
Passive sensor			
2002	136.6	−	−
2003	120.3	−	−

[a] SUM00 = sum of all hourly ozone concentrations; SUM060 = sum of all hourly ozone concentrations ≥0.060 ppm; N80 = number of hours ≥0.080 ppm. All indices for the electronic monitor adjusted for missing hours according to Lee et al. (1991).
[b] SUM00 for passive sensors adjusted to match length of season for electronic monitor (May 1 thru August 31) and tendency to underestimate co-located active monitor. For details see results for ozone measurement in text.

leaf age/position increased concomitantly, and were responsible for most of the observed decline. Since leaf injury and age/position were confounded, it was not possible to separate out the putative effects of O_3 on gas exchange from those of normal leaf senescence when the data were plotted in this manner. Therefore, we separated the data for sensitive and tolerant plants and plotted mean gas exchange rates versus leaf age/position (Fig. 2).

When leaf age/position was analyzed using means comparison (ANOVA) we found, contrary to our first hypothesis, that A_n of sensitive plants was lower ($p < 0.05$) for all leaves except the very youngest and the very oldest (which did not differ) compared to tolerant plants (Fig. 2a). Net photosynthesis of sensitive plants
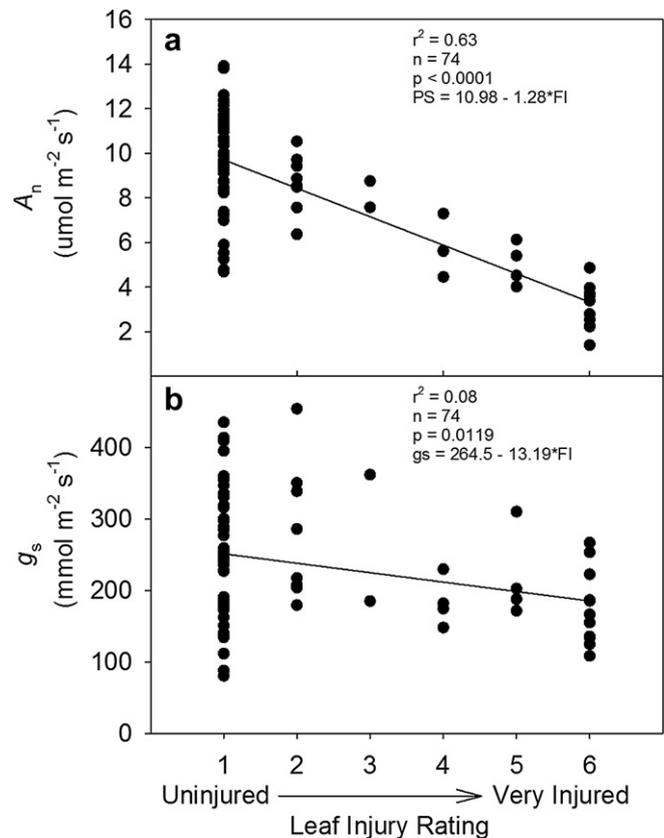
Fig. 1. (a) Net photosynthesis (A_n) and (b) stomatal conductance (g_s) versus leaf injury rating for 5 sensitive and 5 tolerant plants at Purchase Knob. FI = Foliar Injury Rating.
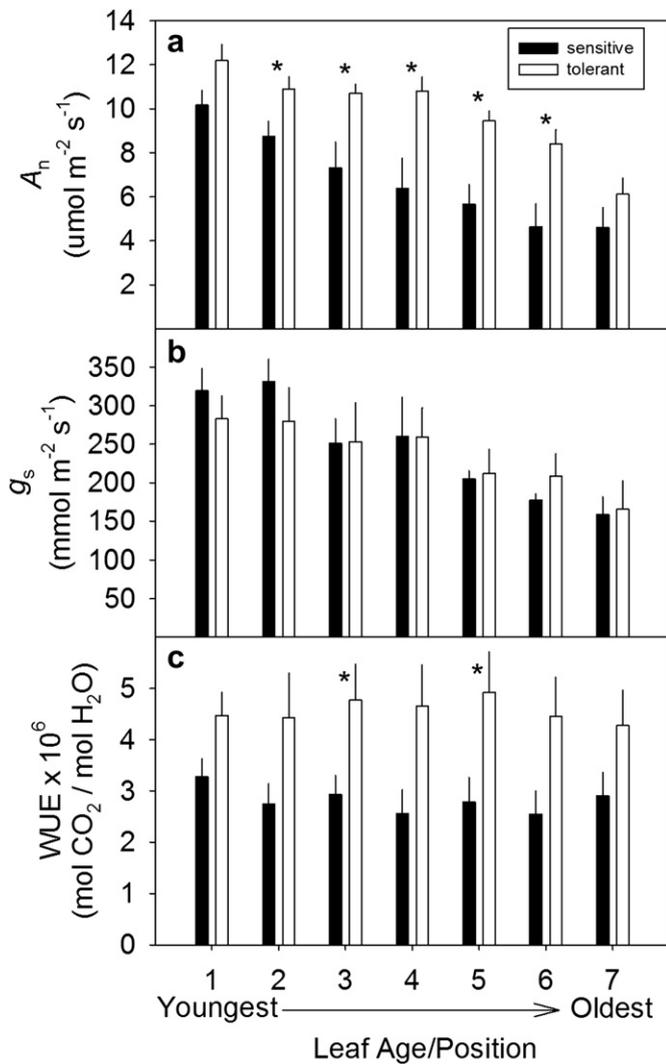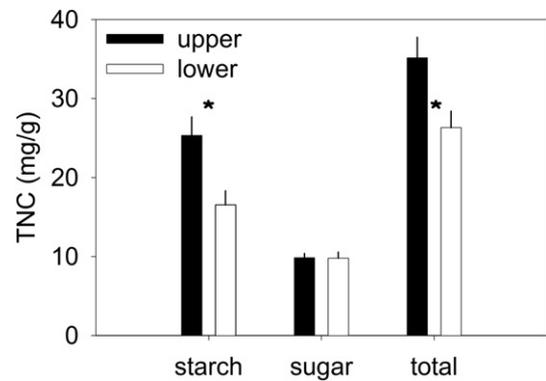
Fig. 2. Comparisons of (a) net photosynthesis (A_n), (b) stomatal conductance (g_s) and (c) water use efficiency (WUE) by leaf age/position for sensitive and tolerant plants at Purchase Knob. Asterisks indicate significant differences ($p < 0.05$) between sensitive and tolerant plants of each leaf position. Bars are mean ± se. $n = 5$.

