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Response of selected warm-season turfgrasses and ornamental monocots to short-term, high concentration, ozone fumigation

Lou Ann McKnight
Louisiana State University and Agricultural and Mechanical College, lmckni2@tigers.lsu.edu

RESPONSE OF SELECTED WARM-SEASON TURFGRASSES AND ORNAMENTAL MONOCOTS TO SHORT-TERM, HIGH CONCENTRATION, OZONE FUMIGATION

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Lou Ann McKnight
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DEDICATION

To my family, past and present, who give and have given me purpose in life. To my grandfather, Bruce Russell, who loved science and nature and who taught me how to float a needle on water. To my grandmother, Marguerite Russell, the greatest person I have ever known. To my children who challenge me in every aspect of life. To my brilliant and awesome grandchildren, Judah and Koenn, who bring me the greatest joy in life. And finally to my husband John, you make friendship and love the easiest thing in the world.

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ABSTRACT

Ozone (O_3), one of the most powerful oxidants known, is phytotoxic at high levels in the troposphere, or ground-level. Effects of acute ozone exposure for two consecutive days was examined on Bermudagrass (*Cynodon dactylon*), centipedegrass (*Eremochloa ophiuroides*), zoysiagrass (*Zoysia japonica*), St. Augustinegrass (*Stenotaphrum secundatum*), *Liriope muscari* 'Big Blue', *Liriope muscari* 'Aztec', and *Ophiopogon japonicus*. Zoysiagrass, St. Augustinegrass, *Liriope muscari* 'Big Blue' were used in the second study based on the differential responses found in the study.

Ozone induced severe visual damage to St. Augustinegrass with symptoms appearing as chlorotic streaks. St. Augustinegrass and *Liriope muscari* had a significant reduction in the maximum quantum yield of PSII electron transport as measured by Fv:Fm ratio, which would indicate no correlation between the visual injury and Fv:Fm. Zoysiagrass and centipedegrass proved to be tolerant to ozone.

The objectives of the second study were to evaluate: 1) response to ozone due to cutting; 2) the use of the SPAD-502 chlorophyll meter as an objective measure of ozone-induced injury; 3) xanthophyll cycle involvement in dissipating light energy due to increased oxidative stress; 4) the relationship of chlorophyll fluorescence coefficients, chlorophyll content, and xanthophyll cycle in the regulation and protection of photosynthesis. Cutting had no significance on any of the parameters in this study.

Centipedegrass with significantly more β-carotene and a quicker engagement of the xanthophylls cycle than the other species in this study was tolerant to increased ozone. This suggests that closing the stomata to exclude ozone is important but does not repair or detoxify the ozone and/or reactive oxygen species that have already entered the leaf. Visual injury

differences in the ozone sensitive St. Augustinegrass may be due to the large thin leaves. Liriope with thick fibrous leaves is sensitive to increased ozone but lacked visual injury.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The atmosphere can be divided into several distinct vertical layers. The two major layers are the stratosphere and the troposphere. The troposphere extends from the earth's surface to about 8-16 km (4.97-9.94 miles) above the earth's surface and is where ground-level ozone is found. Ozone (O_3), one of the most powerful oxidants known, is a naturally occurring allotrope of oxygen that is phytotoxic at high levels in the troposphere (Heath, 1975). It is a secondary pollutant formed through complex photochemical oxidation reactions of carbon monoxide (CO), volatile organic compounds (VOC), and nitrogen oxides ($NO_{x)}$ in the presence of sunlight and high temperatures (U.S. EPA, 1996). The complex chemical formation of ozone is a nonlinear function involving the intensity and wavelength of sunlight, atmospheric mixing, the concentrations of the precursors in ambient air, and the rates of chemical reactions of the precursors (U.S. EPA, 2006a).

The majority of ozone, about 90%, is found in the stratosphere where it is produced by the photolysis of molecular oxygen. Some vertical mixing of stratospheric ozone does occur generally increasing ground-level ozone by less than 20 parts per billion (ppb). The current levels of troposheric ozone are rising as a direct result of anthropogenic pollutants (Colvile, 2002). Chemistry transport models indicate that increased NO_x emissions from fossil fuel combustion have had the greatest effect on ozone concentrations in the lower troposphere since the 1970's (Fusco and Logan, 2003). Comparison of present day ozone measurements to those taken at Montsouris, France that began in 1876 and continued for 34 years, indicate that ground-level ozone has more than doubled in the last 100 years (Volz and Kley, 1988).

The Clean Air Act of 1970 requires the U. S. Environmental Protection Agency to establish, review, and revise air pollution standards. The criteria for setting these standards reflect the latest scientific research on the effects of air pollutants to the environment. These standards are revised when pertinent new research has been conducted to warrant an examination of ozone exposure-related effects with possible changes in the current standards. In 1979, the primary and secondary standards were set at a daily maximum 1-hour average of ozone concentrations did not exceed 120 ppb. The national ambient air quality standards (NAAQS) were revised in July 1997 by the U.S. EPA, from a 1-hour average 120 parts per billion (ppb) to an 8-hour standard that is met when the 3-year average of the annual fourth highest daily maximum 8-h average concentration of ozone is less than or equal to 80 ppb.

Air quality standards are established to minimize the risk to human health and the environment from air pollution. There are many oxidizing air pollutants in the troposphere but the most significant in terms of health and the environment is ozone (Ashmore and Bell, 1991; Lefohn, 1992; U.S. EPA, 1996). Primary standards set limits to protect the public health, including vulnerable groups; such as children, elderly, and asthmatics. High ambient levels of ozone have been reported to cause lung inflammation, decrease immunity against infectious lung disease, acutely limit lung function, heart disease, and chronic lung disease (U.S. EPA, 2006a). Secondary standards are set to protect all other aspects of the public's interest, which includes damage to animals, crops, vegetation, and buildings (Federal Register 44 FR 8202, 1979). All air pollutants combined do not cause as much damage to plants as tropospheric ozone (Gimeno et al., 1999).

There is considerable scientific evidence in the peer-reviewed literature that ozone adversely affects vegetation (Reich and Amundson, 1985; Tingey and Hogsett, 1985; Cooley and

Manning, 1987; Reich, 1987; Heck et al., 1988; Krupa and Manning, 1988; U.S. EPA, 1996; Pell et al., 1997; Black et al., 2000; Clark et al., 2000; Elagoz and Manning, 2002; Kangasjarvi et al., 2005). Plant injury due to ozone is the result of sequential biochemical and physiological processes that result in visible foliar injury, reduced stomatal conductance, and/or reduced photosynthetic rate leading to reduced growth and yield of crops (Guderian et al., 1985). Plants can be impacted by ozone without the occurrence of visible injury thus making non-visible damage assessment methods of plant responses to ozone exposure critical (Tingey and Taylor, 1982). This includes biomass parameters of plant weight and leaf area, gas conductance, net photosynthesis, as well as the probability of future changes in appearance and marketability of ornamental plants.

Species, and even individuals within species, are known to differ in their response to ozone (Karnosky and Steiner, 1981; Berrang et al., 1986). Little research has been conducted, however, on the response of ornamental monocot species to ozone and even less on warm-season C4 turfgrass species. C4 plants could offer an advantage over C3 plants in environmental stress research because of physiological differences in photosynthesis and CO_2 assimilation. Plants with a C4 metabolism have a CO_2 compensation point at or very near zero indicating very low levels of photorespiration. The very low photorespiratory rate of C4 plants results in less competition for the reductants produced through photosynthesis. Research indicates that the ratio of the quantum yield of photosystem II ($\Phi_{PS\ II}$) to the quantum yield of CO_2 (Φ_{CO2}) assimilation of C4 plants are nearly linear even when conditions of CO_2, light, and temperature vary (Edwards and Baker, 1993). Therefore, changes in quantum yield of a C4 plant under environmental stresses are more directly attributable to these stresses. A disruption of this ratio

in C4 plants would therefore indicate a drop in electron transport involving photosystem II or carbon assimilation of CO_2 and not photorespiration.

Assessment for ozone damage to vegetation requires the detection and quantification of potential impacts. The objectives of these studies where to determine the tolerance of several commonly grown warm-season turfgrass species and two ornamental monocot groundcovers to ozone by evaluation of foliage level visible injury, chlorophyll *a* fluorescence, chlorophyll content, and carotenoid content after acute ozone exposure. Characterization of ozone induced changes in non-photochemical quenching (NPQ) in relation to changes in xanthophyll cycle pigments was investigated in species with differential ozone sensitivities. The influence of mowing on the tolerance of these species was also investigated.

1.2 Ozone Chemistry

Unlike CO, which is directly emitted into the atmosphere, ozone is a secondary pollutant formed through reactions of precursors that are emitted through natural and anthropogenic sources. Meteorology, chemical rate of reactions, half-life, type and amount of precursors determine the amount of ozone that will be formed. Computer based models have been developed to predict ozone concentrations from this complex set of factors (Angevine et al., 2006).

The major classes of compounds involved in tropospheric ozone photochemistry are CO, NO_x, and VOCs (Seinfeld, 1989). Nitric oxide (NO) and nitrogen dioxide (NO_2) rapidly inter-convert so this close association is often grouped together and referred to as NO_x. VOC refers to all carbon containing gas-phase compounds except for CO and CO_2. This includes compounds as simple as methane to more complex compounds such as isoprene and aromatic species. Important organic compounds involved in ozone formation include alkanes, alkenes, aldehydes,

ketones, alcohols, peroxides, and alkyl halides. Vegetation emits biogenic VOCs, such as isoprene, pinene, and terpenoid compounds. VOCs, such as methane, are emitted from fossil fuel combustion as well as from decomposing plant material such as leaves on the ground and dead roots in the soil. Biogenic VOCs can react with NOx emitted from anthropogenic sources, such as cars and industrial plants, to produce ozone. Many biogenic VOCs are highly reactive and are even more efficient in forming ozone than those emitted from cars and industrial plants (Neiburger et al., 1982).

Chapman (1930) first identified the basic photochemical mechanism leading to the production of ozone. Ozone is produced in this Chapman mechanism by UV radiation photolysis of O_2. Although the Chapman mechanism explains stratospheric ozone it does not account for much of the ozone found in the troposphere since most UV radiation is found in the stratosphere. Haagen-Smit and co-workers in the 1950's established that ozone formation was due to reactions of organic compounds and nitrogen oxides in the presence of solar radiation (Haagen-Smit, 1952; Haagen-Smit and Fox, 1954). The basic reactions for the formation of tropospheric ozone is referred to as photochemical smog reactions and involves thousands of chemical reactions and thousands of stable and reactive species (Finlayson-Pitts and Pitts, 2000).

Photochemical smog is a complex brew of secondary pollutants that arises from reactions involving hydrocarbons and NO_x. Some of the major components of smog are ozone, peroxyacetyl nitrate (PAN), aldehydes, and alkyl nitrates in a mixture of air borne particles and free radicals (Finlayson-Pitts and Pitts, 1986). Photolysis of NO_2 produces NO and is one of the most important reactions involved in the formation of air pollution.

1.3 Ozone Properties

Ozone is a naturally occurring allotrope of oxygen (Figure 1.1). The resonance structure is composed of one single bond and one double bond. The weak single bond is responsible for the formation of free radicals. The strong double bond is equivalent to molecular oxygen (O_2) and therefore quite stable.

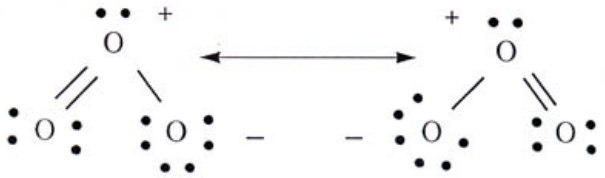


Figure 1.1 Ozone resonance structures

At standard temperature and pressure, ozone is a blue colored gas that has the distinctive smell that occurs after a thunderstorm. Ozone decomposes rapidly in pure water and is 15 times more soluble in water than oxygen (Rohschina and Roshchina, 2003). Ozone absorbs strongly in the region of 200-300-nm, or Hartley bands. It is this region that is responsible for the limiting of harmful UV-radiation reaching the earth's surface.

1.4 Oxidants and Ozone

Oxidation state refers to the net gain or loss of an electron from an atom relative to the number of electrons in its valence shell. The oxidation state of both hydrogen atoms in a water molecule is +1 because hydrogen shares its electron with the oxygen atom. The oxidation state of the oxygen atom is -2 because oxygen has gained an electron from each of the hydrogen atoms. The oxidation number for oxygen atoms is normally assigned as –2 even though the charge is not a full –2 as in O^{2-}. This convention allows for the determination of the other atoms in association with oxygen. Ozone has an oxidation state of 0 making it a strong oxidant because of its power to attract electrons thereby decreasing the oxidation state of at least one of the oxygen atoms.

The reduction of ozone results in the release of molecular oxygen and the formation of an oxygen atom having a -2 oxidation state which means that ozone has a reduction potential of 2.07 V (Figure 1.2). This value is greater than the reduction potentials of almost all other materials and second among elements only to fluorine. Therefore, the ability of ozone to oxidize almost all other species is thermodynamically favorable.

$$2H^+ + 2e + O_3 \rightarrow O_2 + H_2O \quad E^o = 2.07 \text{ V}$$

Figure 1.2 Reduction of ozone

1.5 Ozone in the Troposphere

The major constituents of the tropospheric layer's atmosphere are nitrogen, oxygen, and argon. These elements constitute 99.9% of the atmosphere and are not significantly influenced by human activity. Trace gases, however, such as carbon dioxide (CO_2), methane (CH_4), ozone (O_3), and nitrogen oxides (NO_x) have been increasing due to anthropogenic processes. Changes in land use, population, and the industrial revolution have significantly increased the emission of trace gases during the last 150 years (Seiler, 1974; Crutzen, 1995). The largest contributor to the NO_x budget is fossil fuel burning (Table 1.1). Emissions from the burning of fossil fuels produce the precursors that lead to the formation of the air pollutant ozone.

Ozone is not emitted but formed through complex reactions involving free radicals and solar radiation (Figure 1.3). The main sources of ozone in the stratosphere are ultraviolet irradiation of the atmosphere and electrical discharge during thunderstorms (Fisherman et al., 1979). This layer of ozone in the stratosphere absorbs ultraviolet radiation in the range of 200-360 nm wavelengths that is dangerous for life on earth and also protects the thermal balance of the planet by its absorption of infrared energy radiated from the earth (Baird, 1995).

Table 1.1 NO_x emission sources in United States in 1999.

Source of Precursor	Emissions of NO_2 (10^{12} g/yr)	% Breakdown of Source
Fossil fuel combustion	9.1	Electric utilities 57%; industry 31%,; commercial, institutional, and residential combustion 12%
On-road vehicle exhaust	7.8	Gasoline vehicles 58%, diesel vehicles 42%
Non-road vehicle exhaust	5	Diesel vehicles 49%, gasoline vehicles 3%, railroads 22%, marine vessels 18%, other 8%
Natural sources[1]	3.1	Lightning 50%, soils 50%
Industrial processes	0.76	Mineral products 43%, petrochemical products 17%, chemical manufacturing 16%, metal processing 11%, other industries12%
Biomass burning	0.35	Residential wood burning 11%, open burning 8%, wildfires 81%
Waste disposal	0.053	Non-biomass incineration 100%

[1]Estimated on basis of data from Guenther et al., 2000. Source: U. S. EPA, 2006b.

