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Physiological and foliar injury responses of *Prunus serotina*, *Fraxinus americana*, and *Acer rubrum* seedlings to varying soil moisture and ozone

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"Capsule": *High soil water availability favors ozone uptake, increases foliar injury, and exacerbates the negative ozone effect on gas exchange of seedlings of deciduous tree species.*

Abstract

Sixteen black cherry (*Prunus serotina*, Ehrh.), 10 white ash (*Fraxinus americana*, L.) and 10 red maple (*Acer rubrum*, L.) 1-year old seedlings were planted per plot in 1997 on a former nursery bed within 12 open-top chambers and six open plots. Seedlings were exposed to three different ozone scenarios (ambient air: 100% O_3; non-filtered air: 98% ambient O_3; charcoal-filtered air: 50% ambient O_3) within each of two different water regimes (nine plots irrigated, nine plots non-irrigated) during three growing seasons. During the 1998 and 1999 growing season, leaf gas exchange, plant water relations, and foliar injury were measured. Climatic data, ambient- and chamber-ozone-concentrations were monitored. We found that seedlings grown under irrigated conditions had similar (in 1998) but significantly higher gas exchange rates (in 1999) than seedlings grown within non-irrigated plots among similar ozone exposures. Cherry and ash had similar ozone uptake but cherry developed more ozone-induced injury ($<34\%$ affected leaf area, *LAA*) than ash ($<5\%$ *LAA*), while maple rarely showed foliar injury, indicating the species differed in ozone sensitivity. Significantly more severe injury on seedlings grown under irrigated conditions than seedlings grown under non-irrigated conditions demonstrated that soil moisture altered seedling responses to ambient ozone exposures.

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Keywords: Ozone; Soil moisture; Open-top chamber; Foliar injury; Physiological response

1. Introduction

Tropospheric ozone air pollution poses a serious threat to the health and productivity of our forest ecosystems (US EPA, 2000; Skelly, 2000). Ozone enters plants through leaf stomata, diffuses into the cellular membrane, and enters into metabolic processes (Reich, 1987). Therefore, ozone injury is related not only to stomatal conductance but also to biochemical processes within the leaf. For example, Fredericksen et al. (1996) reported that ozone-induced foliar injury on cherry seedlings was negatively correlated to net photosynthesis (P_n) but not to stomatal conductance (g_{wv}). Samuelson (1994) reported that visible foliar injury was accompanied by reduced P_n and g_{wv}, indicating that ozone reduced P_n not only through reductions in g_{wv}, but also through reductions or limitations in leaf biochemical processes within the mesophyll.

Furthermore, because many environmental factors (such as soil water content, light, etc.) influence stomatal

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conductance, visible injury of some species grown under the same ozone exposure can vary due to microclimate differences. Studies in the Shenandoah National Park in Virginia (SHEN) and the Great Smoky Mountains National Park (GSMNP) in North Carolina have documented a positive correlation of visible foliar injury to cumulative ozone exposures on black cherry and white ash under ambient 12-h average ozone concentrations of 45–60 ppb. Foliar injury was closely associated with SUM60 cumulative ozone concentrations (Chappelka et al., 1992; Neufeld et al., 1995; Fredericksen et al., 1996; Skelly et al., 1996). Hildebrand et al. (1996) observed foliar ozone-induced injury during the 1991–1993 seasons to be associated with site differences; trees on putatively wetter sites experienced higher ozone injury compared to trees on drier sites with two-thirds of the trees on wet sites being consistently more symptomatic than the trees on the dry sites. Various other studies with hybrid poplar (Harkov and Brennan, 1980) and with field crops (Dean and Davis, 1967; Olszyk and Tibbitts, 1981; Tingey et al., 1982; Showman, 1991; Fuhrer, 1995) have shown greater visible ozone injury on plants grown in moist soil over those grown in drier soil.

Black cherry, white ash, and red maple are major components in the mid-Atlantic temperate hardwood forest and valued for commercial wood products and wildlife browse (Marquis, 1990; Schlesinger, 1990; Walters and Yawney, 1990). Black cherry and white ash have been shown to be amongst the most sensitive eastern hardwood tree species to tropospheric ozone as based on visible foliar injury (Steiner and Davis, 1979; Davis and Skelly, 1992; Simini et al., 1992; Skelly et al., 1992, 1996; Neufeld et al., 1995; Hildebrand et al., 1996; Lee et al., 1999, 2002). Because of its sensitivity to ozone, black cherry is considered to be a field bioindicator species (Chappelka et al., 1999a; Skelly, 2000). Within eastern forests, red maple has become a highly competitive 'super-generalist' (Abrams, 1998); the species is considered to be relatively tolerant to ambient ozone exposures (Davis and Skelly, 1992; Simini et al., 1992).

The objective of the current research was to determine the interrelationships between soil moisture, ozone exposure and leaf physiological responses that may lead to differences in ozone injury on two 'ozone-sensitive' and one 'ozone-tolerant' hardwood tree species grown under controlled open-top chamber conditions.

2. Materials and methods

2.1. Study site and experimental design

Twelve open-top chambers (Heagle et al., 1973) and six open plots were established during early summer 1997 within Penn Nursery as located in central Pennsylvania (40°46′ N, 77°37′ W, elev. 466 m). Experiments were conducted using two controlled soil moisture regimes and three ozone exposure treatments. Each plot was randomly assigned one of two levels of soil moisture (irrigated and non-irrigated) and one of three ozone exposures. The three ozone treatments involved 50% ambient ozone via charcoal-filtered air supplied to the open-top chambers, 98% ambient ozone via non-filtered open-top chambers, and 100% ambient ozone in open plots. Within each plot, 36 (16 cherry, 10 ash, and 10 maple) 1-year-old bare-root seedlings were planted in early August 1997. The seed source for white ash and red maple were random nursery seedlings of unknown origin. The seed source for black cherry originated from the nursery seed orchard and seedlings were selected for uniformity in size and coloration (Lee et al., 1999, 2002). The seedlings were planted using a repeated pattern by species, ca. 30 cm apart within two concentric circles resulting in a 1/2 of the plot occupied by cherry, and $\frac{1}{4}$ each by ash and maple respectively. The outer circle consisted of 22 trees and the inner circle consisted of 14 trees.

2.2. Soil moisture treatment

Soil moisture was controlled via two levels of watering. In addition to natural precipitation, the irrigated plots were frequently watered using drip irrigation at each seedling and as connected to the nursery's water supply. Soil moisture measurements were taken every 4–7 days during 1998 and continuously recorded with a data logger every 2 h during the 1999 season with three Gypsum Soil Moisture Probes (Soil Moisture Tester Model KS-D1, Delmhorst Instr. Co., Towaco, NJ) per plot; moisture probes were placed at depths of 10–20 cm in 1998 and 25–30 cm in 1999. Depending on the meteorological conditions and based on the soil moisture measurements the irrigated plots were watered every 2 weeks up to twice a week to maintain soil moisture close to field capacity; i.e. soil water potential of approximately 0 MPa.

