Journal of Agriculture and Environmental Sciences
December 2018, Vol. 7, No. 2, pp. 189-197
ISSN 2334-2404 (Print) 2334-2412 (Online)
Copyright © The Author(s). All Rights Reserved.
Published by American Research Institute for Policy Development
DOI: 10.15640/jaes.v7n2a18
URL: https://doi.org/10.15640/jaes.v7n2a18

Ozone Sensitivity of Green Ash Selections from Midwestern USA

Dennis R. Decoteau[1], Richard P. Marini[2]and Donald D. Davis[2]

Abstract

Green ash seeds were collected from various sites in midwestern USA and shipped to The Pennsylvania State University for evaluation of resultant seedlings' potential use as ozone bioindicators. Seeds were germinated in pots and seedlings maintained for one year in a greenhouse containing carbon-filtered-air (5 – 8 ppb ozone). Seedlings were then planted in the field within four open-top chambers. The air in two of the chambers was charcoal-filtered and contained 24 to 34% of ambient ozone (Filt), whereas two chambers received non-filtered ambient air (NF). After 2.5 years in the chambers, ozone injury symptoms were not observed on seedlings in the Filt treatment. In contrast, ozone-induced leaf injury (dark adaxial stipple) was observed in the NF treatment on seedlings from seed collected in Missouri (MO1 and MO3), Wisconsin (WI2 and WI2), Nebraska (NE) and North Dakota (ND). However, only seedlings from MO3 and ND had ozone injury levels that were significant for all foliar injury metrics, suggesting that seedlings from these two seed sources may serve as ozone-sensitive bioindicators. Seedlings from seed source WI13 exhibited significant reductions in ozone-induced growth effects, even though they exhibited no foliar injury.

Keywords: Ozone, *Fraxinus,* Ash, Air Pollution, Bioindicator

1. Introduction

1.1. Tropospheric Ozone

Tropospheric ozone is the air pollutant of concern to the health and productivity of forest ecosystems in the USA (Skelly, 2000).The U.S. Environmental Protection Agency (EPA) designated ozone as one of six criteria air pollutants that must be regulated to reduce the risk of harmful effects of ozone to human beings, agricultural crops, forest ecosystems, and other resources in the USA (USEPA, 2018).Ozone is a secondary air pollutant formed under conditions of bright sunlight and warm temperatures as a result of photochemical reactions involving primary air pollutants nitrogen oxides and hydrocarbons. This air pollutant is of regional-scale importance in the USA due to long-range transport within slow-moving, stagnant high-pressure systems.

1.2. Ozone Bioindicators and Their Use

Ozone-sensitive plant species have been used for 60 years as bioindicators because of their sensitivity to phytotoxic levels of ambient ozone (Middleton et al., 1950; Noble and Wright, 1958). Bioindicators are plant species that exhibit typical foliar injury symptoms when exposed to ambient ozone under environmental conditions suitable for ozone uptake and injury induction. These characteristic ozone-induced foliar symptoms are diagnostic for ozone injury and have been verified in ozone exposure/response studies under experimental conditions (Krupa and Manning, 1988; Manning and Feder, 1980). Therefore, bioindicator plant species are considered reliable indicators of phytotoxic levels of ambient ozone.

---

[1] Professor, The Pennsylvania State University, Department of Plant Science, University Park, PA 16802 (USA). Email: drd10@psu.edu Tel: 814-865-5587 FAX: 814-863-6139

[2] Professor, The Pennsylvania State University, Department of Plant Pathology and Environmental Microbiology, Penn State Institutes of Energy and the Environment, University Park, PA 16802 (USA),

In addition, quantifying the intensity or extent of foliar injury on known bioindicators can be used to estimate the relative air quality of ambient levels of atmospheric ozone for a particular location or region (Manning and Feder, 1980).

Within some areas of the USA, plants are subjected to acute exposures to ground-level ozone consisting of relatively high ozone concentrations (e.g.,>80 ppb) from a few consecutive hours to days (Krupa et al., 2001). Plants may also be exposed to chronic exposures, which consist of relatively low ozone concentrations (e.g., <40 ppb) for the entire life of a plant. Both acute and chronic ozone exposures can result in foliar injury symptoms on sensitive plant species.

Open stomata provide the pathway for ozone entry into the leaf. Once inside the leaf, ozone immediately forms toxic derivatives that react with various components of the leaf cells (Smith et al., 2007). Ozone-induced symptoms include chlorotic fleck, stipple, chlorosis, and accelerated senescence, as well as reductions in photosynthesis, growth, and yield (Krupa et al., 2001). However, the most diagnostic symptom induced by ozone on sensitive broadleaved plants such as green ash, is a dark stipple on the upper leaf surface (Skelly, 2000). In addition to inducing visible symptoms, ozone can alter plant sensitivity to biotic and other abiotic stresses and reduce resource allocation to the roots (Cooley and Manning, 1987; Davison and Barnes, 1998).

Plant susceptibility to ozone varies with genus, species, and/or subspecies of host plant, age, phenological stage of development, and environmental factors. Some plants are naturally sensitive to ozone, whereas others show resistance to ozone pollution or are able to tolerate the pollutant (Davis and Wilhour, 1976; Dowsett et al., 1992; Neufeld et al., 1992). Numerous studies have reported that variation in plant response to ozone is both interspecific and intraspecific, indicating that ozone-sensitivity has a genetic component (Davis and Coppolino, 1974; Davis and Wilhour, 1976; Karnosky and Steiner, 1981; Kline et al., 2009; Steiner and Davis, 1979).