Fig. 3. The effects of leaf age/position on starch and soluble sugar content (TNC) in leaves at Purchase Knob. Upper leaves are stem leaves on the top half of the plant while lower leaves are stem leaves on the bottom half of the plant. * = significance at $p < 0.05$. $n = 4$.

4. Discussion

Both O_3-sensitive and -tolerant cutleaf coneflower plants exhibited typical age-related reductions in A_n and g_s (Reich, 1984; Sobrado, 1994; Clark et al., 1996; Zhang et al., 2001; Kitajima et al., 2002). Leaves with O_3-induced stipple, though, had substantially lower A_n when compared to asymptomatic leaves of equivalent age/position, in contrast to g_s, where such differences were not detected. These declines in A_n relative to those in tolerant plants (which we interpret as being primarily due to O_3) began in leaves of sensitive plants as young as leaf age/position 2, when foliar injury was still minimal. This indicates that even small amounts of stipple can be associated with significant declines in carbon assimilation in this species.

averaged 51% less across all leaf age/positions and ranged from 23% less at leaf/age position 2 to 83% less at leaf/age position 7. Stomatal conductance did not differ between sensitive and tolerant plants across any leaf age/position (Fig. 2b), and did not begin declining until leaf/age position 5. Conductance eventually decreased by nearly two-thirds at the highest foliar injury level. Mean WUE were consistently lower for sensitive as compared to tolerant plants across all leaf age/positions (Fig. 2c), but statistically significant differences only occurred at leaf age/positions 3 and 5.