2.3. Ozone and meteorological monitoring

Ambient ozone concentrations were monitored at a station within 50 m of the open-top chamber facility. Ozone concentrations within each chamber were continuously monitored at a 1 m height at the center of each chamber using a solenoid sampling design (Simini et al., 1992). Data were collected using the guidelines of the Pennsylvania Department of Environmental Protection, Bureau of Air Quality and the United States Environmental Protection Agency. Meteorological data inclusive of temperature (°C) and precipitation (mm) were recorded using a Campbell meteorological data system (Campbell Scientific Inc., Logan, UT).

2.4. Physiological measurements

During the 1999 growing season, two Pressure Chambers (PMS Instruments, Inc., Corvallis, OR) were used to measure leaf water potential (ψ_L). Daytime (09:00–16:00 EST) ψ_L was measured eight times from six randomly selected leaves per treatment and species. During the driest period of the 1999 growing period, predawn (01:00–04:00 EST) ψ_L was recorded twice in addition to the daytime ψ_L in order to determine if seedlings were experiencing drought stress. Leaf gas exchange measurements were measured from 19 May to 13 September 1998 and from 4 June until 13 September 1999 with two LiCor 6200 portable photosynthesis systems (LiCor Inc., Lincoln, NE). The two LiCor 6200 systems were cross-calibrated to one another and to known concentrations of CO_2 (0 and 360 ppm) at the beginning of each measurement day.

In 1998, 13 gas exchange measurement periods were conducted every 6–13 days. For each gas exchange measurement period (09:00–12:00 EST) and (13:00–16:00 EST), one randomly selected, upper, full sun exposed leaf from eight randomly selected cherry, and from five selected ash or maple seedlings was used per plot. In 1999, nine measurement periods were conducted every 5–19 days, depending upon weather conditions. Due to the increased number of leaves per seedling available as well as increased stem height and a shading effect, one upper crown and one lower crown leaf was selected from four randomly selected seedlings per species and plot. Seedlings were chosen from six non-irrigated plots and from six irrigated plots representing two replicates across three ozone- and two soil moisture-treatments. During 1998, a total of 3744 cherry leaves, 2016 ash leaves, and 1440 maple leaves and in 1999, 1728 leaves per species were measured. Physiological measurements were limited to certain environmental conditions and restricted to a previously determined response range in order to avoid outliers and values recorded under extreme ambient conditions. Measurements taken at light levels below in situ photosynthetically saturated light intensity (PAR < 600 μmol m^{-2} s^{-1}) and high ambient CO_2 concentrations were eliminated from our analysis.

For the monthly cumulative foliar ozone uptake (U), the products of monthly 7-h (09:00–16:00 EST) average of g_{wv} and the accumulative monthly 7-h ozone exposure (SUM0) were added for the period between full leaf expansion until the end of the vegetation period (Zhang et al., 2001). An estimate of the seasonal U was obtained by multiplying the seasonal mean of g_{wv} by the seasonal 7-h SUM0 ozone exposure. The values were then corrected to conductance to ozone by dividing by 1.68, the ratio of diffusivities of ozone and water (Reich, 1987; Wieser and Havranek, 1993). Ozone concentrations in the intercellular space were assumed to be zero (Laisk et al., 1989).

2.5. Visible foliar injury induced by ozone

Two ratings of percentage adaxial stipple were estimated visually on each seedling every 10–14 days: (i) The percentage of leaves showing foliar injury estimated in 5% classes and (ii) the Horsfall–Barratt rating system (Horsfall and Barratt, 1945) as modified by Nash et al. (1992) for affected area were used to determine an average percent of leaf area symptomatic of ozone injury. Ratings were made from the time of full leaf expansion in mid May until early September (18 weeks).

2.6. Data analysis

All data were tested for normality and homogeneous variance before the analysis of variance was performed (SAS Inc., 1989). For the gas exchange data set, values outside of determined plant physiological response limits were omitted, resulting in an unbalanced data set. A split–split plot design analysis was employed for leaf water potential, leaf gas exchange and foliar injury data using the General Linear Model procedure of the Statistical Analysis System with soil moisture as the main plot, ozone exposure as the subplot and species as the sub-subplot. Repeated measures analysis of the gas exchange data resulted in a loss of data due to unbalanced observations and was not used.

3. Results

3.1. Ozone exposure

Seasonal 24-h ambient ozone concentrations averaged 42.1 ppb in 1998 and 40.6 ppb in 1999 (Table 1). The ozone exclusion treatment resulted in 54.6 and 92.9% of ambient ozone for charcoal-filtered, and non-filtered chambers below approximately 85 ppb. Above 85 ppb ambient ozone concentrations, the charcoal filtration efficiency was not found to be proportional and filtration may have been below 50%. One-hour peak values of ambient ozone were slightly higher in 1998 compared to 1999. The highest 1-h peak in 1998 was recorded on 13 May at 113 ppb ozone and in 1999 on 17 August at 109 ppb ozone. During the 1998 and 1999 seasons, seedlings within non-filtered chambers were never exposed to ozone concentrations above 111 ppb hourly average and seedlings within charcoal-filtered chambers were never exposed to concentrations above 62 ppb ozone hourly average.

3.2. Environmental conditions and soil moisture

From May to September the monthly total precipitation was lower in 1998 than in 1999 but individual monthly totals varied between years (Table 2, Fig. 1).

Table 1
Monthly and seasonal average (ppb) and cumulative ozone exposures (ppm h) based on 1-h measurements within the open-top chamber exclusion treatments and ambient open plots during the 1998 and 1999 seasons from May[a] to September at Penn Nursery, Bureau of Forestry, Centre County, PA

Statistics	Ambient (AA)		Non-filtered (NF)		Charcoal-filtered (CF)	
	1998	1999	1998	1999	1998	1999
May[a]						
24-h average	45.9	46.6	40.6	44.2	28.0	25.0
7-h average	56.7	52.7	51.6	50.6	36.9	30.1
1-h peak	113.0	89.0	111.0	89.0	57.0	62.0
24-h Sum0	27.9	21.3	25.0	20.2	14.7	11.2
24-h Sum60	12.1	7.2	8.7	6.0	0.0	0.1
June						
24-h average	38.4	43.0	35.5	39.0	19.3	19.0
7-h average	45.9	49.4	43.0	45.6	25.0	23.5
1-h peak	89.0	100.0	82.0	95.0	45.0	41.0
24-h Sum0	28.8	31.0	25.3	28.6	13.5	12.7
24-h Sum60	3.1	10.5	2.1	8.1	0.1	0.1
July						
24-h average	39.1	43.5	36.1	39.0	17.2	18.0
7-h average	51.7	53.2	48.1	48.5	26.2	22.0
1-h peak	98.0	98.0	92.0	87.0	46.0	36.0
24-h Sum0	33.7	32.4	26.8	29.3	12.8	12.7
24-h Sum60	7.6	11.3	6.2	7.8	0.0	0.0
August						
24-h average	45.2	37.0	42.2	34.0	24.7	17.0
7-h average	57.5	40.7	53.8	38.0	32.3	18.9
1-h peak	104.0	109.0	97.0	103.0	60.0	52.0
24-h Sum0	33.7	26.9	31.4	25.1	18.3	12.0
24-h Sum60	12.4	5.1	10.0	3.9	0.1	0.0
September						
24-h average	42.1	33.0	39.3	32.0	20.5	16.0
7-h average	52.0	36.5	48.5	35.3	28.2	17.7
1-h peak	96.0	76.0	60.0	73.0	31.0	35.0
24-h Sum0	30.3	23.6	28.5	22.8	14.9	11.5
24-h Sum60	11.1	1.5	8.8	1.3	0.0	0.0
Season						
24-h average	42.1	40.6	38.7	37.6	21.9	19.0
7-h average	52.8	46.5	49.0	43.6	29.7	22.4
1-h peak	113.0[b]	109.0[c]	111.0[b]	103.0[c]	60.0[d]	62.0[e]
24-h Sum0	154.4	135.2	137.0	126.0	74.2	60.1
24-h Sum60	46.3	35.6	35.8	27.1	0.2	0.2

[a] Beginning on 6 May 1998 and 13 May 1999.
[b] 13 May 1998.
[c] 29 May 1999.
[d] 21 August 1998.
[e] 17 August 1999.