The ability of stratospheric ozone to protect the temperature and block harmful ultraviolet radiation is extremely important to the planet. Tropospheric ozone, however, is harmful to living organisms due to its high oxidizing potential.

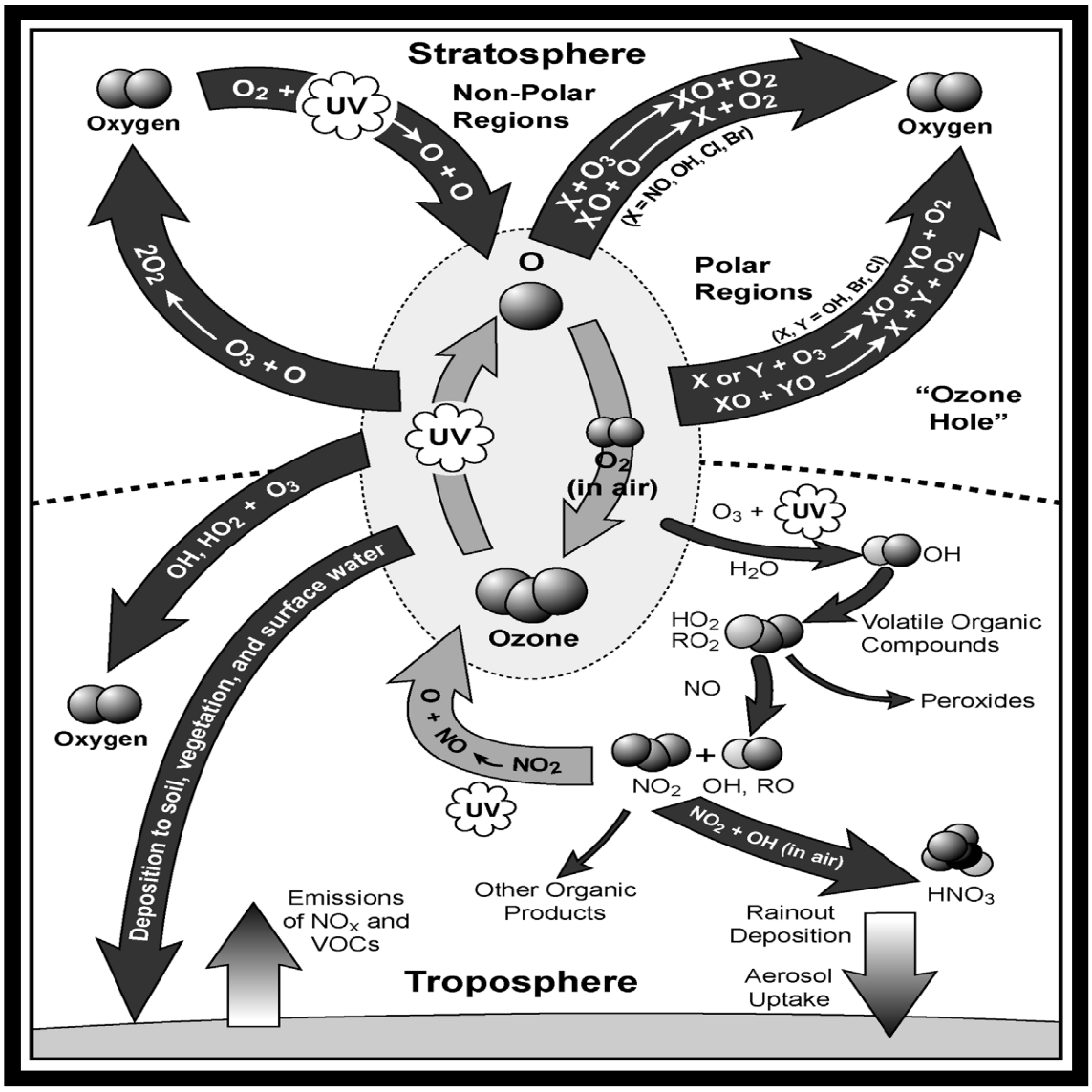


Figure 1.3 Diagram of O_3 photochemistry cycle in the atmosphere. Source: U.S. EPA, 2006b.

Atmospheric Ozone Concentrations. Air pollution has probably been a concern for as long as there have been cities. References dating back to 1257 A.D. in medieval England indicate that air contamination was a problem of great concern in London that was later attributed to the burning of coal, open sewers, and decaying refuse (Brimblecombe, 1976). By the 1930's instruments were being developed that enabled scientists to determine the trace gases involved in air pollution and to understand the mechanisms involved in urban air pollution (Haagen-Smit, 1952).

Unlike CO_2, ozone due to its reactivity and short half-life of a few hours or days is not trapped in ice to give us a record of levels prior to 200 years ago (Pritchard and Amthor, 2005). Researchers estimate that the current level of ground-level ozone has increased anywhere from 36% to 500% during the last 150 years (Volz and Kley, 1988; Hough and Derwent, 1990; Marenco et al., 1994; Prather et al., 2001). It is a generally accepted conclusion that anthropogenic sources have caused significant increases in ground-level ozone concentrations.

Emissions from biogenic sources and stratospheric injection result in a natural background level of tropospheric ozone. Background ozone concentrations are used to make decisions and policies for the national ambient air quality standards (NAAQS). The U.S. EPA, Office of Air Quality Programs and Standards (OAQPS) refers to this as Policy Relevant Background (PRB) ozone concentrations by the. Background levels distinguish between pollution levels that are from natural sources and therefore uncontrollable from those that can be controlled by U. S. governmental regulation or through diplomatic agreements with other nations.

Temporal and Spatial Ozone Variability. Ozone reactions are not limited to the location where the precursors are emitted due to meteorological processes that can transport these precursors for many miles. Lifetimes of the reactants and meteorological processes, such as air movement, lead to a very non-homogeneous distribution of ozone in the global atmosphere. This causes varying ozone concentrations that are spatial and temporal. Ozone levels vary in urban, rural and agricultural areas. The concentration of ozone also varies with the season, year, and during a 24-hour period even at the same site. The complexity of ozone chemistry and variation of concentrations at any given site make characterizing ozone concentrations at any given site difficult.

The photochemical reactions of ozone production are enhanced by summer weather in the northern hemisphere due to the increased solar radiation. Higher temperatures associated with summer weather also increase the rates of reactions involved in ozone production. The maximum ozone concentrations normally occur between June and August in areas that are influenced by precursors emitted by anthropogenic sources, such as heavy traffic or urban areas (U.S. EPA, 2006a). This is an important factor for Baton Rouge, Louisiana due to transport from other industrial areas in the region.

The May to September median of the daily 8-hour maximum ozone concentrations in the United States from 2000 to 2004 for all the counties in the United States was 49.0 ppb. Median values of daily 1-hour maximum ozone concentrations were on average much higher in large polluted urban areas, such as Houston. The Ship Channel region of Houston is one of the largest petrochemical processing complexes in the world. Houston also has the highest hourly average ozone recorded in the United States for the last five years of over 250 ppb ozone (U.S.EPA, 2006a).

The two largest sources of NO_x are electric power generation plants and motor vehicles. However, lightning, fertilized soils, and wildfires are the major natural sources of NO_x in the United States. Agricultural areas can contribute significant amounts of the NO_x precursors. Precursors that are emitted from plants and animals in an agricultural capacity are considered an anthropogenic source (U.S.EPA, 2006b). The amount of nitrification from agricultural fertilizers depends on many things such as the type of fertilizer, type of crop, soil moisture, and temperature. The best management practice of no-till cultivation could greatly decrease the amount of NO_x emitted from agricultural soils (Civerolo and Dickerson, 1998)

Another feature of the spatial and temporal pattern of ozone concentration is the diurnal rise and fall of ozone formation. Areas with ozone formation associated with anthropogenic sources experience maximum values in the early afternoon (Lefohn, 1992). The 8-h daily maximum usually occurs between 10 a.m. and 6 p.m. in this situation (U.S. EPA, 2006b).

1.6 Regional Ozone

Most sites across the country, with the exception of California due areas of extremely high pollution, have similar ozone distributions at the 95th percentile (Hogsett et al., 1987). In Baton Rouge, Louisiana from 2001 to 2005, 95% of the hourly ozone concentrations were 60 ppb or less (Figures 1.4).

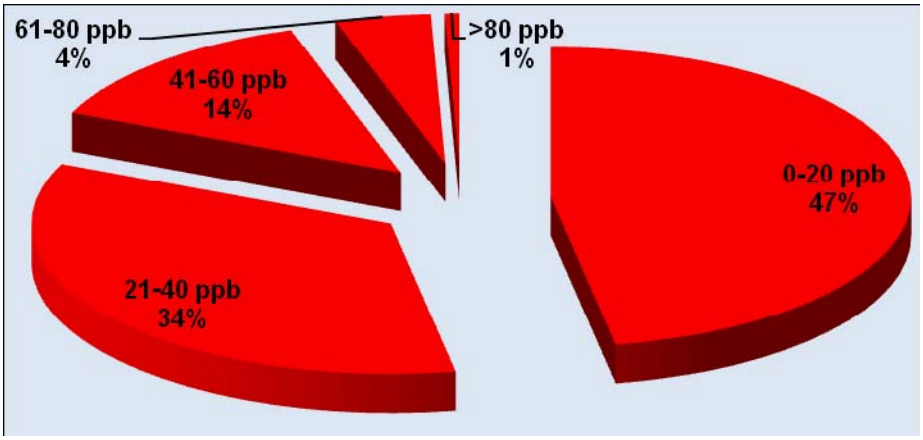


Figure 1.4 Percentage of ozone levels (ppb) at specified levels in Baton Rouge, Louisiana, 2001-2005. Source: LA DEQ (Louisiana Department of Environmental Quality) Air Quality Division Database.

Concentrations of ozone above 80 ppb are rare, 1.27% of the average hourly concentrations in Baton Rouge from 2001-2005 (Table 1.2). These higher concentrations, or episodes, last for only a few hours and are followed by long respite periods.

Table 1.2 Frequency of 1-hour ozone averages for specified ppb at each hour from 2001-2005 in Baton Rouge, Louisiana taken from LSU monitoring site data. Occurrences of over 80 ppb are noted in box.

Hour	0-20 ppb	21-40 ppb	41-60 ppb	61-80 ppb	81-100 ppb	101-120 ppb	121-140 ppb	141-160 ppb	161-180 ppb	Total hourly occurrence
0	1074	498	65	1	0	0	0	0	0	1638
1	1151	435	49	2	0	0	0	0	0	1637
2	1194	407	34	2	0	0	0	0	0	1637
3	1250	360	24	3	0	0	0	0	0	1637
4	1300	323	11	3	0	0	0	0	0	1637
5	1365	263	6	3	0	0	0	0	0	1637
6	1327	298	9	2	0	0	0	0	0	1636
7	1093	477	59	6	0	0	0	0	0	1635
8	748	677	178	29	2	0	0	0	0	1634
9	441	779	322	82	5	2	0	0	0	1631
10	261	794	401	149	169	5	0	0	0	1779
11	191	747	454	187	42	7	0	0	0	1628
12	163	713	520	226	41	10	2	1	0	1676
13	161	673	501	225	50	9	2	1	0	1622
14	184	659	510	215	40	11	1	0	1	1621
15	226	668	489	196	32	7	1	1	0	1620
16	337	685	417	149	22	4	2	0	0	1616
17	546	625	353	76	13	2	0	0	0	1615
18	726	614	233	34	3	1	0	0	0	1611
19	858	601	133	14	1	0	0	0	0	1607
20	937	546	103	8	0	0	0	0	0	1594
21	972	505	94	1	0	0	0	0	0	1572
22	970	471	72	1	0	0	0	0	0	1514
23	692	319	44	0	0	0	0	0	0	1055
Total	18167	13137	5081	1614	420	58	8	3	1	38489

Source: LA DEQ (Louisiana Department of Environmental Quality). Air Quality Division Database, 2005.

However, even though rare, these episodes of over 80 ppb are the reason Baton Rouge is in non-attainment of the EPA's ozone standard for allowable levels of ozone concentration. In Baton Rouge most episodes last one hour but can be up to five hours in duration.

1.7 Plant Response

Several terms are in common usage when discussing air pollution. The symptoms of ozone injury and damage are characterized as acute or chronic are two such terms. Although there is no definitive ozone concentration level that distinguishes acute and chronic ozone levels, chronic is generally defined as levels exceeding the background concentration up to 100 ppb ozone and acute levels as those surpassing 100 ppb. Many research investigations have used 75 ppb when investigating chronic ozone exposures and two times ambient, generally 150-200 ppb, as the criteria for an acute ozone level (Blum and Heck, 1980; Lefohn, 1992; Black et al., 2000). It is generally accepted that injury refers to any abnormal plant response while damage is reserved for more devastating effects such as reduced yield and market value. Injury includes changes in plant metabolism that decrease plant quality (Guderian, 1977). Damage includes any quality that reduces the value of a plant such as yield, storage life, or appearance.

Any effect of ozone on plants is species dependent. With that qualification, it must also be noted that any plant will be affected if the concentration and exposure time are sufficiently high enough to disrupt cell metabolism. For each species it is a matter of the level and duration of ozone exposure at which injury begins to occur. Injury due to acute ozone exposure involves the death of the cells and develops within a few hours or days after ozone exposure. Chronic ozone exposure symptoms may include stippling, premature leaf senescence, and early leaf fall that develops within a few days or weeks following exposures to elevated ozone (Skelly et al., 1999).

Short-term oxidative stress caused by ozone results in visual injury to plants (Becker et al., 1989; Chappelka and Samuelson, 1998; Bungener et al., 1999). Long-term oxidative stress will result in reduced root and shoot growth as well as lower yields (Davison and Barnes, 1998;

Black et al., 2000). Exposures of a few hours or less at low levels of 50 to 100 ppb effect cell permeability and cell wall disruption in extremely sensitive species. Several days of low levels or a few hours of greater than 100 ppb cause damage to primary and secondary metabolism.

Chronic ozone exposure usually results in reduced plant growth and early senescence that may be due to the breakdown of chlorophyll or its metabolites (Skelly et al., 1999). Acute ozone exposure for short periods of time is known as ozone episodes. Acute exposure usually results in visible foliar injury to sensitive plants and may included chlorotic mottle, fleck, stipple, chlorophyll degradation, premature senescence, or the death of the cells leading to necrotic areas, which develop within a few hours or days after ozone exposure (Arbaugh et al., 1998; Staszak et al., 2004). Although visual injury is usually assessed as an indicator of ozone sensitivity that could lead to decreased growth and yield, in the case of ornamentals visual injury is of itself an important economic consideration.