With regard to ozone, plants are generally categorized as “sensitive” (plants that show visible foliar responses to ozone) or “tolerant” (plants that sustain injury or growth loss in the absence of visible symptoms) (Bennett et al., 1992; Reich and Amundson, 1985). Genetic variation in response to ozone also implies that ozone pollution is capable of influencing plant populations through natural selection, by which plants develop tolerance to ozone in areas of high ambient ozone (Berrang et al. 1986,1989,1991; Johnston et al., 1983).

The Forest Health Monitoring (FHM) and the Forest Inventory and Analysis (FIA) programs of the USDA Forest Service has collected data regarding the health of USA forests for more than 70 years (McRoberts et al., 2004). Within these programs, field crews collect more than 300 variables related to land ownership, tree species, tree size, and tree condition (including a broad suite of forest health indicators) (Bechtold and Patterson, 2005). In 1994, a national ozone biomonitoring program was implemented in the USA that used ozone-sensitive bioindicators. This bioindicator program was designed to address specific questions about the area and percent of forest land subjected to levels of ozone pollution that may adversely affect forest ecosystems (Smith et al., 2008).

1.3. Green Ash

Green ash (Oleaceae, *Fraxinus pennsylvanica* Marsh.), also called red ash, swamp ash, and water ash, is a medium-sized deciduous tree native to eastern North America. Green ash is widespread in eastern USA and Canada, where the species is found in mesophytic hardwood forests from Nova Scotia west to southeastern Alberta and eastern Colorado, south to northern Florida, and southwest to eastern Texas. The species is one of the most widely distributed of the native North American ashes (Kennedy, 1990).

During the mid-1960s, Penn State began a series of studies to determine the effects of ozone on woody plants with an emphasis on forest tree species (Wood and Coppolino, 1972). In these early studies, green ash was found to be sensitive to ozone (Davis and Wilhour, 1976), based on ozone-induced foliar injury (“stipple”), and was a test species favored by researchers for many years. For example, in 1979green ash seedlings exposed to non-filtered ambient air in Virginia were observed to exhibit “typical” ozone-induced symptoms (presumably stipple) (Skelly et al., 1983). Also in 1979, Steiner and Davis reported that ozone-induced leaf symptoms on green ash were influenced by provenance. Jensen (1981) and Duchelle et al. (1982), reported that ozone decreased green ash height growth. Kress and Skelly (1982) reported that ambient ozone decreased green ash biomass, and Chappelka et al. (1998) reported that ozone reduced green ash shoot elongation. However, depending on factors such as the type of exposure chamber used, ozone concentrations and length of exposure, and seed source, some researchers reported various results regarding the ozone-sensitivity of green ash. For example, Elliott et al. (1987) reported that ozone had little or no effect on green ash, and Loats and Rebbeck (1999) reported that ozone had little effect on green ash physiology.

1.4. Research Objectives

The primary objective of this study was to evaluate the ozone-sensitivity of green ash seedlings derived from 14 seed sources in midwestern USA, in order to identify seed sources that might yield bioindicator-seedlings that could detect phototoxic concentrations of ambient ozone. Two secondary objectives were to describe ozone-induced foliar symptoms induced on green ash seedlings exposed to extremely low concentrations of ozone and to determine if such low levels of ozone adversely influenced seedling growth. To meet these objectives, we exposed green ash seedlings to ambient ozone (non-filtered) and less than ambient ozone (slightly filtered) to select parent tree seed sources that might contain potential ozone-sensitive green ash bioindicators.

2. Methods

2.1. Plant Material and Seedling Production

Open-pollinated green ash seeds were collected from native selections during the Fall 2003 and Winter of 2003-2004 from midwestern USA (Table 1) and shipped to The Pennsylvania State University in central Pennsylvania. The 14 seed collection locations were chosen to provide plant material from a broad midwestern geographic regional area containing diverse soils, glaciated and non-glaciated landscapes, and varying climate/growing seasons.

Seeds were surface-sterilized in 0.2% sodium hypochlorite, rinsed in tap water, and stratified at 2°C until planting on 9 May 2004 into 9-L pots containing Metromix 510 (Grace Sierra Horticultural Products Co., Milpitas, CA) supplemented with 5 g Osmocote (15N:15P:15K) controlled release fertilizer (Scotts-Sierra Horticultural Products, Marysville, OH). Resultant seedlings were maintained within a greenhouse in charcoal-filtered air, which reduced ambient ozone concentrations to *ca.* 8 ppb. Potted seedlings were maintained under standard greenhouse conditions until transplanting in the field site on 1 May 2005.

Table 1. Green ash seed collection states, site codes, site identification, latitude, and longitude.