Table 2
Monthly average (μ) and standard error (S.E.) of air temperature (°C), precipitation (mm), and soil water potential (−MPa) from the 1998 and 1999 seasons for the open-top chamber research facility at Penn Nursery, Bureau of Forestry, Centre County, PA

Month	Year	Temp. (°C)	Precipitation (mm)	SWP (−MPa)			
				Wet		Dry	
				μ	S.E.	μ	S.E.
May	1998	16.7	91.7	0.072	0.002	0.070	0.002
	1999	21.2[a]	68.6[a]	0.028	0.000	0.028	0.000
June	1998	18.9	142.2	0.069	0.002	0.072	0.006
	1999	18.3[b]	69.6[a]	0.027	0.000	0.066	0.002
July	1998	19.7	50.8	0.074	0.003	0.066	0.001
	1999	22.3	77.9	0.025	0.000	0.182	0.005
August	1998	17.5	94.0	0.070	0.000	0.065	0.002
	1999	19.1	137.2	0.026	0.000	0.313	0.007
September	1998	16.8	50.8	0.068	0.001	0.129	0.025
	1999	16.0	161.0	0.026	0.000	0.033	0.000
Average	1998	17.9	85.9	0.071		0.080	
	1999	19.4	102.9	0.026		0.124	
Total	1998		429.5	0.071	0.001	0.081	0.006
	1999		514.3	0.026	0.000	0.142	0.002

[a] Pennsylvania State Climatology Center, State College.
[b] 10–30 June 1999.

During the early season of 1999, precipitation was lower in May and June and higher in July, August, and September as compared to 1998. Within the non-irrigated plots in 1998, soil water potential (ψ_{s}) values differed between non-irrigated and irrigated soil moisture treatments only towards the end of the season in late August and throughout September and they never exceeded −0.2 MPa. During the 1999 season, three significant cycles of dry-down were evident where ψ_{s} values were as low as −0.8 MPa in early August (Fig. 1). The soil water content of the irrigated plots was kept close to field capacity and ψ_{s} values stayed close to −0.1 MPa in both years. The seasonal average temperature was higher in 1999 compared to 1998. Thus, for the early growing-season of 1999, weather conditions were on average hotter and substantially drier than for the same time period in 1998 (Table 2).

3.3. Leaf water potential

Cherry, ash, and maple responded specifically to the soil moisture treatment. Seedlings responded most dramatically to the differing soil moisture conditions during predawn measurements of ψ_{L} (Fig. 2). During predawn, seedlings grown on the non-irrigated plots showed a more negative seasonal average of ψ_{L} than seedlings grown on the irrigated plots for all three species in 1999. Differences in predawn ψ_{L} between non-irrigated and irrigated plots were lowest for maple with −0.17 MPa, followed by cherry with −0.31 MPa and maple with

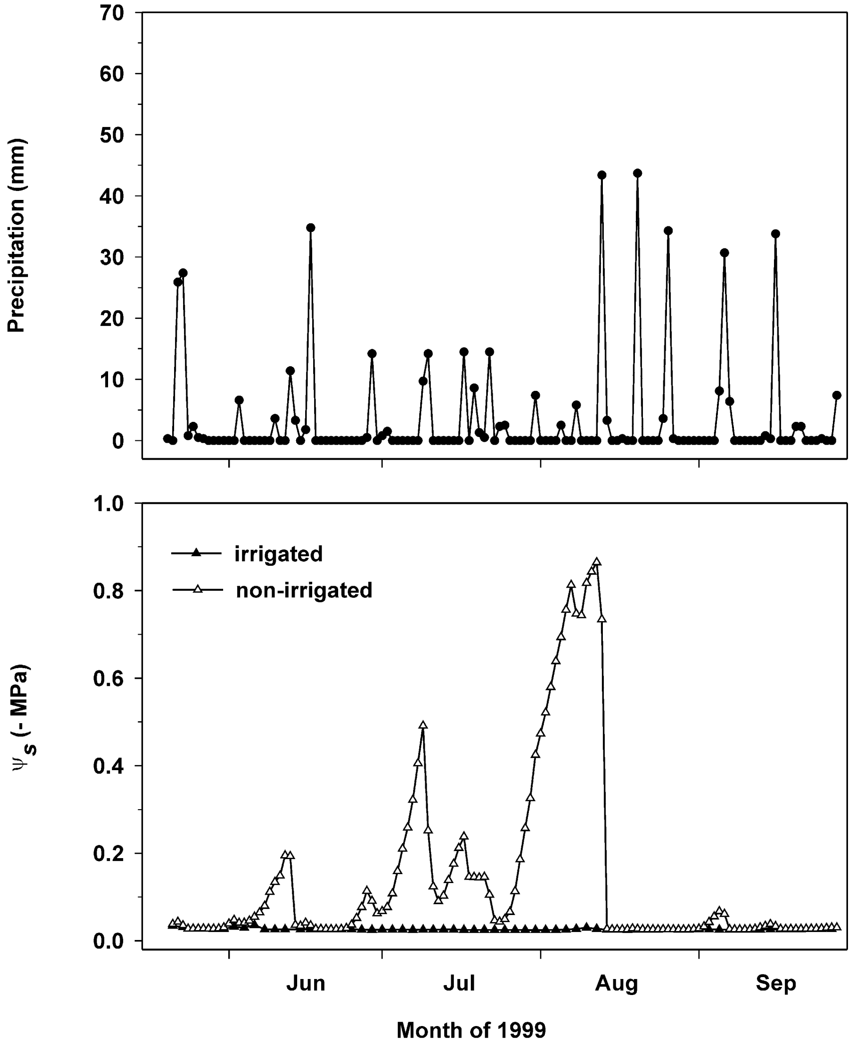

Fig. 1. Seasonal precipitation (mm) and soil water potential (Ψ_s) measured at a rooting depth of 25–30 cm during the 1999 seasons for all species exposed to irrigated and non-irrigated soil moisture conditions at the open-top chamber research facility at Penn Nursery, Bureau of Forestry, Centre County, PA.

−0.32 MPa. Ash expressed the most negative predawn ψ_L of −0.59 MPa, followed by cherry with −0.48 MPa and maple with −0.36 MPa on the non-irrigated plots. Despite the more negative ψ_L measured during predawn on the non-irrigated plots, the differences completely disappeared by mid-afternoon and there were no differences in ψ_L among the species and between the seedlings grown under irrigated or non-irrigated soil moisture conditions (Fig. 2).