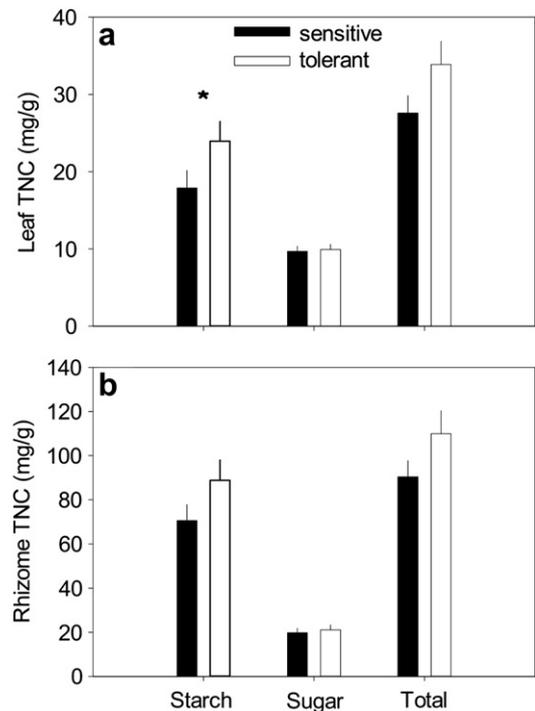### 3.3. Total non-structural carbohydrates

Lower leaves had significantly less starch than upper leaves regardless of injury level (Fig. 3, $p = 0.01$). Starch concentrations were decreased in leaves from sensitive plants as compared to tolerant plants (Fig. 4a, $p = 0.04$), but there were no differences in rhizome starch concentrations (Fig. 4b, $p = 0.17$). Differences in TNC were due entirely to changes in starch levels, as there were no differences in soluble sugars regarding any comparisons tested.

Fig. 4. The effects of ozone injury on starch and soluble sugar content (TNC) in (a) rhizomes and (b) leaves of sensitive and tolerant plants. * = significance at $p < 0.05$. $n = 4$.

The basis for the decline in A_n, though, is not well understood. Brendley and Pell (1998) reported that Rubisco concentrations decreased at a faster rate with leaf age for hybrid poplar (*Populus maximowizii* A. Henry x *trichocarpa* Torr. & A/Grau) exposed to chronic O_3 than in plants grown in charcoal-filtered air. These authors suggested that since Rubisco synthesis rates were unaffected by O_3, increased proteolysis was responsible for the decline. Decreased Rubisco activity and content due to O_3 exposure is often reported (Fiscus et al., 2005; Long and Naidu, 2002). In soybean (*Glycine max* (L.) Merr.) seedlings treated with 120 ppb O_3 for three days, expression of proteins associated with photosynthesis were mostly downregulated while proteins associated with antioxidant, carbon, nitrogen and secondary metabolism were mostly upregulated (Ahsan et al., 2010). This could lead to decreased availability of substrates for metabolism and growth processes, suggesting that starch catabolism is required to supplement demand (Ahsan et al., 2010). Similar effects of O_3 on photosynthesis protein expression has been found in proteomic studies with rice, wheat, bean, maize and poplar (Cho et al., 2011). It seems likely that similar processes are occurring in sensitive coneflowers. In contrast to sensitive plants, tolerant plants were able to maintain near maximum rates of A_n among a larger cohort of leaves, possibly because the lack of O_3 sensitivity delayed the onset of O_3-induced photosynthesis inhibition and premature leaf senescence (Coleman et al., 1995; Grulke et al., 2007; Long and Naidu, 2002). Grulke et al. (2007) suggested that O_3-sensitive plants were genetically more susceptible to O_3 injury than tolerant plants, and that sensitivity was not related to differences in O_3 uptake between the plant lines. Our measurements of g_s would support this finding.

In a previous study, Grulke et al. (2007) performed gas exchange analyses on pairs of sensitive and tolerant individuals growing in full sun at PK, and showed that leaves with extensive foliar injury had substantially lower A_n, g_s and quantum efficiency, higher leaf CO_2 compensation point, and impaired stomatal responsiveness to environmental stimuli (e.g., sluggish stomata; McAinsh et al., 2002; Paoletti and Grulke, 2010) compared to leaves with little or no foliar injury. These measurements were concentrated mainly at leaf age/position 4. However, g_s was measured over a range of leaf age/positions and across all leaf positions was lower for sensitive plants, even in the absence of visible injury (see Fig. 4; Grulke et al., 2007). This differed from our study in which there were no statistical differences between sensitive and tolerant plants at any leaf position, including asymptomatic leaves of sensitive and tolerant plants.