Levels of over 60 ppb ozone can cause distinct visible injury due to cell and tissue death in the mesophyll cells. It is not a coincidence that the resulting necrotic lesions resemble hypersensitive response in appearance because they have many molecular and physiological features in common (Kangasjarvi et al., 1994; Rao et al., 2000). The response of vascular plants to environmental stress involve the plants ability (1) to avoid ozone by stomatal control of entry into the plant intercellular air space (2) detoxify and degradation of ozone and ROS by apoplastic antioxidants (3) control of cell death by regulation of programmed cell death (PCD) and (4) to complete repairs caused by the stressor.

Until the 1940's it was believed that ozone could only be created by photo dissociation of molecular oxygen, which occurs in the stratosphere at wavelengths of 240 nm or shorter (Chapman, 1930). This meant that ozone in the troposphere was thought to be due to mixing of

the stratospheric ozone. In the mid-1940's new types of plant disorders began appearing in the east and west coast of the United States (Middleton et al., 1956). Tobacco in the east developed symptoms called 'weather speck' with similar symptoms being found in spinach, endive, and romaine in the Los Angeles, California area. Other crops and symptoms of lesser extent were also observed that resulted in leaf yellowing, defoliation, and loss of yield. Ozone, found in high concentrations in Los Angeles smog, was found to cause plant damage after severe vegetable damage occurred in the area (Haagen-Smit, 1952). By the late 1950's, ozone injury to plants due to anthropogenic sources, mainly traffic and power plants, was widely accepted in the United States (Heggestad and Middleton, 1959; Millecan, 1971).

Species Tolerance to Ozone. Plants generally react to stress by displaying typical symptoms. Some symptoms are typical regardless of species while others are unique to a species. Nitrogen deficiency, for instance, is presented as chlorosis on younger leaves of plants while plant injury due to chilling depends on the species. Chronic and acute ozone exposure will display differing injury symptoms. Visual symptoms include necrosis, leaf abscission, dwarfing, chlorosis, stippling, mottling, and flecking. Some injury may even be hidden, that is, there may be changes in a plants metabolism without any visual symptoms.

Sensitive species can display ozone injury on leaves after only a few hours of exposure to levels as low as 50 ppb ozone. Many horticultural crops were screened by the early 1970's and found to be sensitive to ozone. These include navel oranges (*Citrus sinensis*), muskmelon (*Citrullus lanatus)*, onion (*Allium cepa)*, potato (*Solanum tuberosum*), radish (*Raphanus sativus*), spinach(*Spinacia oleracea*), tomato (*Lycopersicon esculentum*), strawberry (*Frageria ananassa*), aspen (*Populus tremuloides*), oak (*Quercus coccinea*), lilac (*Syringa vulgaris*), petunia (*Petunia integrifolia*), begonia (*Begonia semperflorens*), carnation (*Dianthus*

caryophyllus), grape (*Vitis aestivalis*), cucumber (*Cucumis sativus)*, lettuce (*Lactuca sativa)*, blackberry (*Rubus allegheniensis*), and chrysanthemum (*Dendranthema grandiflorum*) (Thompson and Taylor, 1969; Jacobson and Hill, 1970; Ormrod et al., 1971; Adedipe et al., 1972;). Many field crops such as corn (*Zea mays*) and cotton (*Gossypium hirsutum*) are also severely impacted by elevated ozone. Cotton shoot biomass is reduced by 75% at 150 ppb and even lower levels can reduce leaf biomass by 50% (Shrestha and Grantz, 2005). These are in addition to the species that are so sensitive to ozone that they were first to indicate a problem with elevated ozone. The most sensitive plants, affected by levels as low as 50 ppb, such as tobacco (*Nicotiana tabacum*) and lettuce, have been used extensively in remote areas as indicator plants.

It has been postulated that faster growing species are more sensitive to increased ozone levels (Harkov and Brennan, 1982; Reich, 1987; Poorter, 1998). Species with a high relative growth rate are assumed to take up more ozone than slower growing species. This would translate into a higher level of plant damage by increased ozone. Species with large thin leaves might also have a higher level of damage due to the higher internal air volume in the stomatal cavity causing more ozone to reach the apoplast. This theory has found some support in various studies (Bungener et al., 1999; Franzaring et al., 2000). A weak relationship between leaf area and growth rate has also been observed (Grime and Hunt, 1975; Davison and Barnes, 1998). This indicates that leaf morphology, such as leaf thickness, may also play a role in the sensitivity of plants to elevated ozone.

Studies conducted from the early 1950's to the 1970's found that there are marked differences in ozone tolerance among turfgrass species (Bleasdale, 1952; 1973). Visual symptoms include chlorosis, mottling, stippling, browning, and necrosis. Quackgrass (*Elymus*

repens), red fescue (*Festuca rubra*), bromegrass (*Bromus commutuatus*), and zoysia (*Zoysia japonica*) were found to be the most insensitive to ozone exposure (Brennan and Halisky, 1970). Annual bluegrass (*Poa annua*) and bentgrass (*Agrostis palustris*), which are cool-season turfgrasses, are the most sensitive of the turfgrass species (Brennan and Halisky, 1970). Sensitivity of these species was found to be correlated with temperature as warmer temperatures decreased the amount of time for symptoms to develop. These changes were also found to be correlated with the opening of stomata. Brennan and Halisky (1970) also found that bermudagrass and zoysia, both warm-season grasses, were the most tolerant to ozone exposure.

Little research has been conducted, however, on the response of ornamental monocot species and other warm-season C4 turfgrass species to elevated ozone levels. A factor that has also received little attention and may alter the response of turfgrasses is the practice of mowing. Mowing is one of the most important cultural practices of turfgrass. The frequency and intensity of mowing affect every other cultural practice. The amount of fertilizer and irrigation are directly influenced by the mowing regime. Each turfgrasss species has a range of tolerance for the optimal mowing height. Mowing below this range creates a turf that is weaker and more sensitive to environmental stresses and diseases.

Stomata and Leaf Surface. Ozone penetrates the leaves and stems of plants by a diffusion gradient of concentrations into open stomata and enters the intercellular space where it contacts the mesophyll cells (Heath, 1975). Reduction of stomatal conductivity reduces the amount of ozone damage to plants (Ormrod and Hale, 1995). The primary route for ozone penetration into plants is the stomata. Stomatal closure would provide a mechanism for the avoidance of ozone flux but would also cause stress to the plant by limiting CO_2 uptake. Interacting factors make it difficult to distinguish between direct effects of ozone on the guard cells and indirect ones

caused by lowering gas exchange and therefore photosynthesis. It is a generally held belief due to numerous studies that have failed to provide evidence of a direct response that stomatal control is regulated by the indirect lowering of photosynthesis (Sheng and Chevone, 1988; Winner et al., 1988). Another entrance route into the plant is by the direct penetration of the epidermal cuticle into the mesophyll cells. Ozone entrance into every cell can only be accomplished by penetration through a cell wall, an extracellular space between the cell wall and the plasma membrane, and finally through the plasmalemma to reach the cytoplasm.

Ozone exposure causes a decline in stomatal conductivity but the effect is determined by many factors (Guderian et al., 1985). Stomatal control is influenced by internal CO_2 levels in the substomatal cavity, water status of the leaf, fluxes of ions such as K^+, and the phytohormones abscisic acid (ABA), and indoleacetic acid (IAA) (Mansfield and Freer-Smith, 1984). Varying degrees of stomatal closure and conductance following ozone exposure have been reported (Lehnherr et al., 1987; Guidi et al., 2001). Increased stomatal opening occurs when there is increased humidity and decreases with decreased water availability to plants (Otto and Daines, 1969; Treshow, 1984). Research has found that after ozone exposure of rice (*Oryza sativa*) the endogenous levels of abscisic acid (ABA) are increased resulting in stomata close (Fletcher et al., 1972). Ozone-tolerant plant species have been found to have higher endogenous level of this plant hormone (Jeong et al., 1980).

Cuticular permeability and the resulting rate of ozone destruction have been determined for several plant cuticles (Kerstiens and Lendzian, 1989). The destruction of ozone as it penetrates the cuticle makes leaves with thicker cuticles less susceptible to further damage of internal cell organelles. The rate of ozone absorption through the cuticle as compared to open stomata, however, is about 1/10000 even in the most permeable plant cuticles. This indicates that

ozone-induced changes on a plant's cuticle are minimal and would not be expected to cause much effect even in the most permeable membranes under natural conditions (Kerstiens and Lendzian, 1989).

Apoplast and Membranes. Ozone interaction with membranes is governed by the structure of the membrane. Membranes are a diverse arrangement of lipids and proteins held together by non-covalent bonds. Organelles are compartments within the cell. Each membrane, cellular or organelle, has a different composition of lipids and proteins specific to the operation of that membrane. Membranes are semi-permeable to solutes and permit energy requiring reactions to occur by active transport across a concentration gradient. The concentration gradient is also harnessed into chemical energy in the form of ATP. Ozone has been found to disrupt this process by inactivating the Mg^{2+}-dependent and K^{+}-stimulated plasma membrane ATPases that are associated with the ion pumps on the membrane, possibly by reacting with the sufhydryl groups on these proteins (Dominy and Heath, 1985).

Ozone reacts with the unsaturated chains of membrane lipids at the double bonds by the Criegee reaction (Criegee, 1975). This reaction forms ozonides from alkenes and ozone by the cycloaddition of ozone into a double bond creating intermediate ozonides that are then broken down into carbonyl compounds and peroxides, which include hydrogen peroxide (H_2O_2), superoxide (O_2^{-}), peroxy radicals ($HO_2^{\cdot)}$, and hydroxyl radicals ($HO^{\cdot}$). These free radicals can then cause lipid peroxidation. Both ozonolysis and lipid peroxidation can produce malondialdehyde. Oleic acid, however, only undergoes ozonolysis, while linoleic and linolenic acids can undergo lipid peroxidation or ozonolysis (Roschina and Roschina, 2003). Ozone can, therefore, initiate a direct attack on membranes or an indirect attack by the formation of free radicals.

The first response of plants to ozone was thought to be reaction with the cell membrane that would cause toxicity by lipid peroxidation and ozonolysis of the plasmalemma (Tomlinson and Rich, 1969). Research indicates, however, that this may happen only with extremely high ozone levels of 500 ppb or higher (Chimiklis and Heath, 1975). Ozone first encounters the water lined cell wall where it is quickly converted to oxy radicals and peroxides (Laisk et al., 1989). After entrance into the leaf air space ozone reacts with compounds or is dissolved into the water lining the cell wall.

Ozone damage results from the creation of reactive oxygen species (ROS) after the ozone has entered the plants apoplast (Melhorn et al., 1990). These chemical species, such as superoxide (O^-_2), the hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2) are normally present in plant cells as part of normal plant metabolism. Thus, oxidative stress is a normal function in plants. Plants are equipped to deal with this stress by means of antioxidants, enzymes, and mitochondrial dismutation of superoxide to hydrogen peroxide. Environmental stresses such as air pollution, high irradiation, salinity, and cold add to the oxidative stress experienced by plants and elicit an oxidative response. It would not be surprising to find these systems being overwhelmed by the added pressure of these environmental stresses.

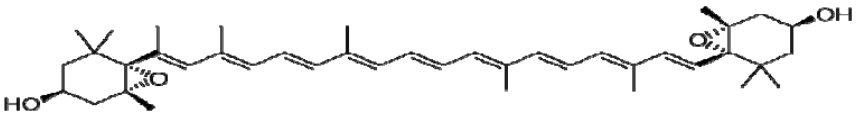
Carotenoids and Their Role in Oxidative Stress. Carotenoids are C_{40} tetraterpenoids built from eight C_5 isoprenoids joined so that the sequence is reversed in the middle of the molecule. There are over 900 carotenoids resulting from the cyclization, hydrogenation, double-bond migration, oxygenation, and isomerization of the basic C_{40} unit. Carotenoids are classified as carotenes and xanthophylls. Carotenes are pure carbohydrates and the xanthophylls are oxygenated carotenoids. Hydrocarbon carotenoids include α-carotene, β-carotene, and lycopene. Oxygenated xanthophylls include violaxanthin, zeaxanthin, and lutein (Zaripheh and Erdman, 2002) (Figure

1.5). The many roles of carotenoids include light harvesting, chlorophyll triplet quenching, singlet oxygen scavenging, dissipation of excess energy, and stabilization of the light-harvesting complex (Croce et al., 1999).

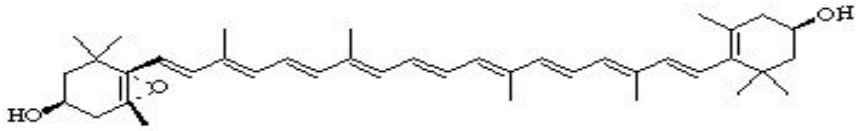
Members of both classes, along with chlorophyll, are components of the light-harvesting complex (LHC) of chloroplasts. The pigment-protein complexes are organized around the reaction centers, known as photosystem I (PSI) and photosystem II (PSII) in the thylakoid membrane (Figure 1.6). The carotenoids of the LHC act as 'funnels' in the light harvesting antennae to channel energy to chlorophyll and also away from chlorophyll during times of excessive light energy to protect the photosynthetic apparatus.

The xanthophyll cycle is ubiquitous in higher plants and for a very good reason. Plants have evolved measures to ensure protection of the photosynthetic apparatus under conditions of high light that exceeds the plants ability to use that energy in photosynthesis (Pogson et al., 1998; Niyogi et al., 1999). Excess energy transfers electrons to ground-state oxygen that leads to the production of superoxide, hydrogen peroxide, and hydroxide. These highly reactive oxygen species oxidize lipids, proteins, and pigments that lead to the destruction of thylakoid membranes and damage to structural proteins (Melis, 1999.).

Violaxanthin, antheraxanthin, and zeaxanthin, which constitute the xanthophyll cycle, play an essential role in the photoprotection of plants by the rapid promotion of thermal energy dissipation (Deming-Adams and Adams, 1992; Niyogi, 1999). This energy dissipation is often referred to as non-photochemical quenching (NPQ) of chlorophyll fluorescence (Maxwell and Johnson, 2000).



a) violaxanthin



b) antheraxanthin

c) zeaxanthin

Figure 1.5. Molecular structure of xanthophyll cycle carotenoids a) double epoxide groups on violaxanthin b) de-epoxidation of violaxanthin results in antheraxanthin c) zeaxanthin results from further de-epoxidation of antheraxanthin. Source: Demmig-Adams, 2003.

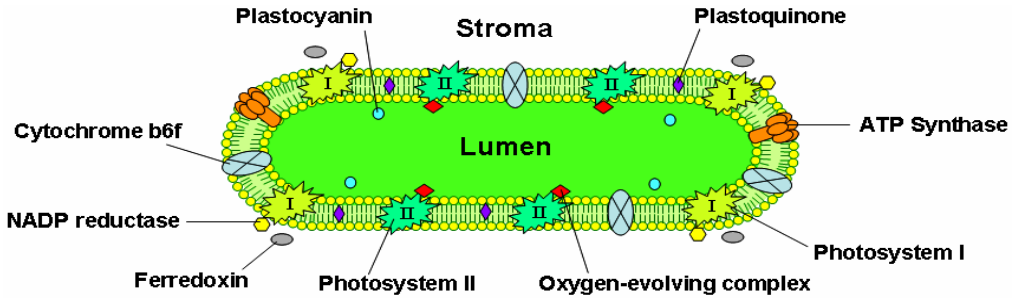


Figure 1.6. Thylakoid with embedded and peripheral enzyme/protein complexes. Source: Klass, 2004.