3.4. Physiological gas exchange

Due to insignificant soil moisture differences between non-irrigated and irrigated plots in 1998, a soil moisture effect on stomatal conductance (g_{wv}) and net photosynthesis (P_n) was only detectable in 1999 for all three species (Tables 3 and 4). As differences in soil moisture increased from early June to mid August 1999, seedlings grown under irrigated conditions showed higher gas exchange than those grown under non-irrigated conditions ($P < 0.05$, Table 3, Fig. 3). Cherry exhibited the highest gas exchange followed by ash and maple. Across all species and ozone exposures, stomatal conductance was 15% and net photosynthesis was 23% higher for seedlings grown on the irrigated plots compared to the seedlings grown on the non-irrigated plots. The highest difference was shown by ash with a 30% increase of net photosynthesis, followed by maple (22%) and cherry (19%) under irrigated conditions.

Across the 1999 season and across all three species, higher ozone exposures reduced g_{wv} and P_n significantly (Fig. 3). Cherry did not seem to be as strongly affected as ash and maple when grown within non-filtered chambers (98% ambient ozone). But when exposed to similar ozone concentrations and grown within open plots (100% ambient ozone), ash and maple showed a gas exchange rate as high as under filtered (50% ambient ozone) conditions, indicating a negative response in

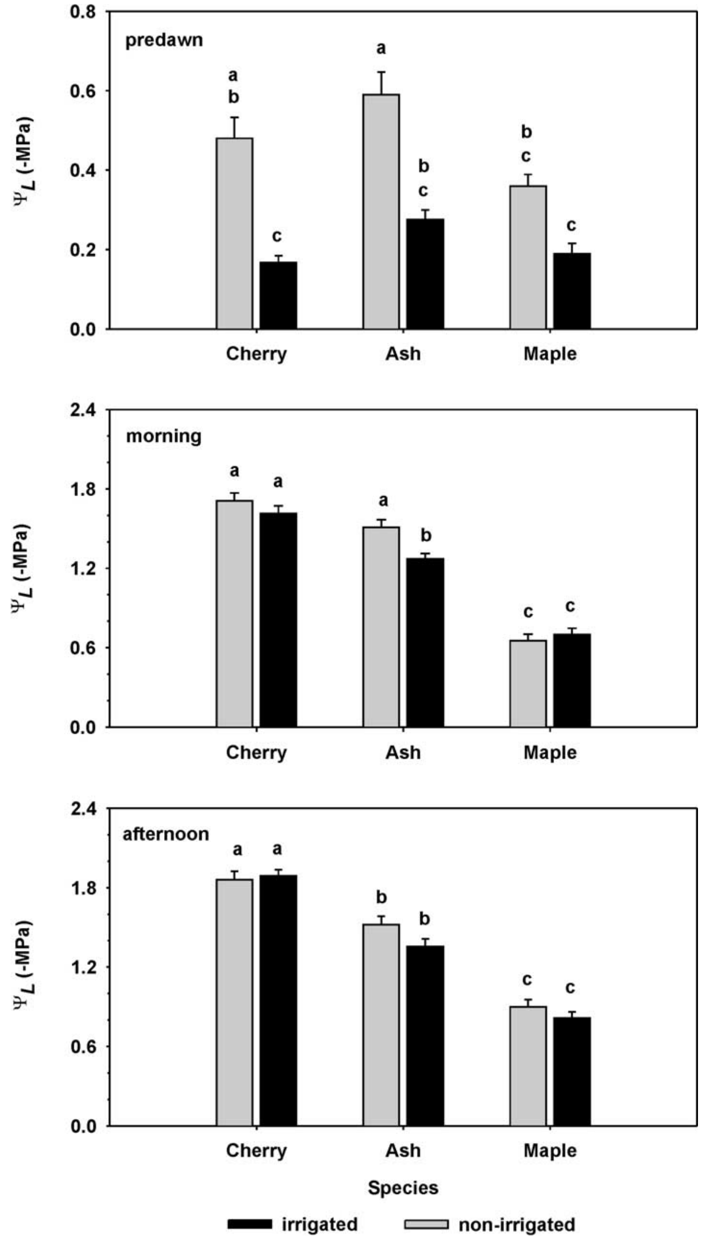

Fig. 2. Seasonal average of predawn-, morning-, and afternoon-leaf water potential (Ψ_L) measured in 1999 on upper crown leaves of black cherry, white ash, and red maple seedlings grown under irrigated and non-irrigated soil moisture conditions and averaged over all three ozone exposure treatments within open-top chambers and open plots. Significant differences are designated by differing lower case letters based on Bonferroni's *t*-21test ($\alpha = 0.05$).

gas exchange to the chamber climate (Fig. 3). When measurements started on 4 June 1999 and differences in soil moisture were still small, the rates of gas exchange were similar on both irrigated and non-irrigated plots with an average of 0.1–0.2 mol m^{-2} s^{-1} for g_{wv} and 9.0–13.0 μmol m^{-2} s^{-1} for P_n (Fig. 4). Watering seedlings during the hotter and drier mid-season resulted in consistently higher gas exchange of the irrigated seedlings in comparison to the non-irrigated seedlings. Towards the end of the season when precipitation increased and differences in soil water potential decreased, gas exchange values for irrigated and non-irrigated seedlings became similar again.

Across the 1999 season, the seasonal average of cumulative *U* was consistently higher for the irrigated seedlings compared to the non-irrigated seedlings (Fig. 5). At the end of the season, cumulative uptake of ozone was highest on the irrigated open plots, with 29.8 mmol m^{-2} ozone for cherry, 30.0 mmol m^{-2} for ash and 22.0 mmol m^{-2} for maple at the end of the season.

3.5. *Visible foliar ozone injury*

Foliar injury development on cherry and ash was clearly affected by soil moisture and ozone exposure

Table 3
Degree of freedom (d.f.), mean square (MS), and *F* values (*F*) for the split–split-plot design of analysis of variance for stomatal conductance to water vapor (g_{wv}), net photosynthesis (P_n), and foliar injury among three species, exposed to three different ozone exposures and grown under irrigated and non-irrigated soil moisture conditions within open-top chambers and open plots in 1999

Source of variation	d.f.	g_{wv} (mol m^{-2} s^{-1})		P_n (μmol m^{-2} s^{-1})		Foliar injury	
		MS	*F*	MS	*F*	MS	*F*
Block (*A*)	2	0.15		42.54		22.72	
Soil moisture (*B*)	1	3.16	6.6	3772.11	22.8*	533.16	12.2
AB (whole plot error)	2	0.48		165.29		43.73	
O_3 treatment (C)	2	0.16	2.6	514.15	10.5**	182.83	15.3**
BC	2	0.02	0.3	23.46	0.5	122.43	10.3**
ABC (subplot error)	8	0.06		48.79		11.93	
Species (*D*)	2	1.52	49.5***	1049.51	22.5***	786.17	35.9***
BD	2	0.11	3.7*	149.59	3.2	547.12	25.0***
CD	4	0.05	1.8	73.80	1.6	177.88	8.1***
BCD	4	0.01	0.3	34.49	0.7	124.78	5.7**
ABCD (sub-subplot error)	24	0.03		46.65		21.92	
Error	3705[a]	0.01		20.89		25.12	