Leaf anatomical features, such as stomatal densities or greater internal air spaces and cell sizes, which have been suggested by some researchers to be linked to increased sensitivity (Bennett et al., 1992; Evans et al., 1996; Plöchl et al., 2000) do not differ between sensitive and tolerant genotypes in this species (Dolan, 2011). Furthermore, leaves of both sensitive and tolerant coneflower plants have negligible quantities of reduced apoplastic ascorbate, while the intercellular wash fluid from injured leaves shows a significantly greater total antioxidant capacity than that for uninjured leaves (Burkey et al., 2006). Leaves with higher antioxidant capacity also had more injury, suggesting a relationship between synthesis and accumulation: whether these differences in extracellular antioxidant capacity serve to distinguish sensitive and tolerant genotypes of coneflowers remains unknown at this time.

Some studies suggest greater plant sensitivity to O_3 in the shade (Topa et al., 2001; Wei et al., 2004). Roberts (2007) studied these same plants in 2004 (a particularly wet year) and found no statistically significant differences in foliar injury on plants in the forest compared to those in the field, although it should be noted that this was a near record low O_3 year, and maximum levels of injury were modest. Interestingly, using a Li-Cor model 1600 steady state porometer, she found that maximum g_s was lower (320 ± 25 mmol m^{-2} s^{-1}) for well-watered plants in the forest compared to those in the field (450 ± 29 mmol m^{-2} s^{-1}; $p < 0.05$).

There was no evidence of a compensatory photosynthetic response in the youngest leaves of sensitive plants, as found under more controlled conditions with other plant species (Pell et al., 1994). This, combined with the O_3-induced enhancement of leaf senescence and declines in A_n for the other leaves strongly suggests that total net carbon uptake by sensitive plants relative to tolerant plants is reduced by exposure to ambient levels of O_3 currently occurring in GRSM.

Leaves on cutleaf coneflower plants that had level 5 or 6 injury at Clingman's Dome (another high elevation site in GRSM) exhibited a 61% decrease in A_n relative to leaves without visible injury (Neufeld, unpublished data), which is comparable to the differences found at PK. These fairly large reductions occurred during the relatively lower ambient O_3 exposure of year 2003 and further corroborate our earlier findings (Neufeld et al., 1992; Chappelka et al., 2003; Davison et al., 2003; Burkey et al., 2006; Roberts, 2007) as well as those in this study, that certain individuals of these plants (both var. *digitata* and var. *laciniata*) are extremely sensitive to ambient O_3.

The greater decline in A_n with increasing foliar injury in sensitive plants, coupled with the lack of a difference in g_s among the two sensitivity classes, resulted in a reduced WUE for sensitive plants compared to tolerant ones. Although only two leaf positions exhibited a statistically significant reduction in WUE (Fig. 2c), the pattern is clear that sensitive plants tend to lose more water per unit of carbon assimilated. However, decreased WUE resulted from declines in A_n rather than increases in g_s, so absolute water loss would not change. Any O_3-induced water stress would more likely result from sluggish stomata (Grulke et al., 2007), which would allow more water loss from sensitive plants than tolerant ones, especially considering that cutleaf coneflowers favor moist sites (Radford et al., 1968), are shallowly rooted (Roberts, 2007) and their stomata are highly sensitive to VPD (Davison, unpublished results). McLaughlin et al. (2007a) and Paoletti and Grulke (2010) reached a similar conclusion for plants from a variety of physiognomic classes, and McLaughlin et al. (2007b) even extended such observations to landscape level hydrological impacts.

The loss of photosynthetic capacity in this species, coupled with the lack of a compensatory response in younger, asymptomatic leaves must ultimately result in a reduced supply of photosynthates over the growing season. Because lower leaves (which are the more severely injured) would tend to translocate most of their carbon belowground (Pate et al., 1979; Coleman et al., 1995; Jahnke et al., 1998) and O_3 is known to impair translocation of photosynthates (Grantz and Farrar, 1999, 2000; Zheng et al., 2000; Fiscus et al., 2005) it is possible that roots and rhizomes are being adversely affected in these plants. However, it is difficult to measure belowground growth responses for perennial wildflowers in the field and confirmation of these impacts would require studies under more controlled conditions. Peoples (2005) did not find any significant impacts on root growth when cutleaf coneflowers were exposed in small open-top chambers to non-filtered and filtered air, but the levels of exposure were fairly low. Szantoi et al. (2009), in contrast, did find reduced root growth in potted plants exposed in OTCs to $2\times$ ambient O_3 concentrations, as well as alterations in cell wall chemistry such as reduced digestibility and increased lignin and N.