Objectives. Although visual damage is usually assessed as an indicator of ozone sensitivity that could lead to decreased growth and yield, in the case of ornamentals visual damage is of itself an important economic consideration. Damage assessment of ozone to vegetation requires the detection and quantification of potential impacts. Photosynthesis is a good indicator of a plant's

stress tolerance to environmental changes. The objective of the preliminary study was to evaluate the sensitivity of commonly grown warm-season turfgrasses and two ornamental monocot groundcovers by means of visual assessment and chlorophyll fluorescence analysis.

The objectives of the second study were to:

1. Evaluate and compare the modification of ozone response due to cutting on PS II efficiency, chlorophyll content, and visible injury in three monocot species having differential sensitivities to ozone exposure.

2. Evaluate the use of the SPAD-502 chlorophyll meter as an objective measure of ozone-induced injury.

3. Determine if the xanthophyll cycle is involved in dissipating light energy as a consequence of increased oxidative stress due to ozone exposure.

4. Evaluate the relationship of chlorophyll fluorescence quenching coefficients, chlorophyll content, and carotenoid derived xanthophyll cycle pigments in the regulation and protection of photosynthesis when the plants are under oxidative stress.

1.8 Literature Cited

Adedipe, N., R. E. Barrett, and D. P. Ormrod. 1972. Phytotoxicity and growth responses of ornamental bedding plants to ozone and sulfur dioxide. Journal of the American Society of Horticultural Science 97: 341-345.

Angevine, W. M., M. Tjernstrom, and M. Zagar. 2006. Modeling of the coastal boundary layer and pollution transport in New England. Journal of Applied Meteorology and Climatology 45:137-154.

Arbaugh, M.J., P. R. Miller, J. J. Carroll, B. Takemoto, T. Procter. 1998. Relationships of ozone exposure to pine injury in the Sierra Nevada and San Bernardino Mountains of California, USA. Environmental Pollution 101: 291-301.

Ashmore, M. R. and J. N. B. Bell. 1991. The role of ozone in global change. Annals of Botany 67: 39-48.

Baird, C. 1995. Environmental Chemistry. Freeman Company, New York, New York, p. 484.

Becker, K., M. Saurer, A. Egger, J. Fuhrer. 1989. Sensitivity of white clover to ambient ozone in Switzerland. New Phytologist 112: 235-243.

Berrang, P., D. F. Karnosky, R. A. Mickler, and J. P. Bennett. 1986. Natural selection for ozone response in *Populus tremuloides*. Canadian Journal of Forestry Research 16: 1214-1216.

Black, V. J., C. R. Black, J. A. Roberts, and C. A. Stewart. 2000. Impact of ozone on the reproductive development of plants. New Phytologist 147: 271-447.

Bleasdale, J. K. A. 1952. Atmospheric pollution and plant growth. Nature 169: 376-377.

Bleasdale, J. K. A. 1973. Effects of coal smoke pollution gases on the growth of ryegrass (*Lolium perenne* L.). Environmental Pollution 5: 275-285.

Blum, U. and W. W. Heck. 1980. Effects of acute ozone exposures on snap bean at various stages of its life cycle. Environmental and Experimental Botany 20: 73-85.

Brennan, E, and P. M. Halisky. 1970. Response of turfgrass cultivars to ozone and sulfur dioxide in the atmosphere. Phytopathology 60: 1544-1546.

Brimblecombe, P. 1976. Attitudes and responses towards air pollution in medieval England. Journal of the Air Pollution Control Association 26: 941-945.

Bungener, P., S. Nussbaum, A. Grub, and J. Fuhrer. 1999. Leaf injury characteristics of grassland species exposed to ozone in relation to soil moisture condition and vapor pressure deficit. New Phytologist 142: 271-282.

Chapman, S. 1930. A theory of upper-atmospheric ozone. Royal Meteorological Society 3: 103-125.

Chappelka, A. H. and L. J. Samuelson. 1998. Ambient ozone effect on forest trees of the eastern United States: a review. New Phytologist 139: 91-108.

Chimiklis, P. E. and R. L. Heath. 1975. Ozone-induced loss of intracellular potassium ion from *Chlorella sorokiniana*. Plant Physiology 56: 723-727.

Civerolo, K. and R. R. Dickerson. 1998. Nitric oxide soil emissions from tilled and untilled cornfields. Agriculture and Forest Meteorology 90: 307-311.

Clark, A. J., W. Landolt, J. B. Bucher, and R. J. Strasser. 2000. Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence index. Environmental Pollution 109: 501-507.

Colvile, R. N. 2002. Emissions, dispersion and atmospheric transformation. In: J. N. B. Bell and M. Treshow (Eds.), Air Pollution and Plant Life. John Wiley and Sons, England.

Cooley, D. R. and W. J. Manning. 1987. The impact of ozone on assimilate partitioning in plants: a review. Environmental Pollution 47: 95-113.
Criegee, Rudolf. 1975. Angewandte Chemie International Edition 14(11): 745-752.

Croce, R., S. Weise, and R. Bassi. 1999. Carotenoid binding sites of the major light-harvesting complex II of higher plants. Journal of Biological Chemistry 224: 29613-29623.

Crutzen, P. J. 1995. On the Role of Ozone in Atmospheric Chemistry. In: A. R. Bandy (Ed.), The Chemistry of the Atmosphere- Oxidants and Oxidation in the Earth's Atmosphere. The Royal Society of Chemistry, Cambridge, United Kingdom.

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

Demmig-Adams, B. 2003. Linking the xanthophyll cycle with thermal energy dissipation. Photosynthesis Research 76: 73-80.

Demmigs-Adams, B. and W. W. Adams III. 1992. Photoprotection and other responses of plants to high light stress. Annual Review of Plant Physiology and Plant Molecular Biology 43:599-626.

Dominy, P. J. and R. L. Heath, 1985. Inhibition of the K^+-stimulated ATPase of the plasmalemma of pinto bean leaves by ozone. Plant Physiology 77: 43-45.

Edwards, G. E. and N. R. Baker. 1993. Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosynthesis Research 37: 89-102.

Elagoz, V. and W. J. Manning. 2002. Ozone and bean plants: morphology matters. Environmental Pollution 120: 521-524.

Federal Register. 1979. National primary and secondary ambient air quality standards; revisions to the national ambient air quality standards for ozone. 44FR:8202-8237.Finlayson-Pitts, B. J. and J. N. Pitts. 1986. Atmospheric Chemistry. Fundamental and Experimental Techniques. Wiley, New York.

Finlayson-Pitts, B. J. and J.N. Pitts. 1986. Atmospheric Chemistry. Fundamental and Experimental Techniques. Wiley, New York.

Finlayson-Pitts, B .J. and J. N. Pitts. 2000. Chemistry of the Upper and Lower Atmosphere - Theory, Experiments, and Applications. Academic Press, San Diego, CA.

Fisherman, J., V. G. Brackett, and K. Fakhruzaman. 1979. Distribution of tropospheric ozone from satellite and ozone sonde measurements. Journal of Atmospheric and Terrestrial Physics 54: 589-597.

Fletcher, R. A., N. O. Adedipe, and D. P. Ormrod. 1972. Abscisic acid protects bean leaves from ozone-induced phytotoxicity. Canadian Journal of Botany. 50: 2389-2391.

Franzaring, J., A. E. G. Tonneijck, A. W. N. Kooijman, and T. A. Deuck. 2000. Growth responses to ozone in plant species from wetlands. Environmental and Experimental Botany 44: 39-48.

Fusco, A. C. and J. A. Logan. 2003. Analysis of 1970-1995 trends in tropospheric ozone at Northern Hemisphere midlatitudes with the GEOS-CHEM model. Journal of Geophysical Research 108: 10.1029/2002JD002742.

Gimeno, B. S., V. Bermejo, R. A. Reinert, Y. Zheng, andJ. D. Barnes. 1999. Adverse effects of ambient ozone on watermelon yield and physiology at a rural site in Eastern Spain. New Phytologist 144: 245-260.

Grime, J. P. and R. Hunt. 1975. Relative growth-rate: its range and adaptive significance in a local flora. Journal of Ecology 63: 393-422.

Guderian, R. 1977. Air pollution. Phytotoxicity of acidic gases and its significance in air pollution control. Ecological Studies 22: 127-136.

Guderian, R., D. T. Tingey, and R. Rabe. 1985. Effects of photochemical oxidants on plants. In: Air Pollution by Photochemical Oxidants. Springer-Verlag, Berlin. Pp. 127-333.

Guidi, L., C. Nali, G. Lorenzini, F. Filippi, and G. F. Soldatini. 2001. Effect of chronic ozone fumigation on the photosynthetic process of poplar clones showing different sensitivity. Environmental Pollution 113: 245-254.

Haagen-Smit, A. J. 1952. Chemistry and Physiology of Los Angeles Smog. Industrial Engineering Chemistry 44: 1342-1346.

Haagen-Smit, A. J. and M. M. Fox. 1954. Photochemical ozone formation with hydrocarbons and automobile exhaust. Journal of the Air Pollution Control Association 4: 105.

Harkov, R. S. and E. Brennan. 1982. An ecophysiological analysis of the responses of woody and herbaceous plants to oxidant injury. Journal of Environmental Management 15: 251-261.

Heath, R. L., 1975. Ozone and Responses of Plants to Air Pollution. Mudd, J.B., Kozlowski, T.T. (Eds). Academic Press, London, N.Y., pp. 23-25.

Heck, W. W., O. C. Taylor, and D. T. Tingey. 1988. Assessment of Crop Loss from Air Pollutants. Elsevier, London.

Heggestad, H. E. and J. T. Middleton. 1959. Ozone in high concentrations as cause of tobacco leaf injury. Science 129: 208-210.

Hogsett, W. E., D. Olszyk, D. P. Ormrod, G. E. Taylor, and D. T. Tingey (Eds.). 1987. Air Pollution Exposure Systems and Experimental Protocols: Volume 1: A Review and Evaluation of Performance. EPA-600/3-87/037a, U. S. Environmental Protection Agency, Environmental Research Laboratory Office of Research and Development, Corvallis, Oregon.

Hough, A. M. and R. G. Derwent. 1990. Changes in the global concentration of tropospheric ozone due to human activities. Nature 344: 645-648.
Jacobson, J. S. and A. C. Hill. 1970. Recognition of air pollution injury to vegetation: a pictorial atlas. Air Pollution Control Association 8: 136-139.

Jeong, J. H., H. Nakamura, and J. Ota. 1980. Physiological studies on photochemical oxidant injury in rice plants: Varietal difference of abscisic acid content and its relations to the resistance to ozone. Japanese Journal of Crop Science 49: 456-460.

Kangasjarvi, J., J. Talvinen, M. Utriainen, and R. Karjalainen. 1994. Plant defense systems induced by ozone. Plant Cell and Environment 17: 783-794.

Kangasjarvi, J., F. Jaspers, and H. Kollist. 2005. Signaling and cell death in ozone-exposed plants. Plant Cell and Environment 28: 1021-1036.

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

Kerstiens, G. and K. J. Lendzian.1989. Interactions between ozone and plant cuticles. 1. Ozone deposition and permeability. New Phytologist 112: 13-19.

Klass, J. van Wijk. 2004. Plastid proteomics. Plant Physiology and Biochemistry 42(12): 963-977.

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

Laisk, A., O. Kull, and H. Moldau.1989. Ozone concentration in leaf intercellular air spaces is close to zero. Plant Physiology 90: 1163-1167.

Lefohn, A. S. 1992. Ozone Standards and Their Relevance for Protecting Vegetation. In: A. S.

Lefohn (Ed.), Surface Level Ozone Exposures and Their Effects on Vegetation. Lewis Publishers, Chelsea, MI, p. 325.

Lefohn, A. S. and J. K. Foley. 1992. NCLAN results and their application to the standard-setting process: Protecting vegetation from surface ozone exposures. Journal of Air and Waste Management Association 42: 1046-1052.

Lehnerr, B., A. Grandjean, F. Machler, and J. Fuhrer. 1987. The effect of ozone in ambient air on ribulosebisphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. Journal of Plant Physiology 130: 189-200.

Lesley, J. W. and O. C. Taylor. 1973. Temperature and air pollution effects on early fruit production of F_2 tomato hybrids. California agriculture 1973-1972: 13-14.

Mansfield, T. A. and P. H. Freer-Smith. 1984. The role of stamata in resistant mechanisms. In: M. J. Koziol and F. R. Whatley (Eds.), Gaseous Air Pollutants and Plant Metabolism. Butterworths, London, pp. 131-146.

Marenco, A., P. Gouget, P. Nedelec, J.-P. Pages, and F. Karcher. 1994. Evidence of long-term increase in tropospheric ozone from Pic du Midi data series: Consequences: Positive radiative forcing. Journal of Geophysical Research 99: 16617-16632.

Maxwell, K. and G. N. Johnson. 2000. Chlorophyll fluorescence- a practical guide. Journal of Experimental Botany 51: 659-668.

Melhorn, H. 1990. Ethylene-promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone, and paraquat. Plant, Cell, and Environment 13: 971-976.

Melis, A. 1999. Photosystem II damage and repair cycle in chloroplasts: What modulates the rate of photodamage *in vivo*? Trends in plant Science 4: 130-135.

Middleton, J. T., A. S. Crafts, R. F. Brewer and O. C. Taylor. 1956. Plant damage by air pollution. California Agriculture, June, pp. 9-12.

Millecan, A. A. 1971. A survey and assessment of air pollution damage to California vegetation in 1970. APTD-0694, Air Pollution Control Office. United States Environmental Protection Agency. Research Triangle Park, North Carolina.

Neiburger, M., J. G. Edinger, and W. D. Bonner. 1982. Understanding our Atmospheric Environment. W.H. Freeman & Company, San Francisco, CA.

Niyogi, K. 1999. Photoprotection revisited: genetic and molecular approaches. Annual Review of Plant Physiology and Plant Molecular Biology 50: 333-359.

Ormrod, D. P., O. Adedipe, and G. Hofstra. 1971. Responses of cucumber, onion, ad potato cultivar to ozone. Canadian Journal of Plant Science 51: 263-288.

Ormrod, D. P. and B. A. Hale. 1995. Physiological responses of plants and crops to ozone stress. In: M. Pessarakli (ed.), Handbook of Plant and Crop Physiology, Marcel Dekker Inc., New York, pp. 735-760.

Otto, H. W. and R. H. Daines. 1969. Plant injury by air pollutants: Influence of humidity on stomatal apertures and plant response to ozone. Science 163 (3872): 1209-1210.

Pell, E. J., C. D. E. Schlagnahfer, and R. N. Arteca. 1997. Ozone-induced oxidative stress; mechanisms of action and reaction. Plant Physiology 100: 264-273.