[a] Due to a different sample size the error term for foliar injury equals 1032.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 4
Seasonal average (μ) and standard error (S.E.) of net photosynthesis (P_n), stomatal conductance for water vapor (g_{wv}) and total affected leaf surface area by ozone induce injury (%) for black cherry, white ash and red maple seedlings exposed to ambient (AA), non-filtered (NF) and charcoal-filtered (CF) air and grown under irrigated and non-irrigated soil moisture conditions within open-top chambers and open plots for 1998 and 1999

Species	Year	O_3	Non-irrigated						Irrigated					
			P_n		g_{wv}		Foliar injury		P_n		g_{wv}		Foliar injury	
			μ	S.E.	μ	S.E.	μ	S.E.	μ	S.E.	μ	S.E.	μ	S.E.
Cherry	1998	AA	8.76	0.207	0.195	0.005	16.51	2.490	8.23	0.179	0.205	0.005	17.38	2.524
		NF	8.03	0.224	0.170	0.006	12.46	1.97	7.88	0.180	0.170	0.004	15.87	2.619
		CF	9.02	0.210	0.175	0.006	4.86	1.210	9.18	0.194	0.206	0.007	1.39	0.478
	1999	AA	12.01	0.324	0.222	0.007	15.06	2.654	13.67	0.390	0.284	0.008	33.83	3.08
		NF	11.23	0.311	0.210	0.008	9.13	1.429	13.63	0.393	0.298	0.009	30.17	3.176
		CF	11.39	0.340	0.209	0.008	0.18	0.083	14.09	0.368	0.302	0.010	0.10	0.037
Ash	1998	AA	8.36	0.339	0.169	0.013	1.69	0.320	8.77	0.312	0.167	0.010	1.70	0.512
		NF	7.48	0.335	0.102	0.009	2.79	1.340	7.87	0.323	0.133	0.011	3.72	1.120
		CF	8.40	0.392	0.126	0.013	2.19	0.836	7.44	0.321	0.115	0.008	2.53	1.134
	1999	AA	11.48	0.302	0.201	0.009	0.00	0.000	15.12	0.311	0.287	0.010	0.22	0.100
		NF	9.45	0.243	0.141	0.006	0.00	0.000	11.91	0.300	0.233	0.010	0.60	0.600
		CF	10.81	0.325	0.171	0.008	0.00	0.000	14.13	0.328	0.280	0.011	0.00	0.000
Maple	1998	AA	8.00	0.307	0.147	0.008	0.00	0.000	6.02	0.229	0.112	0.006	0.00	0.000
		NF	7.08	0.314	0.131	0.011	0.00	0.000	7.26	0.321	0.128	0.009	0.00	0.000
		CF	7.64	0.321	0.147	0.012	0.00	0.000	5.61	0.282	0.101	0.007	0.00	0.000
	1999	AA	10.73	0.286	0.161	0.006	0.00	0.000	12.42	0.306	0.209	0.006	0.00	0.000
		NF	8.65	0.301	0.135	0.006	0.60	0.600	10.67	0.285	0.176	0.005	0.00	0.000
		CF	9.52	0.280	0.142	0.006	0.00	0.000	12.42	0.283	0.213	0.006	0.00	0.000

Assessed at the end of the season on 4 August 1998 and 17 August 1999.

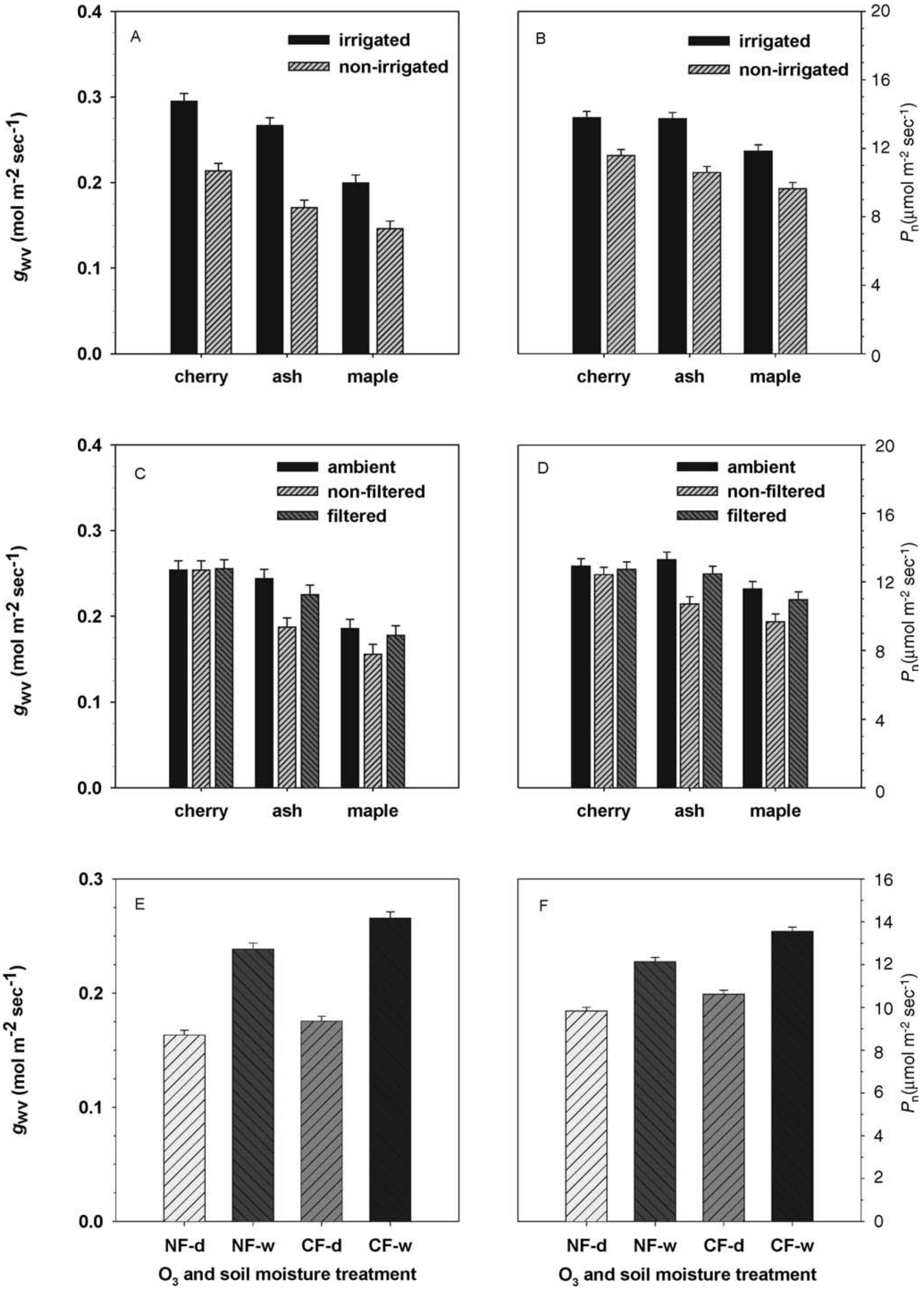

Fig. 3. Seasonal average of stomatal conductance (g_{wv}) and net photosynthesis (P_n) in 1999 for irrigated (w) and non-irrigated (d) seedlings (A, B), exposed to ambient (100% O_3), non-filtered (NF, 98% O_3), and charcoal-filtered (CF, 50% O_3) air (C, D), and for all three species combined, exposed to non-filtered and filtered air on irrigated and non-irrigated plots (E, F).