State	Site Code	Site Identification	Latitude	Longitude
Iowa	IA1	Kennedy Memorial Park – Webster County, Fort Dodge	42.585374°	-94.186742°
Iowa	IA2	Kendall Young Park, Webster City	42.481116°	-93.805788°
Illinois	IL1	Carlyle Lake State Park, Carlyle	42.585374°	-94.186742°
Illinois	IL2	Carlyle Lake State Park, Carlyle	42.585374°	-94.186742°
Illinois	IL3	Clinton Lake State Recreational Area, DeWitt	40.162010°	-88.790271°
Illinois	IL4	Rend Lake State Fish and Wildlife Area, Bonnie	38.200922°	-89.003961°
Missouri	MO1	Akers Ferry Campground, Jadwin	37.376382°	-91.562492°
Missouri	MO2	Akers Ferry Campground, Jadwin	37.376382°	-91.562492°
Missouri	MO3	Akers Ferry Campground, Jadwin	37.376382°	-91.562492°
Nebraska	NE	Louisville Lakes State Recreation Area, Louisville	41.004187°	-96.169934°
North Dakota	ND	Sheyenne FWA, Sheyenne	47.827722°	-99.120346°
Wisconsin	WI1	Lake Farm County Park, Madison	43.026423°	-89.344334°
Wisconsin	WI2	Tenney Park, Madison	43.095014°	-89.370512°
Wisconsin	WI3	Tenney Park, Madison	43.095014°	-89.370512°

2.2. Field Site and Open-Top Chamber Treatments

The field study site was at the Penn State Plant Pathology Research Farm, a rural location 13 km southwest of State College, PA, USA, at an elevation of 366 m. Pots were removed and green ash seedlings planted within open-top exposure chambers (Heagle et al., 1973) into a Hagerstown silt loam soil (fine-loamy, mixed mesic Typic Hapludalfs). Climatic data (temperature and precipitation) was obtained from an EPA monitoring site (USEPA AIRS Site #42-027-0100) located 9.6 km from the study site.

The experimental design consisted of two blocks with two open-top chambers (Heagle et al. 1973), permitting two ozone levels assigned to each block. Each block comprised a carbon-filtered air chamber ("Filt" = ca. 0.7 x ambient ozone concentration) and a chamber with non-filtered air ("NF" = ambient ozone concentration). Three seedlings derived from each of the 14 seed selection sites (Table 1) were assigned to each Filt and NF chamber. Plants from the various selections were tagged and arranged randomly in the soil within each chamber. Ozone treatments began on 1 May in 2005 and 2006. After the first season, the plastic sides (Heagle et al., 1989) of the open-top chambers were removed, leaving the plants exposed to ambient weather and air pollutants until 1 May 2006 at which time the plastic sides were returned to the chambers and ozone treatments began for the second season. In 2005, treatments ended on 30 October and on 6 September in 2006.

2.3. Ozone Monitoring

Ozone concentrations were recorded in the Filt and NF chambers from 10 July to 15 September in 2005, and from 1 June to 30 September in 2006. Ozone measurements were made at 5-minute intervals with a TECO Model-49 ozone analyzer (Thermo Environmental Instrumentals, Inc., Franklin, MA, USA) with data stored via Odessa DSM 3260 data logger (Odessa Engineering, Austin, TX, USA). A six-point calibration (0, 50, 100, 150, 200, 300 ppb) was performed on the monitors at each site every 10-14 days using a Thermo Electron Corporation Model 49PS calibrator and a source of compressed air with hydrocarbons and external pollutants removed. The calibrator was checked against a National Standard on an annual basis at the US Environmental Protection Agency Laboratory in Edison, NJ, USA.

2.4. Ozone-induced Foliar injury

Foliar symptoms of ozone-induced injury on green ash seedlings derived from each of the 14 seed selection locations were evaluated at the termination of the experiment on 6 September 2006. Percentage foliar injury, expressed as adaxial stipple, was estimated visually using the Horsfall-Barratt classification scale based on training with the Forest Health expert system for rating ozone injury (Horsfall and Barratt, 1945; Nash et al., 1992). Percentage leaves that exhibited leaf injury (LA) was determined, followed by determination of average percentage of leaf area injured on each symptomatic leaf (AA). Foliar injury classes were 0, 3, 6, 12, 25, 50, 75, 88, 94, 97, and 100%. From these component variables, we calculated an Injury Index (II) as the average percentage of leaf injury as combined with the total area of foliage injured (II = LA*AA).

2.5. Plant Growth Measurements

Plant height and stem diameter were recorded on 6 September 2006. Plants were immediately harvested and separated into shoot and root components. Both components were dried to a constant weight at 60°C and dry weights (dwt)recorded. Root + shoot total weights were calculated from the dry weights.

2.6. Statistical Analyses

The experimental design was a split-plot. Ozone treatment was the whole-plot with two replications and the three plants from the 14 seed sources were the split-plot. SAS's PROC GLIMMIX (SAS Institute, Cary, NC) was used to perform an analysis of variance (ANOVA) on each response variable. *P*-values obtained from the ANOVA were used to test the main effect of ozone treatment, pooled over the 14 seed sources, the main effect of seed source pooled over the ozone treatments and the interaction of ozone treatment x seed source. The two ozone treatments within each clone were compared with the SLICEDIFF option in the LS means statement and *P*-values obtained for each comparison.