Pogson, B. J., K. K. Niyogi, O. Bjorkman, and D. DellaPenna. 1998. Altered xanthophyll compositions aversely effect chlorophyll accumulation and nonphotochemical quenching in *Arabiopsis* mutants. Proceedings of the National Academy of Science 95: 1332-13329.
Poorter, H. 1998. Do slow growing species and nutrient stressed plants respond relatively strongly to elevated CO_2? Global Change Biology 4:693-697.

Prather, M, D. Ehhalt, F. Dentener, R. Derwent, E. Dlugokencky, E. Holland, L. Isaksen, J. Katima, V. Kirchhoff, P. Matson, P. Midgley, and M. Wang. 2001. Atmospheric chemistry and greenhouse gases. In: Climate Change 2001: J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell, and C. A. Johnson (Eds)., The Scientific Basis, Cambridge University Press, pp.183-235.

Pritchard, S. G. and J. S. Amthor. 2005. Crops and Environmental Change. Food Products Press, New York, p. 17.

Rao, M. V. and K. R. Davis. 1999. Ozone-induced cell death occurs via two distinct mechanisms in Arapidopsis: the role of salicylic acid. Plant J. 17: 603-614.

Rao, M. V., H, Lee, R. A. Creelman, I. Raskin, J. E. Mullet, and K. R. Davis. 2000. Jasmonate perception desensitizes O_3-induced salicylic acid biosynthesis and progamed cell death in *Arabidopsis*. Plant Cell 12: 1633-1646.

Reich, P. B. 1987. Quantifying plant responses to ozone: a unifying theory. Tree Physiology 3: 63-91.

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

Roshchina, V. V. and V. D. Roshchina. 2003. Ozone and Plant Cell. Kluwer Academic Publishers, Boston.

Seiler, W. 1974. The cycle of atmospheric CO. Tellus 26: 117-135.

Seinfeld, J. 1989. Urban air pollution: state of the science. Science 243: 745-752.

Sheng, S. and B. I. Chevone. 1988. Gas exchange response of soybean cultivars to short term exposure of sulfur dioxide and ozone. Phytopathology 78: 1513.

Shrestha, A. and D. A. Grantz. 2005. Ozone impacts on tomato and nutsedge: Competition above- and below-ground. Crop Science 45: 1587-1595.

Skelly, J. M., J. L. Innes, J.E. Savage, K. R. Snyder, D. Vanderheyden, J. Zhang, and M. J. Sanz. 1999. Observation and confirmation of foliar ozone symptoms of native species of Switzerland and southern Spain. Water, Air, and Soil Pollution 116: 227-234.

Staszak, J., N. E. Grulke, and W. Prus-Glowacki. 2004. Genetic differences of *Pinus ponderosa* trees tolerant and sensitive to ozone. Water, Air, and Soil Pollution 153: 3-14.

Thompson, C. R. and O. C. Taylor. 1969. Effects of air pollutants on growth, leaf drop, and yield of citrus trees. Environmental Science and Technology 3: 934-340.

Tingey, D. T. and G. E. Taylor. 1982. Variation in plant response to ozone: a conceptual model of physiological events. In: M. H. Unsworth and D. P. Ormrod (Eds.), Effects of Gaseous Air Pollution in Agriculture and Horticulture. Butterworth, London, pp. 111-138.

Tingey, D. T. and W. E. Hogsett. 1985. Water stress reduces ozone injury via stomatal mechanism. Plant Physiology 77: 944-947.

Tomlison, H. and S. Rich. 1969. Relating lipid content and fatty acid synthesis to ozone injury of tobacco leaves. Phytopathology 59: 1284-1286.

Treshow, M. 1984. Diagnostics of the influence of air pollution and similarity of symptoms. In: Air Pollution and Plant Life. Wiley, New York. Pp. 126-143.

U.S. EPA (United States Environmental Protection Agency). 1996. Air quality criteria for ozone and other photochemical oxidants, Vol. II. EPA-600/P-93004, U. S. Environmental Protection Agency, U. S. Environmental Criteria and Assessment Office, Research Triangle Park, N. C.

U.S. EPA (United States Environmental Protection Agency). 2006a. Air quality criteria for ozone and other photochemical oxidants, Vol. I. EPA-600/R-05/004bF, U. S. Environmental Protection Agency, U. S. Environmental Criteria and Assessment Office, Research Triangle Park, N. C.

U.S. EPA (United States Environmental Protection Agency). 2006b. Air quality criteria for ozone and other photochemical oxidants, Vol. II. EPA-600/R-05/004bF, U. S. Environmental Protection Agency, U. S. Environmental Criteria and Assessment Office, Research Triangle Park, N. C.

Volz, A and D. Kley. 1988. Evaluation of the Monsouris series of ozone measurements in the nineteenth century. Nature 332: 240-242.

Winner, W. E., C. Gillespie, W. S. Shen, and H. A. Mooney. 1988. Stomatal responses to SO_2 and Ozone. In: S. Schulte-Hostede, N. M. Darrall, L. W. Blank, and A. R. Wellburn (Eds.), Air Pollution and Plant Metabolism. Elsevier, London, England, pp. 255-271.

Zaripheh, S. and J. W. Erdman. 2002. Factors that influence the bioavailability of xanthophylls. Journal of Nutrition 132(3): 531S-534S.

CHAPTER 2: SELECTED TURFGRASS AND ORNAMENTAL SPECIES TOLERANCE TO ACUTE OZONE EXPOSURE

2.1 Introduction

Ozone (O_3), one of the most powerful oxidants known, is a naturally occurring allotrope of oxygen that is phytotoxic at high levels in the troposphere (Heath, 1975). There is considerable scientific evidence in the peer-reviewed literature that ozone adversely effects vegetation (Reich and Amundson, 1985; Tingey and Hogsett, 1985; Cooley and Manning, 1987; Reich, 1987; Heck et al., 1988; Krupa and Manning, 1988; U.S. EPA, 1996; Pell et al., 1997; Chappelka, 2002). Plant injury due to ozone can result in visible foliar injury, reduced stomatal conductance, and reduced photosynthetic rate leading to reduced growth and yield of crops (Guderian et al., 1985). Plants can be impacted by ozone without the occurrence of visible injury making damage assessment of plant responses to ozone exposure critical (Tingey and Taylor, 1982). This is especially true for ornamental plants because visual injury decreases the desirability and marketability of plants. Reduced vigor and decline of plants can also result in extra inputs, such as fertilizers, that increase costs.

Turfgrass usage is extensive, including home lawns, roadsides, athletic fields, golf courses, schools, churches, parks, cemeteries, and commercial properties. Turfgrass usage in North Carolina alone is 2.1 million acres, larger than the combined corn, wheat, tobacco, and peanut acreage of the state (North Carolina Department of Agriculture, 1999). Managed turfgrass, such as golf courses, accounts for approximately 50 million acres, one-third of the nation's total acreage (National Turfgrass Federation, 2003).

Chl *a* fluorescence analysis is an effective non-destructive tool for the *in vivo* detection of stress to the photosynthetic apparatus. It is used extensively in the evaluation of ozone impacts on the effects to the photosynthetic apparatus (Guidi et al., 1997; Farage and Long, 1999; Chang

and Yu, 2001). The principle of chlorophyll α fluorescence analysis is that the light energy absorbed by chlorophyll undergoes one of three fates: it can be used in photosynthesis, dissipated as heat, or be re-emitted as light. An increase in one of these processes will therefore cause a decrease in the other two. Changes in chlorophyll fluorescence, or re-emission of light, can provide information on changes in the efficiency of photosynthesis (photochemistry) and heat dissipation (non-photochemistry). Because the reduction of photosynthesis would lead to other negative effects, such as reduced levels of carbohydrates and reduced growth, this analysis is useful in the early detection of plant stress induced by ozone (Armond et al., 1980; Fracheboud et al., 1999).

Although visual damage is usually assessed as an indicator of ozone sensitivity that could lead to decreased growth and yield, in the case of ornamentals visual damage is of itself an important economic consideration. Damage assessment of ozone to vegetation requires the detection and quantification of potential impacts. Photosynthesis is a good indicator of a plants stress tolerance to environmental changes. The objective of this study was to evaluate the sensitivity of commonly grown warm-season turfgrasses and two ornamental monocot groundcovers by means of visual assessment and chlorophyll fluorescence analysis.

2.2 Materials and Methods

An ozone fumigation study was initiated on January, 2007 at the Louisiana State University Burden Research Center located in East Baton Rouge Parish, Baton Rouge, Louisiana.

Plant Materials. Bermudagrass (*Cynodon dactylon*), centipedegrass (*Eremochloa ophiuroides*), zoysiagrass (*Zoysia japonica*), St. Augustinegrass (*Stenotaphrum secundatum*), *Liriope muscari* 'Big Blue', *Liriope muscari* 'Aztec', and *Ophiopogon japonicus* were used in this study. The

plants were transplanted into 10.16 cm containers containing 80% sand and 20% peat media two months prior to fumigation. Plants were maintained in an ozone exclusion greenhouse equipped with an ozone destruct unit (Ozone Solutions, Sioux Center, IA), supplemental lighting specifically for plant growth, dehumidifier, heater, and air conditioner to maintain temperature levels between 19°C and 29.5°C.

Ozone Exposure. Custom-built systems for growth and fumigation were built specifically for this research (Figure 2.1). The ozone exclusion greenhouse is a modified open-top field chamber modeled after structures designed for long-term studies of 'Valencia' oranges (*Citrus sinensis*) (Kats et al., 1985) and a large dome chamber designed for studies with various air pollutants (Lucas, 1985).

Figure 2.1. Ozone exclusion chamber located at Burden Research Center, Baton Rouge, Louisiana, November 2006.

UV-resistant polyethylene was used to cover an untreated pine frame. Air was circulated through the chamber by two ½ horsepower attic fans. One fan was placed in a 2.4-meter duct running into the side of the chamber with two charcoal filters placed 46-cm in front of the fan. The other was

placed at the top of the chamber to exhaust air. An air conditioning system placed to the left of the charcoal filtered air duct was added for cooling during warmer months.

The polycarbonate fumigation chamber measured 76.2 cm x by 53.3 cm x 76.2 cm (Figure 2.2). The fumigation chamber was continuously ventilated with one air exchange min^{-1}. A slightly negative pressure was maintained to limit escape of ozone from the exposure chamber into the open-top chamber. Air infiltrated the chamber by a 2.62 cm computer fan placed in a 2.62 cm opening at the top of the chamber and then directed downward through a perforated pegboard ceiling of polycarbonate placed 10 cm from the top of the chamber with 0.6 cm holes spaced 7.6 cm apart. Air was exhausted through a polycarbonate false floor 20 cm from the bottom with 0.6 cm holes spaced 7.6 cm apart and vented with an exhaust fan placed in a 2.62 cm opening in a lower corner on the opposite side of the inlet fan.

The plants were placed on a plastic-coated wire rack placed 5 cm above the lower false floor to allow for air circulation. The fumigation chamber was ventilated with a single pass of charcoal-filtered air from the exclusion chamber using 2.62 cm PVC tubing to an ozone generator box leading to the fumigation chamber. Another polycarbonate chamber housed the OMZ-420 ozone generator and relay unit (Ozone Solutions, Sioux Center, IA). The chamber was 74 cm x 49.5 cm x 35.5 cm with a 2.62 cm inlet fan in the upper left side and a 2.62 cm PVC outlet tube on the lower right side. A single pass of ozone or filtered air was delivered through the 2.62 cm outlet tube connecting the fumigation chamber to the ozone generator chamber.

Figure 2.2. Fumigation chamber designed for 2007 and 2008 ozone studies.

Temperature and relative humidity were measured at the top of the plant canopy during the entire fumigation period using three HOBO U10 loggers (Onset Computer Corporation, Bourne, MA). Ozone was monitored continuously during fumigation using an Aeroqual 500 Ozone monitor (Ozone Solutions, Sioux Center, IA). Vertical and horizontal ozone distributions were measured before the fumigation was conducted.

The fumigation chamber was used for treatments of 200 ppb ozone that was delivered during an 8-hour period (1000 to 1800 hours) and had a control level with an average of 34 ppb ozone between the periods of fumigation. Ozone was delivered for two consecutive days with an average relative humidity of 55 %. The average daylight and nighttime temperature during fumigation was 34.6° C and 17.1° C, respectively. The choice of concentration was determined by a level of ozone that is high enough to cause visible damage to a sensitive species during an acute episode but not to more tolerant species of plants (Heath, 1975). The ozone fumigation was conducted during the daylight hours when photochemical reactions result in the highest daily ozone levels. The fumigation was carried out for two consecutive days in keeping with acute ambient levels experienced in Baton Rouge, Louisiana and other urban areas (Heath, 1994).

Chlorophyll α Fluorescence Analysis. The ratio of variable to maximum chlorophyll *a* fluorescence (F_v:F_m) measurements were taken using a FMS2 modified modulated fluorometer (Hansatech Instruments, Kings Lynn, UK). Fluorescence is excited by a weak modulated beam (<0.05 μM m^1/s^1 of wavelength 655 nm) that is powerful enough to provide a reliable fluorescence analysis but not enough to drive photochemistry. Pulsed actinic light causes a transient closure of all PSII reaction centers allowing the maximum fluorescence (F_m) to be determined. The fluorescence parameters were assessed 48 hours after the start of fumigation on leaves that were dark adapted for 30 minutes. Measurements were taken on one first fully expanded leaf per pot at one-third the way down from the leaf apex.

The maximal quantum yield of PSII photochemistry (F_v/F_m) was calculated fluorescence according to Genty et al. (1989). The maximal quantum yield of PSII photochemistry is calculated as:

$$(F_v/F_m) = (F_m - F_o)/ F_m = \Phi_{PSII}/qP,$$

where F_o is the fluorescence origin, F_v is the variable fluorescence, and qP is the proportion of PSII reaction centers that are open and commonly referred to as the photochemical quenching coefficient.

A change in qP would be the result of closed reaction centers that are not able to donate electrons to the next electron acceptor in the electron transport chain. A change in the efficiency of non-photochemical quenching (i.e. fluorescence) would result in a change in (F_v/F_m). The value of (F_v/F_m) in dark-adapted plant samples is a sensitive indicator of plant photosynthetic performance and the optimal value of most plant species has been found to be near 0.83 (Bjorkman and Demming, 1987).

Visual Symptoms. Visual damage resulting from ozone fumigation was assessed 48 hours after the start of fumigation on each pot. Damage was rated by the average amount of damage to leaves on a scale of 0 for 0% visual damage, 1 for 1-25% visual damage, 2 for 26-50% visual damage, 3 for 51-75% visual damage, and 4 for 76-100% visual damage. Each sample unit had two ratings based on the relative age of the leaves, younger and older leaves.

Statistics. The treatments were arranged in a complete randomized block design with sub-sampling. For ranking and comparison of species, $LSD_{0.05}$ was computed for each treatment combination. There were three sample units (one pot for each unit) for each of the four turfgrasses and the three ornamental monocots. Two treatments consisting of a control with an average of 34 ppb and 200 ppb ozone with four replications resulting in a total of 168 potted plants. Data were tested using Analysis of Variance (ANOVA). Data were analyzed using the SAS® System for Windows version 9.0 (SAS Institute, Raleigh, NC).