(Table 3 and Fig. 6). During both seasons of 1998 and 1999 there were differences in injury expression for each species to varying ozone exposures. Foliar injury was most dramatic on cherry followed by ash with only minor injury noted on maple (Table 4). Foliar injury on cherry and ash advanced through to leaf discoloration and senescence with increasing ozone exposures as the season progressed. Maple developed a very fine adaxial leaf surface stipple within the non-filtered and open plots during the latter part of the 1999 season but injury did not involve more than 1% of the leaf area per seedling.

In 1999, soil moisture became an additional influencing factor and the interaction between soil moisture and ozone exposure was highly significant for the foliar injury development (ozone–soil moisture–species interaction; $P < 0.01$, Table 3), in particular for cherry. At

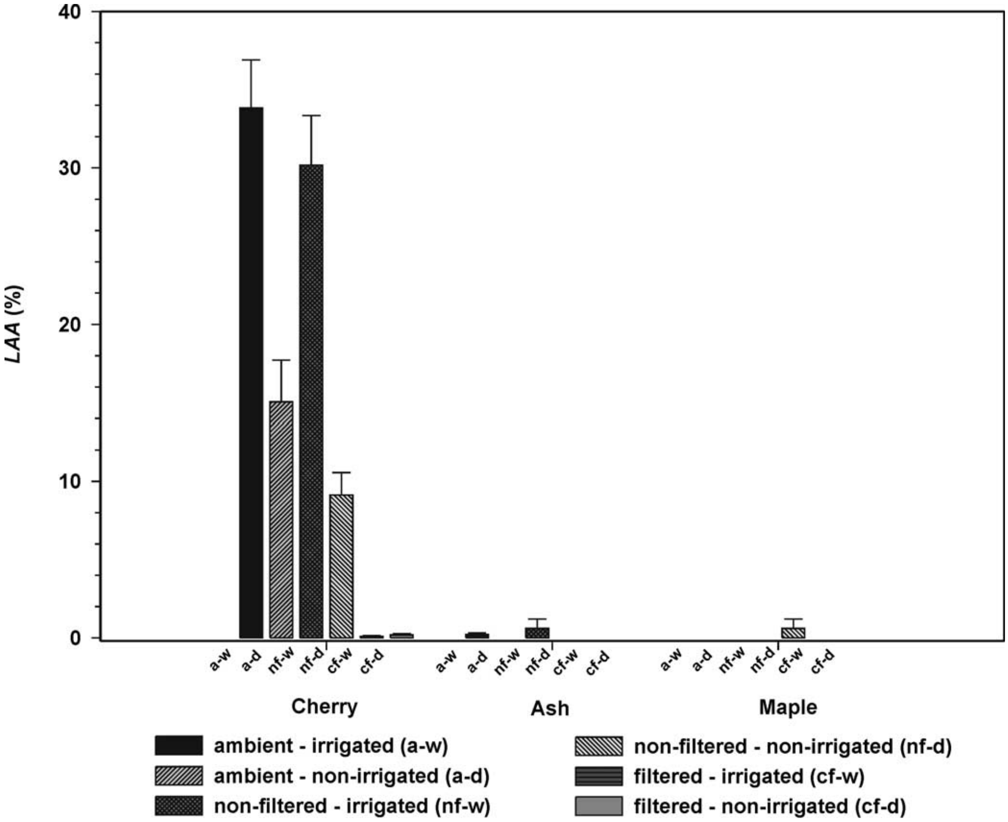

Fig. 4. Average of percentage leaf area (*LAA*) affected by visible foliar injury measured at the end of the season on 17 August 1999. Black cherry, white ash, and red maple seedlings were exposed to ambient (100% O_3), non-filtered (98% O_3), and charcoal-filtered (50% O_3) air on irrigated (w) and non-irrigated (d) soil moisture conditions within open-top chambers and open plots.

the end of the season, cherry seedlings grown on the irrigated plots showed at least twice as much affected leaf area than the seedlings grown under non-irrigated conditions. Under irrigated conditions, cherry showed 34% (open plot) and 30% (non-filtered) injured leaf surface compared to 15% (open plot) and 9% (non-filtered) injured leaf surface on the non-irrigated plots. For cherry exposed to filtered air as well as for ash and maple, the total affected leaf area never exceeded 5% during both seasons (Fig. 6). For the onset of foliar injury, there were no differences between irrigated and non-irrigated plots but the threshold was higher in 1999 compared to 1998. First foliar injury became visible on cherry at the end of June 1998 after being exposed to 15–17 ppm h (Sum0) and at the beginning of July 1999 after being exposed to 18–20 ppm h (Sum0).

4. Discussion

The hypothesis of this research centered on the supposition that tree species growing on wetter sites have higher stomatal conductance, suffer more severe ozone-induced foliar injury and have larger reductions in photosynthetic activity than individuals of the same species when grown under drier soil conditions.

The hypothesis was supported when soil moisture differences actually became significant during the 1999 season. Seedlings grown under irrigated soil moisture conditions had a higher gas exchange rate and ozone-induced visible injury was more severe than for seedlings grown under non-irrigated soil moisture conditions. In addition, higher ozone exposures favored the development of visible foliar injury and resulted in reduced net photosynthesis.

In 1998, the soil water potential reached a minimum of −0.2 MPa and then only at the end of the season. Although the total precipitation was higher during the 1999 measurement period (514.3 mm) than in 1998 (429.5 mm), soil water potential measurements showed three significant cycles of dry down in 1999 reaching values as low as −0.8 MPa.

As a result of the better-established trees in 1999, we believe that the three-year old seedlings demanded a greater supply of soil water in order to provide sufficient nutrient and water flow while maintaining more below and above ground biomass. Although the monthly average of temperature was approximately 1.5 °C and precipitation was 17 mm greater across the 1999 season as compared with the 1998 season; the difference in soil water potential between irrigated and non-irrigated plots was greater in 1999 than in 1998. Thus, for the