3. Results and Discussion

3.1. Ozone and Seasonal Temperature and Precipitation

While seasonal ozone concentrations trends were similar for both 2005 and 2006, monthly mean and maximum ozone concentrations varied by OTC treatments and year (Table 2). In 2005, the 7-h, 12-h, and 24-h monthly ozone averages were greatest in July, whereas in 2006 the averages were highest in June (the first months for each year in which the measurements were recorded) for both treatments in the respective years. September had the lowest monthly 24-h ozone concentration each year. Carbon-filtration reduced the 24-h ozone concentrations in the Filt chambers by *ca.* 37% in 2005 and 28% in 2006 as compared to ozone concentrations monitored within NF chambers. The maximum hourly ozone concentration of 83 ppb and 56 ppb was recorded on 13 July 2005 in the NF chambers and the Filt chambers, respectively, while the maximum in 2006 was 72 ppb and 62 ppb on 17 June, respectively. The 2005 season was slightly warmer and drier later in the monitoring season than 2006, whereas 2005 had a wetter July and a drier September, whereas 2006 had a cooler and wetter September.

Table 2. Average monthly and seasonal ambient ozone concentrations (ppb) in the carbon filtered (Filt) and non-filtered (NF) chambers, as well as temperature and precipitation.

		Open-Top Chamber (OTC) Ozone Treatment							
		Filt[a]	NF[b]	Filt[a]	NF[b]	Filt[a]	NF[b]	Temperature	Precipitation
Year	Month	7-h		12-h		24-h		(°C)	(cm)
2005	July	31.6	45.6	28.7	43.1	19.4	30.6	22.7	12.40
	Aug.	29.1	41.7	26.4	39.6	18.1	28.7	22.0	6.63
	Sept	31.5	46.0	27.5	41.6	16.4	26.4	21.1	2.69
	3-mo. avg.	30.7	44.4	27.5	41.4	18.0	28.6	21.0	21.95
2006	June	33.8	41.9	31.5	41.3	22.6	31.1	18.7	7.67
	July	33.1	40.7	29.9	39.2	21.3	29.4	22.8	7.29
	Aug.	31.4	39.6	28.3	38.0	20.4	28.6	21.5	7.24
	Sept	28.2	35.7	25.3	34.1	18.3	25.6	15.0	10.21
	4-mo. avg.	31.8	40.0	29.2	38.7	21.1	29.2	19.6	32.44

[a] Filtered *ca.* 0.7 x ambient ozone concentration

[b] Non-filtered ambient ozone concentration

On 1 October 2015, the U.S. Environmental Protection Agency (EPA) strengthened both the primary and secondary U.S. National Ambient Air Quality Standards (NAAQS) for ozone, reducing the ozone standard from 75 to 70 ppb (USEPA 2015). The current 70-ppb ozone NAAQS is based on the 4th highest daily maximum ozone concentration, averaged across 3 consecutive years for an averaging time of 8 h. The reduction makes the current ozone NAAQS more stringent to help protect public health and welfare, as well as the health of individual plants and ecosystems (USEPA 2015). Figure 1 illustrates the downward trend in U.S. ambient ozone levels from 1980–2015 in northeastern U.S. and is compared to the current 70 ppb NAAQS for ozone (horizontal dashed line). We present these data and graph to put the ozone levels used in our 2005 and 2006 exposure doses in perspective. The ambient ozone concentrations that we used during both years of this study were likely all less than the current 8-h NAAQS.