2.3 Results

Visual Symptoms. Exposure to 200 ppb ozone for 8 hours on two consecutive days induced severe visual damage to St. Augustinegrass. The symptoms of damage appeared as chlorotic streaks parallel to the leaf blade commonly referred to as stipple (Figure 2.3). Young leaves had less percentage of per leaf damage than older leaves (Table 2.1). The younger leaves had 50% chlorotic streaks on each leaf. The older leaves had at least 80% chlorotic streaks per leaf in all samples. Visual damage on the St. Augustinegrass appeared before the end of the fumigation period. This was the only species in the screening study to exhibit any visual symptoms.

Figure 2.3. Chlorotic streaking on St. Augustine leaf blade due to ozone fumigation of 200 ppb, 15 January, 2007 (left) and 17 January, 2007 (right).

Table 2.1. Visual damage caused by 200 ppb ozone fumigation on various warm-season turfgrasses and ornamental monocots

Species	% leaf injury	
	Young	Old
Centipedegrass	0	0
Zoysia	0	0
Liriope muscari 'Big Blue'	0	0
Ophiopogon japonicus	0	0
St. Augustinegrass	2	4
Liriope muscari 'Aztec'	0	0
Bermudagrass	0	0

Scale: Average leaf area damaged for young and older leaves determined as 0 for 0%, 1 for 1-25%, 2 for 26-50%, 3 for 51-75%, and 4 for 76-100% leaf.

Chlorophyll α Fluorescence. There was a species, ozone, and species x ozone treatment interaction (P≤0.001) indicated by the ANOVA test. After ozone fumigation at the rate of 200 ppb the quantum efficiency value was significantly lowered in St. Augustinegrass, Bermudagrass, *Liriope muscari* 'Big Blue', *Liriope muscari* 'Aztec' and *Ophiopogon japonicas*. Although St. Augustinegrass was the only species with visual damage, it was not the only species that had a significant reduction in the Fv:Fm ratio, which would indicate no correlation between the two parameters (Table 2.2). Centipedegrass and zoysiagrass Fv:Fm ratio of 0.812 and 0.799, respectively, after two days of elevated ozone were not significantly different from the control levels and indicate that these species are not significantly affected by the ozone.

Table 2.2. Ozone effect on photosynthesis of various warm-season turfgrasses and ornamental monocots

Species	Control (Fv:Fm*)	200 ppb ozone (Fv:Fm)
Centipedegrass	0.814a	0.812a[y]
Zoysiagrass	0.812a	0.799a
St. Augustinegrass	0.806a	0.766b
Bermudagrass	0.811a	0.766b
Liriope muscari 'Big Blue'	0.805a	0.753b
Liriope muscari 'Aztec'	0.802a	0.748b
Ophiopogon japonicus	0.809a	0.748b

[y]means within columns and rows with the same letter are not significantly different at P ≤ 0.001. * Fv:Fm= ratio of variable to maximum chlorophyll *a* fluorescence

2.4 Discussion

This study gave evidence of differential responses of the species to ozone with only one species showing visual injury after two 8-hour days of elevated ozone. On the basis of the results obtained it was possible to differentiate their response to ozone. Significant differences were

observed on visual appearance and the Chl *a* fluorescence parameter. On the basis of these results it possible to distinguish between sensitive and tolerant species to acute ozone treatment. St. Augustinegrass is extremely sensitive to ozone, showing visual damage before the end of the treatment and also a significant reduction in the Fv:Fm ratio after elevated ozone exposure as compared to the control level. The decrease in the Fv:Fm ratio indicates impaired PSII electron transport and reduced photochemical efficiency. Zoysiagrass and centipedegrass proved to be tolerant as they not only had no visual damage but also had no reduction in the Fv:Fm ratio after elevated ozone exposure. The other species proved to be affected by ozone but were not as sensitive or tolerant as the other three species.

Both St. Augustinegrass and centipedegrass are C4 plants. Intuitively it would be expected that both C4 plants would be more tolerant to ozone due to their ability to concentrate CO_2 at Rubisco allowing for a higher level of photochemistry at lower stomatal conductance levels. This was not the case since centipedegrass was tolerant to ozone and St. Augustinegrass was very sensitive to ozone. A possible explanation may be the differences in relative growth rates between the two species. Centipedegrass is a very slow growing species and St. Augustinegrass is a fast growing species. Studies indicate that faster growing species are more susceptible to ozone than slower growing species (Reiling and Davison, 1992; Karlsson et al., 1997; Bortier et al., 2000).

Interestingly, the visual damage to St. Augustinegrass appears to be very similar to the St. Augustine Decline stippling caused by panicum mosaic virus. Studies are beginning to indicate that ozone-induced plant responses may be similar to pathogen-induced responses of the hypersensitive response (Kangasjarvi et al., 1994; Sandermann et al., 1998; Schraudner et al., 1998; Pellinen et al., 1999; Rao and Davis, 1999; Wohlgemuth et al., 2002; Dat et al., 2003).

These studies using the ozone-sensitive tobacco cultivar BelW3, birch, and Arabidopsis have shown that ozone induces early bursts of H_2O_2 in the cell walls (Wohlgemuth et al., 2002; Dat et al., 2003). The oxidative burst is one of the earliest actions in the plant-pathogen interactions (Bestwick et al., 1998).

Although visual damage and Chl *a* fluorescence were not correlated and changes in the efficiency of PSII can be found without visual damage, it may be that visual damage would change the Fv:Fm ratio. The fast and non-invasive method of Chl *a* fluorescence appears to be useful in detecting early events in photosynthesis immediately following ozone fumigation. Neither visual damage nor Chl *a* fluorescence are effects on plant growth and productivity that are usually associated with tolerance and sensitivity, however, and as such are not related to the long-term effects of ozone on plants (Pye, 1988).

The results of this study showed that there are differential responses in warm-season turfgrasses to ozone fumigation. It is not possible, however, to extrapolate further what the mechanisms involved are and the extent of the damage to these species. Research involving stomatal control and antioxidants may give insight into differences found between the species. Stomatal resistance is considered the main obstacle to ozone entrance into plant cells. Ozone entrance into the leaf apoplast is detoxified by ascorbate. Antioxidant levels may be a good indicator for ozone tolerance.

2.5 Literature Cited

Armond, P. A.O. Bjorkman, and L. A. Staehelin. 1980. Dissociation of Supramolecular Complexes in Chloroplast Membranes- a Manifestation of Heat Damage to the Photosynthetic apparatus. Biochimica et Biophysica Acta 601: 433-442.

Bestwick, C. S., I. R. Brown, and J W. Mansfield. 1998. Localized changes in peroxidase activity accompanying hydrogen peroxide generation during the development of a nonhost hypersensitive reaction in lettuce. Plant Physiology 118: 1067-1078.

Bjorkman, O. and B. Demming. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence at 77K among vascular plants of diverse origins. Planta 170: 489-504.

Bortier, K., L. De Temmerman, R. Ceulemans. 2000. Effects of ozone exposure in open-top chambers on poplar (*Populous nigra*) and beech (*Fagus sylvatica*): a comparison. Environmental Pollution 109: 509-516.

Chang, Y.-S. and M. R. Yu. 2001. Correlation between ozone resistance and relative chlorophyll fluorescence or relative stomatal conductance of bedding plants. Botanical Bulletin of Academia Sinica 42: 265-272.

Chappelka, A. H. 2002. Reproductive development of blackberry (*Rubus cuneifolius*) as influenced by ozone. New Phytologist155: 249-255.

Cooley, D. R. and W. J. Manning. 1987. The impact of ozone on assimilate portioning in plants: a review. Environmental Pollution 47: 95-113.

Dat, J. F., R. Pellinen, T. Beckman, B. Van De Cotte, C. Langebartels, J. Kangasjarvi, D. Inze, and f. Van Breusegem. 2003. Changes in hydrogen peroxide homeostasis trigger an active cell death process in tobacco. Plant Journal 33: 621-632.

Farage, P. K. and S. P. Long. 1999. The effects of O_3 fumigation during leaf development on photosynthesis of wheat and pea: An *in vivo* analysis. Photosynthesis Research 59: 1-7.

Fracheboud, Y., P. Haldimann, J. Leipner, and P. Stamp. 1999. Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). Journal of Experimental Botany 50: 1533-1540.

Genty, B., J.-M.Briantais, and N. R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990: 87-92.

Guderian, R., D. T. Tingey, and R. Rabe. 1985. Effects of photochemical oxidants on plants. In: Air Pollution by Photochemical Oxidants. Springer-Verlag, Berlin. Pp. 127-333.

Guidi, L., C. Nali, S. Ciompi, G. Lorenzini, and G. F. Soldatini. 1997. The use of chlorophyll fluorescence and leaf gas exchange as methods for studying the different responses to ozone of two bean cultivars. Journal of Experimental Botany 48:173-179.

Heath, R. L., 1975. Ozone and Responses of Plants to Air Pollution. Academic Press, London, N.Y., pp. 23-25.

Heath, R. L. 1994. Alterations of plant metabolism by ozone exposure. In: Alscher, R. G. and Wellburn, A. R. (Eds.). Plant Responses to the Gaseous Environment: molecular, metabolic, and physiological aspects. Chapman and Hall, London, United Kingdom.

Heck, W. W., O. C. Taylor, and D. T. Tingey. 1988. Assessment of Crop Loss from Air Pollutants. Elsevier, London.

Kangasjarvi, J., J. Talvinen, M. Utriainen, and R. Karjalainen. 1994. Plant defense systems induced by ozone. Plant Cell and Environment 17: 783-794.

Karlsson, P. E., E. L. Medin, G. Wallin, G. Selldén, L. Skärby. 1997. Effects of ozone and drought stress on the physiology and growth of two clones of Norway spruce (*Picea abies*). New Phytologist 136: 265-275.

Kats, G. D. M Olszyk, and C. R. Thompson. 1985. Open-top experimental chambers for trees. Journal of Air pollution Control Association 12: 1298-1301.

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

Lucas, P. 1985. Hemispherical Domes for Fumigation of Plants. In: W. E. Hogsett, D. Olszyk, D. P. Ormrod, G. E. Taylor, and D. T. Tingey (Eds.). Air Pollution Exposure Systems and Experimental Protocols: Volume 2: Description of Facilities. EPA-600/3-87/037b, U. S. Environmental Protection Agency, Environmental Research Laboratory Office of Research and Development, Corvallis, Oregon.

National Turfgrass Research Initiative. 2003. National Turfgrass Federation.

North Carolina Turfgrass Industry. 1999. North Carolina Department of Agriculture.

Pell, E. J., C. D. E. Schlagnahfer, and R. N. Arteca. 1997. Ozone-induced oxidative stress; mechanisms of action and reaction. Plant Physiology 100: 264-273.

Pellinen, R., T. Palva, and J. Kangasjarvi. 1999. Subcellular localization of ozone-induced hydrogen peroxide production in birch (*Betula pendula*) leaf cells. Plant Journal 20: 349-356.

Pye, J. M. 1988 impact of ozone on the growth and yield of trees: a review. Journal of Environmental Quality 17: 347-360.

Rao, M. V. and k. R. Davis. 1999. Ozone-induced cell death occurs via two distinct mechanisms in Arapidopsis: the role of salicylic acid. Plant Journal 17: 603-614.

Reich, P. B. 1987. Quantifying plant responses to ozone: a unifying theory. Tree Physiology 3: 63-91.

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

Reiling, K. and A. W. Davlson. 1992. The response of native, herbaceous species to ozone; growth and fluorescence screening. New Phytologist 120, 29–37.

Sandermann, H., D. Ernst, W. Heller, and C. Langebartels. 1998. Ozone: an abiotic elicitor of plant defense reactions. Trends in Plant Science 3: 47-50.

Schraudner, M., W. Moeder, C. Wiese, W. Van Camp, D. Inze, C. Langebartels, and H. Sandermann. 1998. Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. Plant Journal 16: 235-245.

Tingey, D. T. and G. E. Taylor. 1982. Variation in plant response to ozone: a conceptual model of physiological events. In: M. H. Unsworth and D. P. Ormrod (Eds.). Effects of Gaseous Air Pollution in Agriculture and Horticulture. Butterworth, London, pp. 111-138.

Tingey, D. T. and W. E. Hogsett. 1985. Water stress reduces ozone injury via stomatal mechanism. Plant Physiology 77: 944-947.

U.S. EPA (United States Environmental Protection Agency). 1996. Air quality criteria for ozone and other photochemical oxidants, Vol. II. EPA-600/P-93004, U. S. Environmental Protection Agency, U. S. Environmental Criteria and Assessment Office, Research Triangle Park, N. C.

Wohlgemuth, H., K. Mittelstrass, S. Kschieschan, J. Bender, H.-J. Weigel, K. Overmyer, J. Kangasjarvi, H. Sandermann, and C. Langebartels. 2002. Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. Plant Cell and Environment 25: 717-726.

CHAPTER 3: CHARACTERIZATION OF XANTHOPHYLL PIGMENTS, PHOTOSYSTEM II PHOTOCHEMISTRY AND THERMAL ENERGY DISSIPATION DURING OZONE-INDUCED STRESS OF *EREMOCHLOA OPHIUROIDES*, *STENOTAPHRUM SECUNDATUM*, AND *LIRIOPE MUSCARI*

3.1 Introduction

Ozone (O_3), one of the most powerful oxidants known, is a naturally occurring allotrope of oxygen that is phytotoxic at high levels in the troposphere (Heath, 1975). There is considerable scientific evidence in the peer-reviewed literature that ozone adversely effects vegetation (Tingey and Hogsett, 1985; Cooley and Manning, 1987; Pell et al., 1997; Ranieri et al., 2001). Plant injury due to ozone is based on sequential biochemical and physiological processes that can result in visible foliar injury and reduced photosynthetic rate leading to reduced growth and yield of crops (Ranieri et al., 2003). Plants can be impacted by ozone, however, without the occurrence of visible injury, thus making non-visual assessment methods of plant responses to ozone exposure critical (Heath, 1994). This is especially true for ornamental plants because visual injury decreases the desirability and marketability of plants.

Although visual damage is usually assessed as an indicator of ozone sensitivity that could lead to decreased growth and yield, in the case of ornamentals visual damage is of itself an important economic consideration. Visible injury includes leaf surface stippling, chlorotic mottling, or areas of necrotic tissue. All of these symptoms of ozone injury are a result of pigment loss, most notably chlorophyll. The change in chlorophyll content has been investigated frequently in studies on the effects of ozone on plants (Arbaugh et al., 1998 and Staszak et al., 2004). Decreased chlorophyll content and visual injury in turfgrass has been positively correlated (Madison and Anderson, 1963). This suggests that hand-held chlorophyll meter readings may be a more quantitative measure of ozone injury than the usual qualitative visual measure of percentage of leaf damage.