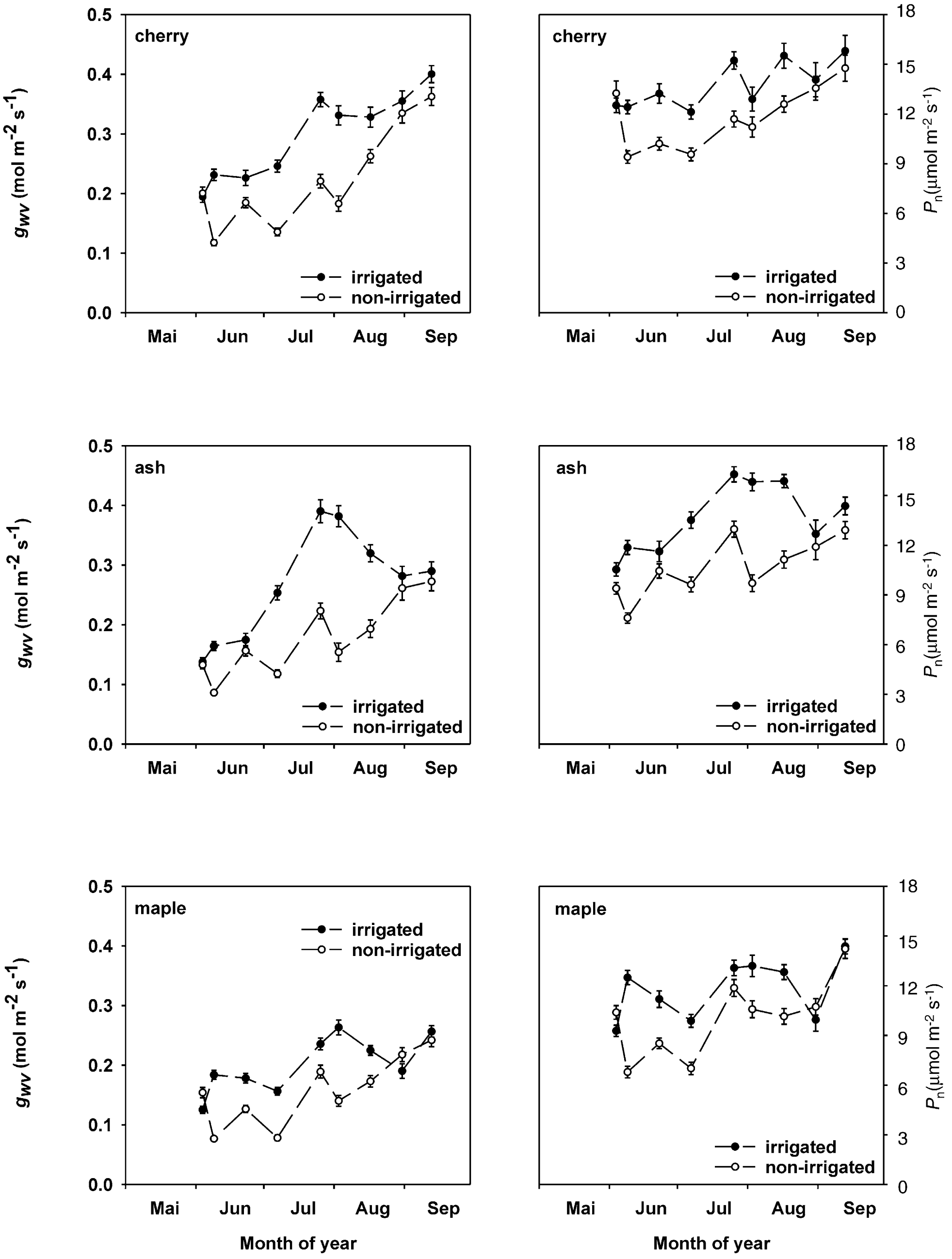

Fig. 5. Daily average of stomatal conductance (g_{wv}) and net photosynthesis (P_n) in 1999 for black cherry, white ash, and red maple seedlings exposed to ambient (100% O_3), non-filtered (98% O_3), and charcoal-filtered (50% O_3) air within irrigated and non-irrigated open-top chambers and open plots. Gas exchange data were averaged over morning (09:00–12:00 EST) and afternoon (13:00–16:00 EST) measurements.

months of June–August 1999, the dry site seedlings experienced more negative soil water potential than the wet site seedlings than for the similar period in 1998. The differences in the soil moisture regimes between the irrigated and non-irrigated plots became a controlling factor in affecting interactions with ambient ozone and subsequent physiological differences leading to alterations in ozone uptake. Very similar findings of having well-established seedlings within field investigations prior to making observations for visible ozone-induced foliar injuries were reported by VanderHeyden et al. (2000).

The results of the 1999 observations more clearly define the influence of soil moisture on the physiological and foliar injury response of seedlings to elevated ambient ozone exposures. For example, for all species combined, seedlings grown on irrigated plots showed consistently and significantly higher gas exchange than seedlings grown on non-irrigated plots. Species with the highest gas exchange were also those species that exhibited the most severe visible injury. As the most severely injured species, black cherry tended to maintain the highest stomatal conductance throughout most of the

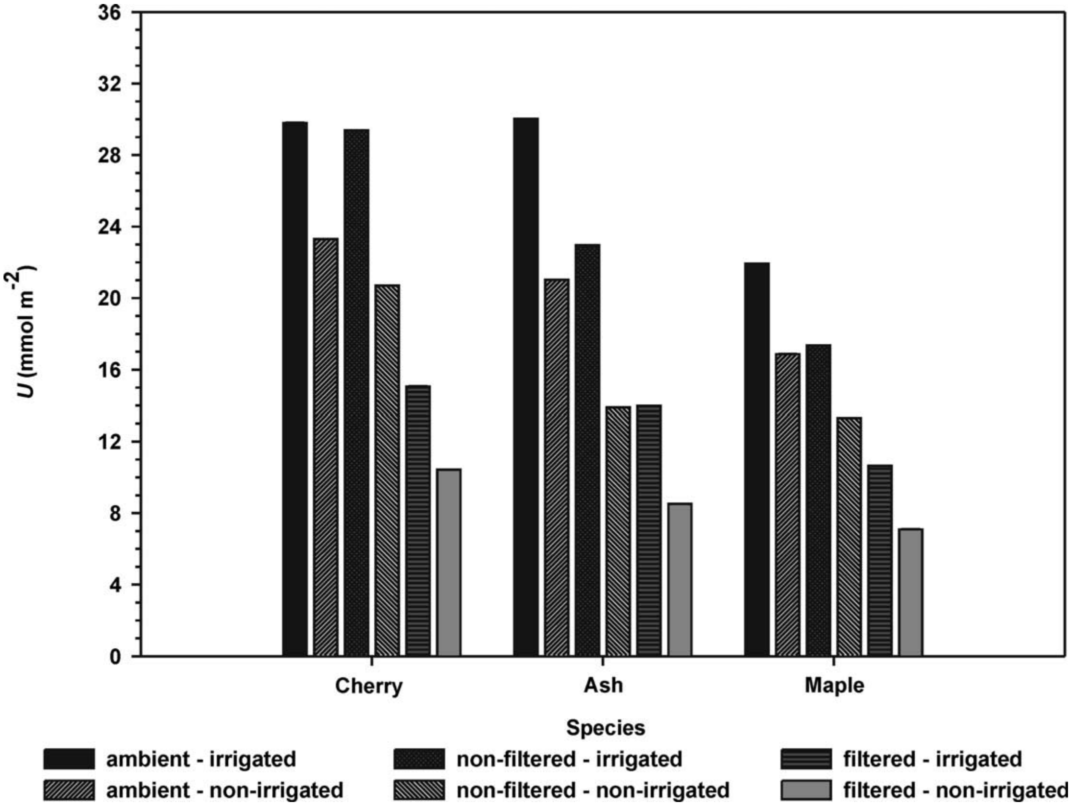

Fig. 6. Seasonal cumulative ozone uptake (*U*) calculated as a product of seasonal average stomatal conductance (g_{wv}) and seasonal cumulative ozone exposures (SUM0) based on 7-h (09:00–16:00 EST) ozone concentrations from 18 May to 30 September 1999 for black cherry, white ash, and red maple seedlings exposed to ambient (100% O_3), non-filtered (98% O_3), and charcoal-filtered (50% O_3) air on irrigated and non-irrigated soil moisture treatments within open-top chambers and open plots.

growing season suggesting that cherry did not seem to be affected as strongly as ash and maple when grown within non-filtered chambers. For each gas exchange measurement period one upper leaf was randomly selected to avoid shading and age effects. Due to longer exposure time, the lower leaves developed the most severe foliar injury. However, cherry showed the most pronounced continuous production of new leaves throughout the growing season. The method of selecting an upper crown and fully developed leaf combined with the pronounced free shoot growth of cherry may explain the disconnection between ozone exposure and over all sensitivity to ozone in terms of gas exchange. To accurately compare ozone exposure with plant response, calculations of ozone exposure need to be weighted by the length of time that different-aged leaves are exposed to ozone (Fredericksen et al., 1996). Nevertheless, our results demonstrate that higher soil moisture conditions favor the seasonal cumulative ozone uptake leading to more severe ozone induced foliar injury.