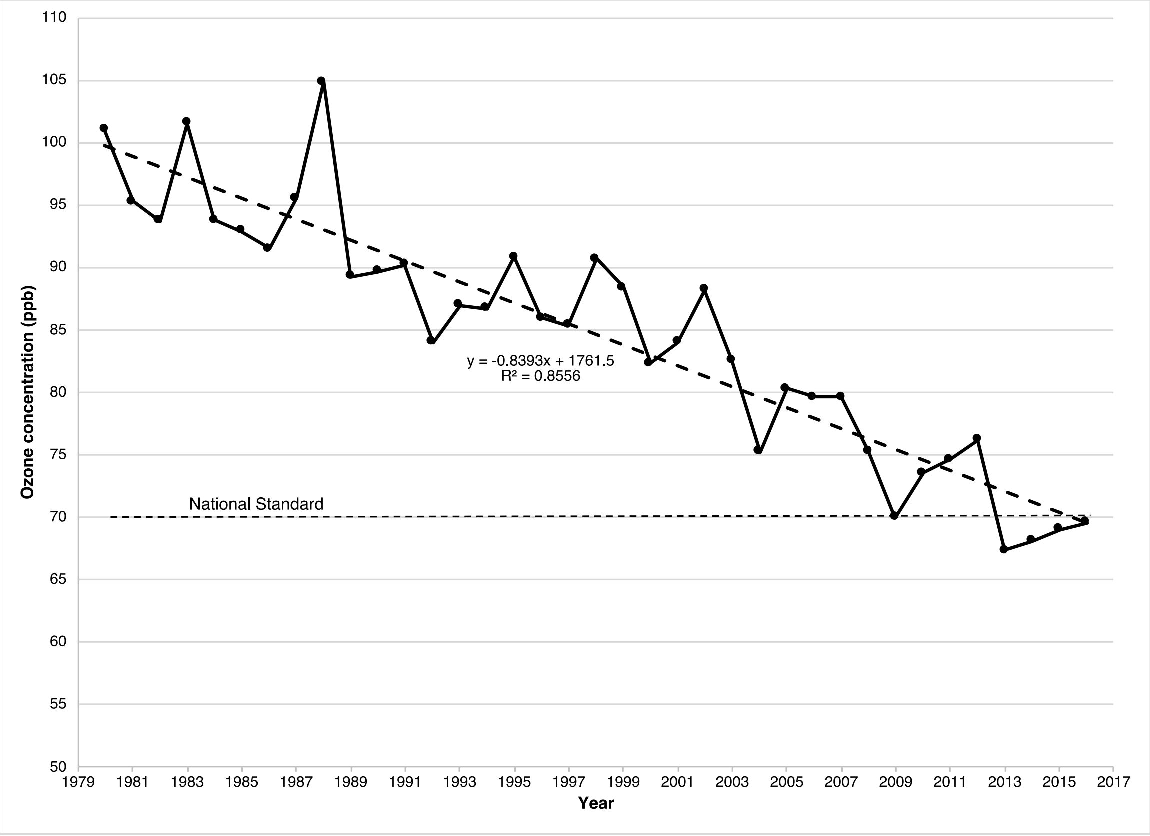

Figure 1. Trend of average ambient ozone concentrations (ppb) during 1980-2016, based on the current NAAQS annual 4th maximum daily 8-h averages. The horizontal dashed line represents the current NAAQS of 70 ppb ozone. Fig 1. illustrates the national trend based on 206 monitoring sites, showing a 31% decrease in average ozone from 1980–2016. The best fit for the trend line was linear (R^2 = 0.86). Data available online at https://www.epa.gov/air-trends/ozone-trends. Accessed 24 June 2018.

3.2. Foliar Injury

Visual ozone injury was not observed on green ash seedlings from any of the 14 seed sources in the Filt treatment during two years exposure (Table 3). In contrast, foliar injury (dark adaxial stipple) was observed after two years, at the conclusion of the experiment, in the NF treatment on seedlings from seed sources MO1, MO3, NE, ND, WI1, and WI2. However, only seedlings from seed sources MO3 and ND had significantly different levels of injury between the Filt and NF treatments for all three foliar injury characteristics (LA, AA, and II). In contrast, MO1 exhibited statistically significant foliar injury between the Filt and NF treatment only for the AA injury rating. Greatest LA, AA, and II injury was observed on seedlings in the NF treatment grown from seed sources MO3 followed by ND and MO1.

Table 3. Effects of charcoal-filtered air (Filt), nonfiltered air (NF) on foliar injury on field-grown green ash seedlings from the various seed sources at completion of experiment.

	Open-Top Chamber (OTC) Ozone Treatment					
	% Leaves Injured (LA)		% Leaf Area Injured (AA)		Injury Index (II)	
Seed Source	Filt[a]	NF[b]	Filt[a]	NF[b]	Filt[a]	NF[b]
IA1	0	0	0	0a	0	0
IA2	0	0	0	0a	0	0
IL1	0	0	0	0a	0	0
IL2	0	0	0	0a	0	0
IL3	0	0	0	0a	0	0
IL4	0	0	0	0a	0	0
MO1	0	3.7	0	1.5b**	0	7.7
MO2	0	0	0	0a	0	0
MO3	0	11.8***	0	3.2c***	0	109.5***
NE	0	0.5	0	0.2a	0	0.5
ND	0	9.8***	0	1.7b*	0	53.5*
WI1	0	1.0	0	0.5ab	0	3.0
WI2	0	1.7	0	0.8ab	0	3.7
WI3	0	0	0	0a	0	0
			P-values from ANOVA			
OTC Treat		0.021		0.002		0.105
Seed Source		0.155		0.023		0.322
Interaction		0.155		0.023		0.322

[a] Filtered *ca.* 0.7 x ambient ozone concentration

[b] Non-filtered ambient ozone concentration

Significant statistical differences for ratings within seed sources and treatments are indicated by *(P<0.05), **(P<0.01), and ***(*P*<0.001). LA = % leaves injured, AA = average % leaf area injured on each symptomatic leaf (AA), and II = LA

The primary objective of this paper was to evaluate the ozone-sensitivity of green ash seedlings derived from 14 seed sources in midwestern USA. Our results suggest that ash seedlings derived from seed sources MO3 and ND may yield bioindicators that are useful to detect phytotoxic levels of ambient ozone, especially since the levels of ozone that induced symptoms were likely less than the current NAAQS for ozone. A secondary objective was to describe ozone-induced foliar symptoms on green ash seedlings and to determine if seedlings from different seed sources exhibited similar or different effects due to ozone. Symptoms of foliar injury induced by ozone appeared as a dark adaxial leaf surface stipple, the classic foliar response of broadleaved plants to ozone (Skelly, 2000). Classic stipple symptoms were similar on all ozone-sensitive seedlings, regardless of seed source.

3.3. Growth

Following two years exposure to ozone, height, shoot dry weight, root dry weight and shoot + root dry weight measurements were related to the 14 seed sources in some cases (Table 4). The tallest seedlings following the NF treatment and Filt treatment were from seed sources IA1 and IA2, respectively. The shortest seedlings in both treatments were from seed source ND and MO1. The greatest shoot dry weights following the NF treatment were from seedlings from seed source IA2, whereas the greatest shoot dry weight in the Filt treatment was from seed source WI3. The greatest root weight and shoot + root weight for both treatments were from seed source W13. WI3 was the only seed source to produce seedlings that exhibited significant height, shoot dry weight, root dry weight, and shoot + root dry weight effects due to ozone treatment. Seedlings from seed source WI3 in the NF treatments were shorter and had less shoot, root and shoot + root biomass as compared to trees from the Filt treatment.