As we predicted, there was a significant reduction in starch in the leaves of sensitive plants, which corroborates findings by other researchers (Miller et al., 1995; Lux et al., 1997; Grantz and Yang, 2000; Braun et al., 2004), but differs from Zheng et al. (2000) who found increased amounts in *Plantago major* (L.) leaves due to impaired export. This suggests that by the time we measured these leaves, A_n was impacted relatively more so than reductions in translocation, resulting in a net decrease in starch in leaves affected by O_3.

We did not find any reduction though, of TNCs in the rhizomes, yet several researchers have found reduced starch amounts in the roots of trees growing in O_3-polluted environments (Grulke et al., 2001; Thomas et al., 2002). The lack of an effect in cutleaf coneflower rhizomes may have been due to a number of causes, including a) small sample size and consequent low statistical power, b) because plants were less affected in 2003 than in 2002 due to the lower O_3 exposure that year, and c) reduced rhizome growth may have eliminated the growth dilution effect on the TNCs. Unfortunately, we were not able to sample plants in 2002 because of the need to make additional physiological measurements on them in 2003. The lower exposure in 2003 may have allowed the plants to recover from past O_3-induced reductions in TNCs, and eliminated the differences between sensitive and tolerant plants. It is also possible that several consecutive years of elevated O_3 may be required before detectable reductions in rhizome TNCs can be found in these perennial wildflowers (Renaud et al., 1997). If ambient concentrations reduce TNC production during high O_3 years, this could adversely affect growth (Andersen et al., 1991), both sexual as well as asexual reproduction (Zheng et al., 2000; Bender et al., 2006; Darbah et al., 2008), and increase susceptibility to cold stress (Lucas et al., 1987; Ranford and Reiling, 2007).

The large O_3-induced decreases in A_n measured in this study might lead one to suggest that sensitive genotypes of this species should be selectively disadvantaged compared to tolerant genotypes, and eventually eliminated from the landscape (Davison and Reiling, 1995). For example, as we noted earlier, coneflowers subjected to O_3 fumigation in Pennsylvania by Kline et al. (2008) were relatively tolerant and showed few symptoms. These plants had been collected from an area with a history of high O_3 concentrations, leading these authors to suggest that there might have been selection against sensitive genotypes that left only tolerant ones. However, persistence of O_3-sensitive genotypes of perennial wildflowers in GRSM may be facilitated by the variability in exposures that occur from year to year (Tong et al., 2006). This may allow a temporary relaxation of the selective pressures, and perhaps even physiological recovery in low O_3 years, like those in 2003, 2004 and 2009, with this last year being a record low year in the Park in terms of the SUM60 exposure index (US National Park Service, 2010). If intervals between high exposures are long and occur frequently enough, sensitive genotypes might be able to persist indefinitely in the wild, particularly if in the absence of O_3 these genotypes perform equally or better than tolerant genotypes (Bell et al., 1991) due to potential physiological "costs" associated with O_3 resistance in tolerant plants (Pitelka, 1988). However, if O_3 concentrations increase again, as is predicted by several models (Fowler et al., 1999; Prather et al., 2003; Vingarzan, 2004), then selection against these sensitive genotypes may intensify, and their persistence could be jeopardized (Dunn, 1958; Barnes et al., 1999; Wolff et al., 2000). The paradoxical consequence of chronically high O_3 exposures coupled with the elimination of sensitive wildflower genotypes is that plants in the Park would actually appear healthier because only tolerant genotypes with little expression of foliar injury would remain. However, populations of cutleaf coneflower in the Park would be genetically less diverse and possibly at greater risk to other stressors, especially if O_3 sensitivity and stress resistance are linked (Koch et al., 1998).

Acknowledgments

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