Hand-held chlorophyll absorbance meters provide a noninvasive optical method for assessing relative leaf chlorophyll levels. The use of these meters has been shown to be a reliable method for assessing photosynthetic pigment content that determine the state of photosynthetic processes in leaves (Maquard and Tipton, 1987; Netto et al., 2002; Griffin et al., 2004). Chlorophyll meters associate the relative chlorophyll content of leaves with the one-dimensional values determined by the green color intensity index of the meter (Markwell et al., 1995). The readings given by the chlorophyll meter refer to quantification of the light intensity absorbed by the sample. Chlorophyll meters measure absorbance of the leaf sample at light wavelengths of 650 nm and 940 nm. The 650 nm wavelength is strongly absorbed by chlorophyll and the 940 nm wavelength is used as a reference to adjust for differences in leaf structure (Markwell et al., 1995).

The efficient use of light by photosystem II (PSII) found in the chlorophyll can be quantified by chlorophyll fluorescence meters. Chlorophyll fluorescence measurements are used to investigate damage caused by various plant stresses. Chlorophyll α fluorescence parameters provide important information on the photochemical process of photosynthesis. At least 95% of chlorophyll fluorescence is derived from the chlorophyll molecules of PS II due to differences in the functions of the pigment groups of PSI and PSII. Measurements of chlorophyll fluorescence, therefore, reflect the efficiency of light absorption that is used to drive PSII photochemistry. The calculation of variable to maximum fluorescence (Fv:Fm) has been used extensively to evaluate the relative state of PSII.

The F_v/F_m (F_m -F_o /F_m) ratio is the most widely used variable of the fluorometer information in research using the fluorescence technique. This ratio is correlated to the photochemical efficiency of the PS II. Values corresponding to high photochemical efficiency

for photochemical processes are 0.800 ± 0.03 (Bjorkman and Demming, 1987). The minimum fluorescence (F_o) variable represents the fluorescence emission from the antenna complex before the energy reaches the photosystem reaction center. In this case, all the reaction centers are oxidized or 'open'. The F_m is the maximum fluorescence emitted when the electron carrier plastoquinone is in a reduced state or 'closed' blocking the transfer of electrons from PSII and energy is then dissipated as fluorescence.

Carotenoid levels as well as chlorophyll fluorescence measurements are a good indicator of damage to the photosynthetic apparatus. Carotenoids are involved, along with chlorophyll, in the transfer of photons to the reaction centers for use in photochemistry. A decrease in photosynthetic capacity can lead to excess energy that can result in damage to the antenna complexes or to the reaction centers (Demmig-Adams and Adams, 1992). Excess energy would cause oxidative damage by forming a triplet state chlorophyll and singlet oxygen. Photoinhibition causes a change in the PSII reaction center that results in excess energy dissipation by means of non-photochemical quenching (NPQ).

Thermal energy dissipation during periods of excessive light absorption has been well characterized in C3 plants. Excess energy can be dissipated by the antenna complexes of PSII as heat in a process known as the xanthophylls cycle, although the precise mechanism by which the xanthophylls control the energy dissipation has yet to be fully elucidated (Demmig-Adams and Adams III, 1996). Light energy moves an electron in chlorophyll to an excited, or singlet state. If this energy is not used in photochemistry it can be dissipated as heat by zeaxanthin in a process known as the xanthophylls cycle. Excess absorbed light energy can result from a number of plant stresses including cold, drought, salinity, and wounding as well as elevated ground-level ozone.

Mowing is one of the most common cultural practices of turfgrass. The frequency and intensity of mowing affect every other cultural practice. The amount of fertilizer and irrigation are directly influenced by the mowing regime. Each turfgrasss species has a range of tolerance for the optimal mowing height. Mowing below this range creates a turf that is weaker, more sensitive to environmental stresses and diseases.

Turfgrass usage is extensive, including home lawns, roadsides, athletic fields, golf courses, schools, churches, parks, cemeteries, and commercial properties. Managed turfgrass, such as golf courses, account for approximately 50 million acres and one-third of the nation's total acreage (North Carolina Department of Agriculture, 1999). Due to their widespread use many turfgrass species are grown in areas where air pollution creates an environmentally stressful condition for plant growth and development.

Studies that were conducted from the early 1950's to the 1970's found that there are marked differences in ozone tolerance among turfgrass species (Bleasdale, 1952; 1973). Several species were exposed to ozone and found to vary in tolerance from insensitive to very sensitive. Annual bluegrass and bentgrass were the most sensitive species of turfgrass, while quackgrass, red fescue, bromegrass, and zoysia were the most insensitive (Brennan and Halisky, 1970).

Although visual damage is usually assessed as an indicator of ozone sensitivity that could lead to decreased growth and yield, in the case of ornamentals visual damage is of itself an important economic consideration. Damage assessment of ozone to vegetation requires the detection and quantification of potential impacts. Photosynthesis is a good indicator of a plants stress tolerance to environmental changes.

The objectives of the study were to:

1. To evaluate and compare the modification of ozone response due to cutting on PSII efficiency, chlorophyll content, and visible injury in three monocot species having differential sensitivities to ozone exposure.

2. To evaluate the use of the SPAD-502 chlorophyll meter as a quantitative measure of ozone-induced injury.

3. To determine if the xanthophyll cycle is involved in dissipating light energy as a consequence of increased oxidative stress due to ozone exposure.

4. To evaluate the relationship of chlorophyll fluorescence quenching coefficients, chlorophyll content, and carotenoid derived xanthophyll cycle pigments in the regulation and protection of photosynthesis when the plants are under oxidative stress.

3.2 Materials and Methods

An ozone fumigation study was initiated in February, 2008 and again in October, 2008 at the Louisiana State University Burden Research Center located in East Baton Rouge Parish, Baton Rouge, Louisiana.

Plant Material. Centipedegrass (*Eremochloa ophiuroides*), St. Augustinegrass (*Stenotaphrum secundatum*), *Liriope muscari* 'Big Blue' were used in this study. *Eremochloa ophiuroides*, and *Stenotaphrum secundatum* were transplanted from a mature field into an 80% sand and 20% peat media. *Liriope muscari* 'Big Blue' was purchased as 10.16 cm (4–inch) potted plants and transplanted into the 80% sand and 20% peat media. The plants were maintained for eight weeks prior to the start of the study in an outdoor open-top ozone exclusion chamber equipped with charcoal filters and an ozone destruct unit (Ozone Solutions, Sioux Center, IA), artificial lighting, heater, and air conditioner to maintain consistent temperature and lighting.

Ozone Exposure. Custom-built systems for growth and ozone exposure were built specifically for this research (see Chapter 2). The ozone exclusion greenhouse is a modified open-top field chamber modeled after structures designed for long-term studies of 'Valencia' oranges (*Citrus sinensis*) (Kats et al., 1985) and a large dome chamber designed for studies with various air pollutants (Lucas, 1985). UV-resistant polyethylene was used to cover an untreated pine frame. Air was circulated through the chamber by two ½ horsepower attic fans. One fan was placed in a 2.4-meter duct running into the side of the chamber with two charcoal filters placed 46-cm in front of the fan. The other was placed at the top of the chamber to exhaust air. An air conditioning system placed to the left of the charcoal filtered air duct was added for cooling during warmer months.

The polycarbonate exposure chamber measured 76.2 cm x 53.3 cm x 76.2 cm. The exposure chamber was continuously ventilated with one air exchange per minute. A slightly negative pressure was maintained to limit the escape of ozone from the exposure chamber into the open-top chamber. Air infiltrated the chamber by a 2.62 cm computer fan placed in a 2.62 cm opening at the top of the chamber and then directed downward through a perforated pegboard of polycarbonate placed 10 cm from the top of the chamber with 0.6 cm holes spaced 7.6 cm apart. Air was exhausted through a polycarbonate false floor 20 cm from the bottom with 0.6 cm holes spaced 7.6 cm apart and vented with an exhaust fan placed in a 2.62 cm opening in a lower corner on the opposite side of the inlet fan.

The plants were placed on a plastic-coated wire rack placed 5 cm above the lower false floor to allow for air circulation. The exposure chamber was ventilated with a single pass of charcoal-filtered air from the exclusion chamber using 2.62 cm PVC tubing to an ozone generator box leading to the exposure chamber. Another polycarbonate chamber housed the

OMZ-420 ozone generator and relay unit (Ozone Solutions, Sioux Center, IA). The chamber was 74 cm x 49.5 cm x 35.5 cm with a 2.62 cm inlet fan in the upper left side and a 2.62 cm PVC outlet tube on the lower right side. A single pass of ozone or filtered air was delivered through the 2.62 cm outlet tube connecting the exposure chamber to the ozone generator chamber.

Temperature and relative humidity were measured at the top of the plant canopy during the entire exposure period using three HOBO U10 loggers (Onset Computer Corporation, Bourne, MA). Ozone was monitored continuously during exposure using an Aeroqual 500 Ozone monitor (Ozone Solutions, Sioux Center, IA). Vertical and horizontal ozone distributions were measured before ozone exposure was conducted.

The exposure chamber was used for treatments of 200 ppb ozone that was delivered during an 8-hour period (10:00 to 18:00 hours) and had a control level with an average of 6 ppb ozone between the periods of exposure. Ozone was delivered for four consecutive days with an average relative humidity of 45%. The average daylight and nighttime temperature during exposure was 34.4° C and 20.0° C, respectively. The choice of concentration was determined by a level of ozone that is high enough to cause visible damage to a sensitive species during an acute episode but not to more tolerant species of plants (Heath, 1975). The ozone exposure was conducted during the daylight hours when photochemical reactions result in the highest daily ozone levels.

Ozone and Cutting Treatments. The exposure chamber was used for two ozone treatment levels consisting of a scrubbed (charcoal filtered and ozone destruct unit) air low ozone control (average 6 ppb) and 200 ppb ozone for 4 days duration. There were three replications and the experiment was repeated four times. There were also three cut and three uncut plants per variety in each treatment for a total of 18 plants in each experiment resulting in a total of 144 potted

plants. Ozone was delivered during an 8-hour period of 10:00 to 18:00 hours for four consecutive days. The plants received scrubbed air for the remaining 16 hours of the day. Plants were cut immediately before the start of the exposure period and cutting heights followed the median recommended mowing height for the turfgrasses. For the coarser St. Augustinegrass this is 3 inches (7.62 cm). Centipedegrass was cut at 1.5 inches (3.81 cm). *Liriope muscari* was cut at approximately 3 inches (7.62 cm).

The experiment was repeated in November 2008 due to the loss of the pigment sample extractions that were being held at -80° C for HPLC analysis after hurricane Gustav caused power outages in Baton Rouge, Louisiana. The experiment was again conducted on *Liriope muscari*, *Eremochloa ophiuroides*, and *Stenotaphrum secundatum* and had two treatment levels of ozone consisting of an air-scrubbed low ozone control and 200 ppb ozone with 4 days duration. There were three replications. There were four cut and four uncut plants of each of the three varieties in each treatment for a total of 48 potted plants. All other factors were the same as the previous experiments.

Visual Injury and Chlorophyll Content. The degree to which species of plants develop visible foliar damage is commonly used to determine sensitivity to ozone (Davis and Coppolino, 1974; Evans et al., 1995; Ferdinand et al., 1999). Visible leaf damage due to stress results in loss of chlorophyll. This loss would be measurable with a chlorophyll meter which can non-destructively measure the total amount of chlorophyll in leaves with a high degree of accuracy (Samdur et al., 2000). Chlorophyll meters measure the ratio of light transmittance at 940 nm to light absorbed by chlorophyll at 650 nm. The results of a chlorophyll meter are a nearly linear relationship between the two wavelengths for a given species. Chlorophyll levels in a leaf are not static and change in response to environmental stresses, including increased ozone levels

(Ommen et al., 1999; Samdur et al., 2000, Lawson et al., 2001). Visible injury to leaves by ozone results in discoloration, loss of chlorophyll, and even cell death that would lead to changes in the spectral quality of the leaves. These changes may be an objective measure that better estimates of visible ozone injury than the commonly used subjective measure of leaf percentages. It may also be a reliable measure for early damage even before any visible signs of become apparent on the leaves.

Visual damage resulting from ozone exposure was assessed on each experimental unit prior to exposure and immediately following the end of exposure on days 2 and 4. Readings were taken on the first fully expanded (young) leaves and on older leaves of each experimental unit. Chlorophyll meter readings were taken immediately prior to Chl *a* fluorescence measurements. Damage was rated by the average amount of damage to leaves on a scale of 0 for 0% visual damage, 1 for 1-25% visual damage, 2 for 26-50% visual damage, 3 for 51-75% visual damage, and 4 for 76-100% visual damage. Each sample unit had two ratings based on the relative age of the leaves, younger and older leaves.

Relative chlorophyll content was determined by using a Minolta SPAD-502 chlorophyll meter (Hydro Agriculture, Immingham, UK). Measurements were taken at the same place on the leaf as the Chl *a* fluorescence measurement, one-third the way down from the leaf apex, with the values of fifteen readings per plant averaged for a single value. Readings were taken on the first fully expanded (new) leaves and on older leaves of each experimental unit. Readings were taken on days 2 and 4 immediately after the visual injury assessment on all experimental units.

Chlorophyll α Fluorescence. After measurements are taken with the SPAD-502 to determine chlorophyll content then Chl *a* fluorescence measurements were taken on all experimental units using a FMS2 modified modulated fluorometer (Hansatech Instruments, Kings Lynn, UK) on the

apical portion, one-third the way down from the leaf apex. Readings were taken immediately after the chlorophyll content determination on days 2 and 4 on all experimental units.

The ratio of variable to maximum chlorophyll *a* fluorescence (F_v:F_m) measurements were taken using a FMS2 modified modulated fluorometer (Hansatech Instruments, Kings Lynn, UK). A weak modulated beam (<0.05 μM m^1/s^1 of wavelength 655 nm) that is powerful enough to provide a reliable fluorescence analysis but not enough to drive photochemistry allows the measurement of the dark-adapted minimum fluorescence (F_o). Pulsed actinic light causes a transient closure of all PSII reaction centers allowing the maximum fluorescence (F_m) to be determined.

The maximal quantum efficiency of PSII photochemistry (F_v/F_m) was calculated according to Genty et al. (1989). The maximal quantum yield of PSII photochemistry is calculated as:

$$(F_v/F_m) = (F_m - F_o)/ F_m = \Phi_{PSII}/qP,$$

with F_o being the fluorescence origin, F_v is the variable fluorescence, and qP is the proportion of PS II reaction centers that are open and commonly referred to as the photochemical quenching coefficient.