A secondary objective of this study was to determine the effects of low ozone levels of ozone on green ash seedling growth. Growth of seedlings from different seed sources slightly different due to ozone. Only those seedlings from seed collected at WI3 (Wisconsin) exhibited growth reduction due to ozone. Whereas ambient ozone induced at least some foliar symptoms on plants from six of the 14 seed sources, ozone caused significant negative impacts on growth only on seedlings derived from only one collection site. This is not surprising since the appearance of visible symptoms in response to ozone does not always coincide with measured growth effects (Kress and Skelly, 1982; Shafer and Heagle, 1989; Taylor et al., 2002).

Table 4: Effects of charcoal-filtered air (Filt), nonfiltered air (NF) on height, dry weights of shoots and roots, and shoot + root on field-grown green ash seedlings from the 14 seed sources at completion of experiment.

Seed Source	Open-Top Chamber (OTC) Ozone Treatment											
	Height (cm)			Shoot (dwt, g)			Root (dwt, g)			Shoot + Root (dwt, g)		
	Filt[a]	NF[b]	Mean	Filt[a]	NF[b]	Mean	Filt[a]	NF[b]	Mean	Filt[a]	NF[b]	Mean
IA1	146.0	146.2	146.1b	67.3	70.2	68.7ab	89.5	86.2	87.8ab	156.8	156.3	156.3ab
IA2	158.5	142.5	150.5b	75.0	77.0	76.0b	80.5	81.5	81.0ab	155.5	158.5	157.0ab
IL1	86.7	107.8	97.2ab	22.2	32.7	27.4a	29.8	43.5	36.7a	52.0	76.2	64.1a
IL2	93.8	116.2	100.8ab	35.8	47.0	41.4ab	56.8	54.5	55.7ab	92.7	101.5	97.1ab
IL3	104.7	99.7	102.2ab	28.5	27.0	27.8ab	33.0	37.5	35.2a	61.5	66.2	63.8a
IL4	89.7	110.7	100.2ab	23.3	35.3	29.3ab	31.0	44.0	37.5a	54.3	79.3	66.8a
MO1	75.3	76.3	75.8a	19.5	19.2	19.3a	25.0	30.3	27.7a	44.5	49.5	47.0a
MO2	103.5	124.3	113.9ab	34.3	40.8	37.6ab	54.0	55.8	54.9ab	88.3	96.7	92.5a
MO3	84.8	76.5	80.7ab	22.5	18.0	20.2a	34.8	30.2	32.5a	57.3	48.2	52.8a
NE	125.5	113.2	119.3ab	53.7	39.2	46.4ab	63.8	59.2	61.5ab	117.5	98.3	107.9ab
ND	86.2	64.8	75.5a	29.5	23.8	26.7a	58.5	53.2	55.8ab	88.0	77.0	82.5a
WI1	114.2	114.8	114.5ab	51.7	53.0	52.3ab	103.0	87.0	95.0b	154.7	140.0	147.3ab
WI2	108.2	110.0	109.1ab	47.0	46.8	46.9ab	101.8	78.8	90.3b	148.8	125.7	137.2ab
WI3	155.8	118.7*	137.2ab	105.5	72.0*	88.8b	139.0	97.7**	118.3b	244.5	169.7**	207.1b
			P-values from ANOVA									
OTC Treatment			0.848			0.861			0.518			0.666
Seed Source			0.006			0.001			0.001			0.001
Interaction			0.957			0.984			0.979			0.986

[a] Filtered *ca.* 0.7 x ambient ozone concentration

[b] Non-filtered ambient ozone concentration

[c] Means for seed source pooled over ozone treatments followed by common letters do not differ at 5% level of significance (Tukey-Krame Asterisks indicate that ozone treatments means within seed sources were significantly different, by SLICEDIFF; *($P<0.10$) and **(P<0.C

4. Conclusions.

It has been documented that plant genetics affects level of ozone-sensitivity (Johnston et al., 1983). In this study, we used 2-year-old selections of green ash from different seed sources to estimate the influence of genetics on the severity of foliar ozone injury and plant growth. Other studies from our laboratory have since shown that open-pollinated plant families may differ genetically in sensitivity to ozone in the seedling stage (Kouterick et al., 1994; Simini et al., 1991; Skelly et al., 1994). The degree of intra specific variability maybe due genetic differences in ozone-sensitivity among geographically scattered populations of the same species (Kline et al., 2009). Such differences may arise randomly or due to selection pressure from spatially different levels of ozone, which select more ozone-tolerant plants in areas of greater ambient ozone (Berrang et al., 1986, 1989, 1991). However, our database was too small and contained too much variation to conduct robust spatial statistical analyses. If our sample size, or number of plants exposed, would have been larger, confidence levels in our results would have increased (Stolte and Mangis, 1992).