Seasonal averages of stomatal conductance were not significantly different between cherry and ash exposed to ambient air and grown under similar soil moisture, but foliar injury was much greater for cherry (<30%) than for ash (<4%). Based on this discrepancy, the same conclusion as Zhang et al. (2001) can be made; i.e., empirical relationships between stomatal conductance and foliar injury are species specific. Because species differ in many characteristics that may be collectively controlling their responses to ozone exposure (Ehleringer, 1991; Winner et al., 1991; Pääkkönen et al., 1998), the interpretation of ozone sensitivity based on only stomatal conductance would seriously over-simplify plant response to ozone.

These findings support the hypothesis of Reich (1987), who suggested that a negative correlation most likely exists between ozone uptake and net photosynthesis and further suggested that water stress and nutrient deficiency may prevent ozone injury by reducing stomatal conductance and ozone uptake. The significant difference in seedling response to elevated ambient ozone exposures under differing soil moisture within this current study makes it difficult to draw an overall conclusion of the potential effect of elevated ambient ozone exposures on the growth and biomass production of cherry, ash, and maple. Small decreases in net photosynthesis compounded over many years may produce significant growth reductions (Teskey, 1995), but small reductions in net photosynthesis may be too difficult to detect because of low experimental replication and greater variance in net photosynthesis among leaves or seedlings than among ozone treatments and/or differing soil moistures, respectively (Samuelson, 1994). Furthermore, because trees may express multiple flushing and indeterminate stem and leaf growth in the seedling stage, single leaf studies do not reflect cumulative and whole plant responses to ozone (Chappelka and Samuelson, 1998).

Ozone concentrations and the duration of exposures were sufficient during both years to induce typical foliar injury on cherry and ash as originally described on grape by Richards et al. (1958) and further illustrated on these species by Davis and Coppolino (1976), Simini et al. (1992), Skelly et al. (1996), Skelly (2000), and Innes et al. (2001). The 7-, 12-, and 24-h averages are similar to the background ozone exposures between 30 to 50 ppb and episodic peaks above 80 ppb ozone that typically occur during the summer months in Pennsylvania (Simini et al., 1992; Comrie, 1990, 1994). Kouterick et al. (2000) reported seasonal 24-h ozone averages for ozone exclusion treatments in central Pennsylvania in 1989 that were similar to the seasonal 24-h averages in 1999. Simini et al. (1992) and later Kouterick et al. (2000) demonstrated significant increases of foliar stipple on cherry with increasing ozone exposures during very similar ozone exclusion experiments within open-top chambers.

Average injury on cherry was greater and developed earlier than foliar injury observed on ash, which correlates with the findings of Davis and Skelly (1992), suggesting that ash is more tolerant to ambient ozone than cherry. The type of foliar injury observed in this study on ash and cherry was generally similar to the symptoms induced by ozone in previous studies described as dark adaxial stipple between the veins and predominantly on the oldest leaves (Karnosky and Steiner, 1981; Davis and Skelly, 1992; Innes et al., 2001).

Results from several studies (Winner et al., 1989; Showman, 1991; Hildebrand et al., 1996; Lefohn et al., 1997; Chappelka and Samuelson, 1998; Chappelka et al., 1999b) indicate that local site and associated environmental factors play an important role in determining overt foliar injury responses of trees to ambient ozone concentrations. For example, Showman (1991) conducted a field survey of foliar injury occurrence on sensitive plants and trees in Ohio and Indiana over a 2-year period. Although ambient ozone concentrations were very high in 1988 little injury was observed. In 1989 ozone concentrations were less, but foliar injury was much higher. The author interpreted that this was due to much lower precipitation in 1988 than in 1989. Similarly, Lefohn et al. (1997) combined ozone exposure information with soil moisture availability across a broad range within the southern Appalachian Mountains for 1983 through to 1990 and reported that the drought conditions of 1985–1988 greatly reduced the numbers of hectares that may have been of concern regarding possible ozone-induced effects.

A significant difference becomes evident when comparing the plant response of cherry seedlings grown under irrigated conditions as recorded during this study in 1999 and the plant response of cherry grown under non-irrigated conditions as likely occurred within several previous studies (Neufeld et al., 1995; Fredericksen et al., 1996; Kouterick et al., 2000). Cherry seedlings grown under constantly irrigated soil conditions clearly showed the lowest SUM0 ozone exposure threshold of 24 ppm h required for visible ozone injury to develop compared to the ozone exposure threshold of cherry grown under natural (drier) soil conditions (36 ppm h). These thresholds are considerably lower for wet sites as compared to most other previous studies ranging from 30 ppm h (Kouterick et al., 2000), 39 ppm h (Neufeld et al., 1995), and upwards to 52 ppm h (Fredericksen et al., 1996). These results clearly demonstrate that environmental conditions, such as varying soil moisture content, can have a significant effect on foliar responses to ozone. In particular, high soil water availability may favor onset, further development, and severity of visible foliar ozone injury.

In 1998, under moist soil conditions and within the open plots and non-filtered chambers, symptoms appeared on black cherry at ozone exposures of SUM $60<12$ and <9 ppm h (12 h daylight 0800–1959 h), respectively. Symptoms occurred within 60 days of the foliage encountering ambient ozone exposures (unpublished data). This result supports the suggestion of Heck and Cowling (1997) that a 3 month 12 h SUM 60 within the range of <8–12 ppm hrs may be necessary to protect against foliar injuries on native plants within natural ecosystems.

When grown under the above described ambient ozone concentrations, maple developed less than 1% leaf area affected by a very fine adaxial leaf surface stipple within non-filtered-air-supplied chambers and open plots at the end of the 1999 season. Samuelson (1994) observed similar visible foliar injury on maple after exposing the seedlings to twice-ambient ozone concentrations. Within their investigations, no foliar injury developed under ambient ozone concentrations. Since a very fine adaxial stipple did develop during the late 1999 season within the open plots and non-filtered-air-supplied chambers, this may be the first report of adaxial stipple to be identified under ambient field conditions for this species.

In conclusion, these results demonstrate the significant influence of site conditions on gas exchange throughout the season, as soon as the physiological processes start. When looking at the interrelationship between cumulative ozone uptake and total leaf area affected by visible foliar injury, it becomes clear that higher soil water availability exacerbates the negative effect of ambient ozone on sensitive seedlings. Because of the ubiquitous nature of ozone and the fact that tree response is altered by soil water availability, soil moisture must be controlled when determining whether or not ambient ozone concentrations significantly affect tree growth and productivity under natural forest conditions.

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