Whereas ambient ozone induced some foliar symptoms on seedlings from six of the 14 seed sources, it caused significantly negative growth impacts on seedlings from only one collection site, W13 in Wisconsin. This is not surprising since the appearance of visible symptoms in response to ozone does not always coincide with measured growth effects (Kress and Skelly, 1982; Shafer and Heagle, 1989; Taylor et al., 2002). Green ash seedlings derived from seed collection sites MO3 in Missouri and ND in North Dakota, exhibited classic ozone-induced stipple, and may prove useful as bioindicators of phytotoxic levels of ozone in midwestern USA.

References

Bechtold, W. A., & Patterson, P. L. (2005). The enhanced Forest Inventory and Analysis program— national sampling design and estimation procedures. General Technical Report SRS-80. USDA Forest Service, Southern Research Station. Asheville, NC, USA.

Bennett, J. P., Rassat, P., Berrang, P., & Karnosky, D. F. (1992). Relationships between leaf anatomy and ozone sensitivity of *Fraxinus pennsylvanica* Marsh. and *Prunus serotina* Ehrh. Environmental and Experimental Botany, 32, 33-41.

Berrang, P., Karnosky, D. F., & Bennett, J. P. (1989). Natural selection for ozone tolerance in *Populustremuloides*: field verification. Canadian Journal of Forest Research, 19, 519-522.

Berrang, P., Karnosky, D. F., & Bennett, J. P. (1991). Natural selection for ozone tolerance in *Populustremuloides*: an evaluation of nationwide trends. Canadian Journal of Forest Research, 21, 1091-1097.

Berrang, P., Karnosky, D. F., Mickler, R. A., & Bennett, J. P. (1986). Natural selection for ozone tolerance in *Populustremuloides*. Canadian Journal of Forest Research, 16, 1214-1216.

Chappelka, A. H., Chevone, B. I., &Burk, T. E. (1988). Growth response of green and white ash seedlings to ozone, sulfur dioxide, and simulated acid rain. Forest Science, 34, 1016-1029.

Cooley, D., & Manning, W. (1987). The impact of ozone on assimilate partitioning in plants: a review. Environmental Pollution, 47, 95-113.

Davis, D. D., & Coppolino, J. B. (1974). Relative ozone susceptibility of selected woody ornamentals. HortScience, 9, 537-539.

Davis, D. D., & Wilhour R. G. (1976). Susceptibility of woody plants to sulfur dioxide and photochemical oxidants. U.S. EPA Ecological Research Series Report Number EPA-600/3-76-102, Corvallis, OR, USA.

Davison, A. W., & Barnes, J. D. (1998). Effects of ozone on wild plants. New Phytologist, 139, 135-151.

Dowsett, S. E., Anderson, R. L., & Hoffard, W. H. (1992). Review of selected articles on ozone sensitivity and associated symptoms for plants commonly found in the forest environment. Tech. Pub. R8-TP 18, USDA Forest Service, Southern Region, Forest Pest Management, Atlanta, GA, USA.

Duchelle, S. F., Skelly, J. M., & Chevone, B. I. (1982). Oxidant effects on forest tree seedling growth in the Appalachian Mountains. Water, Air, and Soil Pollution, 18, 363-373.

Elliott, C. L., Eberhardt, J. C., & Brennan, E. G. (1987). The effect of ambient ozone pollution and acidic rain on the growth and chlorophyll content of green and white ash. Environmental Pollution, 44, 61-70.

Heagle, A. S., Body, D. E., & Heck, W. W. (1973). An open-top field chamber to assess the impact of air pollution on plants. Journal Environmental Quality, 2, 365-368.

Heagle, A. S., Philbeck, R. B., Ferrell, R. E., & Heck, W. W. (1989). Design and performance of a large, field exposure chamber to measure effects of air quality on plants. Journal of Environmental Quality, 18, 361-368.

Horsfall, J. G., & Barratt, R. W. (1945). An improved grading system for measuring plant disease. Phytopathology, 35, 655.

Jensen, K. F. (1981). Ozone fumigation decreased the root carbohydrate content and dry weight of green ash seedlings. Environmental Pollution, 26, 147-152.

Johnston, W., Haaland, R., & Dickens, R. (1983). Inheritance of ozone resistance in tall fescue. Crop Science, 23, 235-236.

Karnosky, D. F., & Steiner, K. C. (1981). Provenance and family variation in response of *Fraxinus americana* and *Fraxinus pennsylvanica* to ozone and sulfur dioxide. Phytopathology, 71, 804-807.

Kennedy, H. E. (1990). *Fraxinus pennsylvanica* Marsh. Green Ash. In: Burns, R. M., & Honkala, B.H., Editors. Silvics of North America, Volume 2, Hardwoods, Agricultural Handbook #654, USDA Forest Service, Washington, DC, USA.

Kline, L. J., Davis, D. D., Skelly, J. M., & D.R. Decoteau. (2009). Variation in ozone sensitivity within Indian hemp and common milkweed selections from the Midwest. Northeastern Naturalist,16, 307–313.

Kouterick, K., Skelly, J., Fredericksen, T., Savage, J., & Snyder, K. (1994). Relationships between foliar ozone injury and physiology among black cherry genotypes. Phytopathology, 84, 544.

Kress, L. W., & Skelly, J. M. (1982). Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. Plant Disease, 66, 1149-1152.

Krupa, S. V., & Manning, W. J. (1988). Atmospheric ozone: formation and effects on vegetation. Environmental Pollution, 50, 101–137

Krupa, S., McGrath, M. T., Andersen, C. P., Booker, F. L., Burkey, K. O. Chappelka, A. H., Chevone, B. I., Pell, E. J. & Zilinskas, B. A. (2001). Ambient ozone and plant health. Plant Disease, 85, 4-12.

Loats, K. V., & Rebbeck, J. (1999). Interactive effects of ozone and elevated carbon dioxide on the growth and physiology of black cherry, green ash, and yellow-poplar seedlings. Environmental Pollution, 106, 237-248.

Manning, W. J., & Feder, W. A. (1980). Biomonitoring Air Pollutants with Plants. Applied Science Publishers, UK.

McRoberts, R. E., McWilliams, W. H., Reams, G. A., Schmidt, T. L., Jenkins, J. C., O'Neill, K. P., Miles, P.D., & Brand, G.J. (2004). Assessing sustainability using data from the Forest Inventory and Analysis Program of the United States Forest Service. Journal of Sustainable Forestry, 18, 23-46.

Middleton, J. T., Kendrick, J. B., & Schwalm, H. W. (1950). Injury to herbaceous plants by smog or air pollution. Plant Disease Reporter, 34, 245–252.

Nash, B. L., Saunders, M. C., Miller, B. J., Bloom, C. A., Davis, D. D., & Skelly, J. M. (1992). Forest Health, an expert advisory system for assessing foliar and crown health of selected northern hardwoods. Canadian Journal of Forest Research, 22, 1770-1775.

Neufeld, H. S., Renfro, J. R., Hacker, W. D., & Silsbee, D. (1992). Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. In Berglund, R.D. (Ed.), Tropospheric Ozone and the Environment II: Effects, Modeling, and Control. Air & Waste Management Association Press pp 594–617, Pittsburgh, PA, USA.

Noble, W. M., & Wright, L. A. (1958). Air pollution with relation to agronomic crops: II. A Bio-assay approach to the study of air pollution. Agronomy Journal, 50, 551.

Reich, P. B., & Amundson, R. G. (1985). Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science, 230, 566–570.

Shafer, S. R., & Heagle, A. S. (1989). Growth responses of field-grown loblolly pine to chronic doses of ozone during multiple growing seasons. Canadian Journal of Forest Research, 19, 821-831.

Simini, M., Skelly, J. M., & Savage, J. E. (1991). Sensitivity of wildtype and half-sib families of black cherry to ambient doses of ozone in northcentral Pennsylvania. Phytopathology, 81, 124.

Skelly, J. M. (2000). Tropospheric ozone and its importance to forests and natural plant communities of the northeastern United States. Northeastern Naturalist, 7, 221-236.

Skelly, J. M., Savage, J. E., Snyder, K. R., Fredericksen, T. S., & Kouterick, K. B. (1994). Response of open-pollinated black cherry families to ambient ozone under open-top chamber conditions. Phytopathology, 84, 547.

Skelly, J. M., Yang, Y. S., Chevone, B. I., Long, S. J., Nellessen, J. E. & Winner, W. E. (1983). Ozone concentrations and their influence on forest species in the Blue Ridge Mountains of Virginia. In: Air Pollution and the Productivity of the Forest. Davis, D. D., Millen, A. A., & Dochinger, L. (Editors). Izaak Walton League of America Publishers, 143-160.

Smith, G. C., Smith, W. D., & Coulston, J. W. (2007). Ozone bioindicator sampling and estimation. General Technical Report NRS-20. USDA Forest Service Northern Research Station., Newtown Square, PA, USA.

Smith, G. C., Coulston, J. W., & O'Connell, B. M. (2008). Ozone bioindicators and forest health: A guide to the evaluation, analysis, and interpretation of ozone injury data in the Forest Inventory and Analysis Program. Gen. Tech. Rep. NRS-34. USDA Forest Service, Northern Research Station, Newtown Square, PA, USA.

Steiner, K. C., & Davis, D. D. (1979). Variation among *Fraxinus americana*, *Fraxinus pennsylvanica* families in foliar response to ozone injury. Canadian Journal of Forest Research, 9, 106-109.

Stolte, K. W., & Mangis, D. R. (1992). Identification and use of plant species as ecological indicators of air pollution stress in national park units. In D. H. McKenzie, D. E. Hyatt, & V. J. McDonald (Eds.), Ecological Indicators: Volume 1 (pp. 373-392). Springer Publishers, Boston, MA, USA.

Taylor, M. D., Sinn, J. P., Davis, D. D., & Pell, E. J. (2002). The impact of ozone on a salt marsh cordgrass (*Spartina alterniflora*). Environmental Pollution, 120, 701-705.

U.S. Environmental Protection Agency (USEPA). (2015). National Ambient Air Quality Standards (NAAQS) for Ozone [Online]. Available: https://www.epa.gov/ozone\pollution/2015-national-ambient-air-quality-standards-naaqs-ozone (3 February 2017).

U.S. Environmental Protection Agency (USEPA). (2018). Criteria Air Pollutants [Online]. Available: https://www.epa.gov/criteria-air-pollutants (June 12, 2018).

Wood, F. A., & Coppolino, J. B. (1972). The influence of ozone on deciduous forest tree species. In: Effects of Air Pollutants on Forest Trees, VII International Symposium of Forest Fume Experts, Vienna, September 1970, pp 233-253.