A change in qP would be the result of closed reaction centers that are not able to donate electrons to the next electron acceptor in the electron transport chain. A change in the efficiency of non-photochemical quenching (heat dissipation) would result in a change in (F_v/F_m). The value of (F_v/F_m) in dark-adapted plant samples is a sensitive indicator of plant photosynthetic performance and the optimal value of most plant species has been found to be near 0.83 (Bjorkman and Demming, 1987). Plants under stress will exhibit lower values indicating photoinhibition. The plants were dark-adapted by covering them for 30 minutes with black

plastic sheeting. After dark adaptation, the F_m, F_o, and F_v/F_m variables was analyzed. Light adapted fluorescence parameters were calculated according to Schreiber et al. (1994). After 15 minutes of illumination, the maximum fluorescence of light-adapted leaf blades (F'_m), steady state fluorescence yield (F_s), and ground level fluorescence (F'_o) were determined. After the dark-adapted analysis, the plants were then illuminated with actinic light (200 μmol m^2/s^1) and saturating flashes of 0.7 seconds duration were applied every 1.5 minute. Non-photochemical quenching (NPQ) measures photoinhibition as a ratio of a change in F_m to the final F_m' and was calculated as:

$$NPQ = (F_m - F'_m) / F'_m.$$

The quantum efficiency of excitation energy capture by open PSII reaction centers was calculated as:

$$F'_v / F'_m = (F'_m - F'_o / F'_m).$$

The quantum efficiency of the PSII electron transport was calculated as:

$$\Phi PSII = (F'_m - F_s) / F'_m.$$

And photochemical quenching was calculated as:

$$qP = (F'_m - F_s) / (F'_m - F'_o).$$

Carotenoid Analysis. Leaf blades of 0.30-0.50 g per plant were collected immediately following chlorophyll fluorescence analysis. Plant pigments were extracted from plant tissue according to McElroy et al. (2006) under dim lighting. Tissue samples were collected for HPLC analysis of the carotenoid pigments of the xanthophyll cycle; β-carotene, violaxanthin, and zeaxanthin. Samples were collected immediately after chlorophyll fluorescence measurements on days 2 and 4 after ozone fumigation.

Tissue samples were kept on ice during extraction to guard against degradation of carotenoids (Kimura and Rodriguez-Amaya, 1999). Samples were stored in microfuge tubes at -80°C until analyzed. Plant pigments were extracted in dimmed light first by grinding tissue samples with 0.1-0.2 g autoclaved sand, 0.8 ml ethyl-β -apo-8'-carotenoate (CaroteNature, Lupsingen, Switzerland), 2.5 ml tetrahydrofuran (THF) stabilized with 2,6-di-tert-butyl-4-methoxyphenyl (BHT)**,** and 4 ml methanol. The sample was then centrifuged for 3 minutes at 500 g. The supernatant was extracted with a pasteur pipette and placed in a conical 15 ml test tube. The pellet was re-suspended in 2 ml THF stabilized with BHT and the extraction procedure was repeated until the supernatant was colorless plus one additional extraction. The pellet was then discarded and the supernatants were combined, placed on ice, and reduced to 0.5 ml under N stream. Samples were then filtered with a 0.20 μm polytetrafluoroethylene filter (Watman PTFE filter, Fisher, DE).

A Waters 2690 HPLC (Waters, Milford, MA) HPLC unit with a photodiode array detector was used for peak separation. Analysis of carotenoids was conducted using a ProntoSIL C30 reverse phase 4.6 x 250 mm column (MAC-MOD Analytical Inc., Chadds Ford, PA) with a 5.0 μm and 200-Å pore size with a 4 x 23 mm guard column (MAC-MOD Analytical Inc., Chadds Ford, PA**).** A thermostated column was used to maintain the column at 30°C. Pigment separation was conducted using an isocratic mixture of methanol/methyl-tert-butyl-ether 89:10% (v/v) plus 1% triethylamine. Eluted compound**s** from a 10 μl injection were detected at 453, 655, and 665 nm, collected, recorded, and integrated. The levels of the carotenoids β-carotene, violaxanthin, and zeaxanthin were determined. Peak assignment was determined by comparing retention times to internal standards and line spectra (250-650 nm) from the photodiode detector with the purchased standards of β-carotene, violaxanthin, zeaxanthin (ChromaDex, Irvin, CA).

Concentrations of the purchased standards were determined using quantitative spectroscopic and mass spectroscopy data (Davies and Kost, 1988). HPLC recovery rates of ethyl- β-apo-8'-carotenoate were used to estimate carotenoid losses during extraction.

Data Analysis. The treatments were arranged in a Randomized Block Design. Three replications of the experiment were conducted. Data from each variable were subjected to Analysis of Variance (ANOVA) with protected LSD at $P \leq 0.05$ for means separation. SPAD measurements correlation to visual ratings and carotenoid content correlation to fluorometer readings were measured using PROC CORR procedures for correlation coefficients (r) rather than (r^2) because the data sets are independent units of measurement (does not imply a dependent and independent variable). Data was analyzed using the SAS® System for Windows version 9.0 (SAS Institute, Raleigh, NC).

3.3 Results

Cutting. The simulated mowing effect had no significance on any of the parameters in this study (data not shown). However, cutting was done immediately prior to ozone fumigation. It is suggested that lawns be mowed the night before when ozone is expected to be high the following day. It may be possible, therefore, that cutting the plants several hours before they are placed in the ozone chambers would impart some measure of protection from the effects of ozone by initiating wounding responses in the plant.

Visible Injury and SPAD Meter Chlorophyll Measurements. St. Augustinegrass was the only species to exhibit foliar symptoms in this study. Exposure to 200 ppb ozone for 8 hours on four consecutive days induced severe visual foliar damage to St. Augustinegrass in all the replications of this study (see Chapter 2).

Correlation coefficients indicate that after two and four days of ozone exposure visual damage to St. Augustinegrass was negatively correlated to the levels of chlorophyll in the leaf and to the species at two days after ozone exposure (visual2) and after four days (visual4) as measured by the SPAD meter (Table 3.1). This is in agreement with other studies that have also found that the levels of chlorophyll are correlated to visible injury (Delgado e al., 1992; Saitanis et al., 2001).

Table 3.1 Correlation coefficients for two (2) and four (4) days after ozone treatment determined by fluorescence parameters and SPAD chlorophyll meter in January 2008 and December 2008.

	species	*O3*	*FvFm2*	*FvFm4*	*Fm2*	*Fm4*	*Fo2*	*Fo4*	*chl2*	*chl4*	*NPQ2*	*NPQ4*
species	1											
O3	0	1										
FvFm2	0.0034	-0.451	1									
FvFm4	0.0607	-0.484	0.851	1								
Fm2	-0.894*	0.1736	-0.06	-0.125	1							
Fm4	-0.8*	-0.175	0.114	0.016	0.839	1						
Fo2	-0.962*	-0.047	0.047	-0.072	0.899*	0.83	1					
Fo4	-0.867*	0.016	0.18	0.102	0.785	0.816*	0.8605	1				
chl2	0.4286*	0.899*	-0.425	-0.514	-0.24	0.384	-0.43	-0.368	1			
chl4	0.456*	0.8447*	-0.507*	-0.527*	-0.26	0.456*	-0.46*	-0.381*	0.905	1		
NPQ2	-0.566	-0.161	-0.186	-0.085	0.391	0.32	0.4961	0.36	-0.46	-0.396	1	
NPQ4	-0.762*	0.2111	-0.035	-0.085	0.649	0.392	0.733*	0.667*	-0.19	-0.185	0.568	1
visual2	0	1*	-0.451	-0.484	0.174	0.175	-0.047	0.016	0.7*	0.745	-0.161	0.211
visual4	0	1*	-0.451	-0.484	0.174	0.175	-0.047	0.016	0.7	0.745*	-0.161	0.211

*Highly significant correlations at $P \leq 0.0001$

The chlorophyll content determined by the SPAD chlorophyll meter revealed differences among the three species used in this study. After two days of 200 ppb ozone exposure St. Augustinegrass and liriope had a decrease in chlorophyll content of 42.6% and 5%, respectively (Figure 3.1). After four days of ozone exposure further decreases in chlorophyll of 9% and 5%, respectively, were observed (Figure 3.2). An increase of 18% and 30% in chlorophyll after two and four days, respectively, of ozone exposure was observed in centipedegrass. This contradicts

most studies that find that chlorophyll has decreased due to ozone injury (Reiling and Davison, 1992; Evans et al., 1995; Netto et al., 2002). It is interesting to note however, that a common effect of plant growth regulators (PGR), which have been found to protect plants from ozone injury, is either an increase in chlorophyll biosynthesis and/or a reduction of leaf expansion with normal rates of chlorophyll biosynthesis (Miller and Armitage, 2002; Steinke and Stier, 2003).

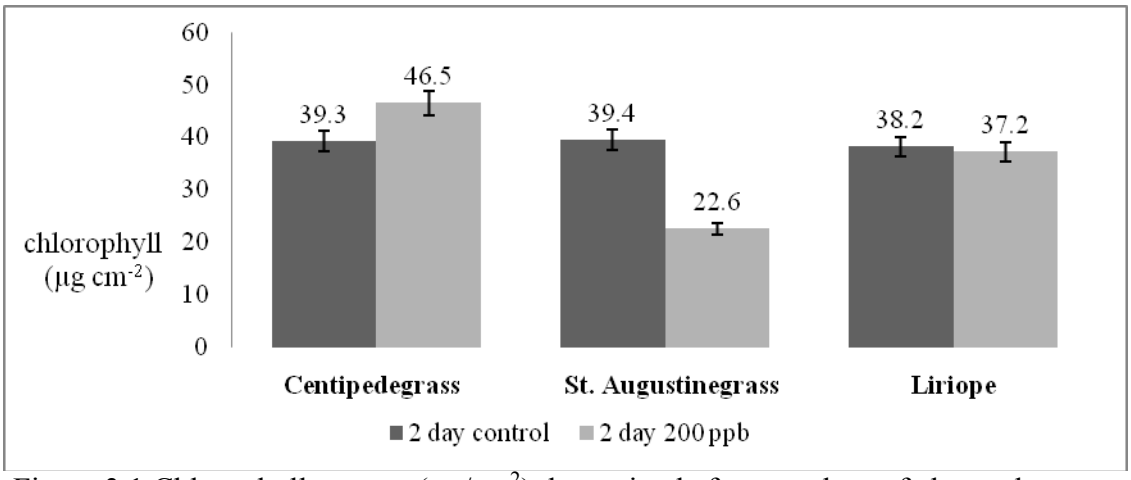


Figure 3.1 Chlorophyll content (µg/cm^2) determined after two days of elevated ozone exposure by SPAD chlorophyll meter of centipedegrass, St, Augustinegrass, and liriope January 2008 and November 2008 total averages. Vertical bars show standard error.

Chlorophyll α Fluorescence. After ozone exposure at the quantum efficiency value, or maximum quantum yield of PSII electron transport as measured by Fv:Fm, was significantly lowered in St. Augustinegrass and liriope (Table 3.2). This indicates that ozone exposure impaired the PSII mediated electron transport of both these species. The centipedegrass Fv:Fm mean ratio at two and four days after ozone exposure of 0.812 and 0.805, respectively, indicated that this species was not significantly affected by the ozone treatment and suggests a greater photochemistry capacity of centipedegrass under elevated oxidative stress due to increased ozone levels.

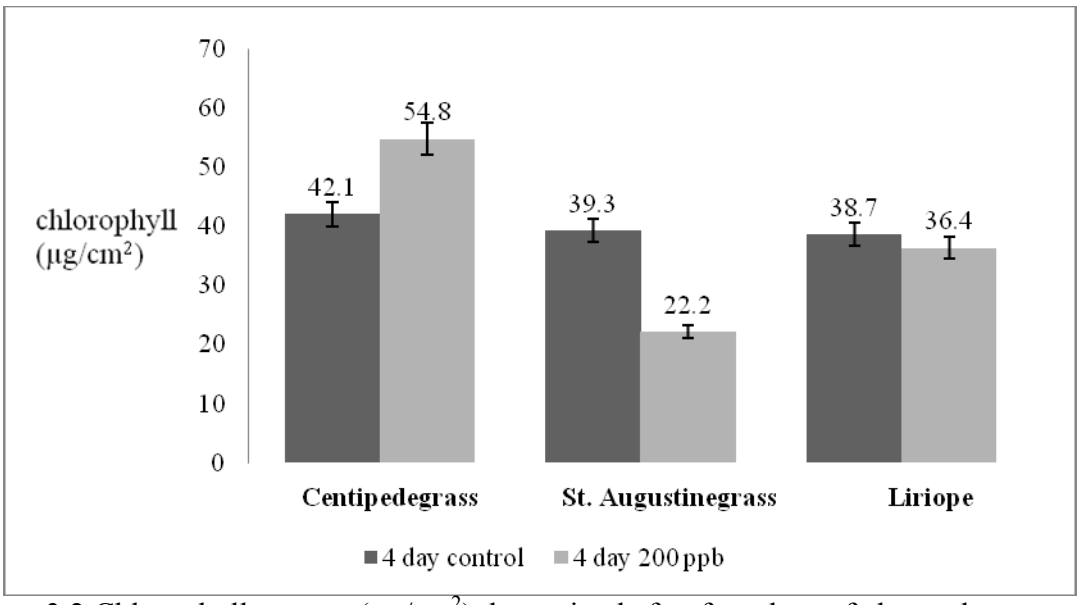


Figure 3.2 Chlorophyll content (µg/cm^2) determined after four days of elevated exposure by SPAD chlorophyll meter of centipedegrass, St, Augustinegrass, and liriope January 2008 and November 2008 total averages. Vertical bars show standard error.

The values of the initial, or ground fluorescence (F_o), was significantly different in the species both before and after ozone fumigation. The F_o in the centipedegrass was significantly lower than the levels of St. Augustinegrass and liriope both before ozone fumigation and after two and four days of ozone exposure. St. Augustinegrass had a significantly lower level F_o than liriope at the control level. The F_o level after two and four days of ozone exposure was significantly lower in centipedegrass and was increased but not significantly different in the other two species. Again, F_o is found to increase with ozone fumigation but is found to decrease with the application of PGR application (Gliozeris et al., 2007).

As seen in the F_o values, the F_m values between the species at the control level were also significantly different. In ascending order, the levels increased from centipedegrass, St. Augustinegrass, to liriope. At four days of ozone exposure there was no significant between the centipedegrass control even though the F_m value was now lower than the control level. Liriope F_m was significantly lower after two and four days of ozone exposure.

Table 3.2 Chlorophyll meter and chlorophyll fluorescence parameters determined from *Eremochloa ophiuroides* (centipedegrass), *Stenotaphrum secundatum* (St. Augustinegrass), *Liriope muscari* 'Big Blue' subjected to 2 and 4 days (200 ppb for 8 h) of ozone and filtered air January 2008 and November 2008.

	Chlorophyll		F_o		F_m		F_v:F_m		NPQ	
	2 day	4 day	2 day	4 day	2 day	4 day	2 day	4 day	2 day	4 day
Centipedegrass										
control	39.3a	42.1a	136.2a	140.0ab	580a	610a	0.821a	0.815a	0.081a	0.125a
ozone	46.5b	54.8b*	81.7b	118.0a*	907b	679a*	0.812a	0.805a	0.130a	0.114a
Liriope										
control	39.2a	40.7a	345.5c	349.3c	1939c	1810a	0.821a	0.807a	0.447bc	0.433b
ozone	37.2a	36.4a	369.2c	357.2c	1710d	1234b*	0.778b	0.711b	0.477bc	0.770c*
St. Augustinegrass										
control	39.4a	39.3a	189.2d	162.0ab	1008e	986c	0.813a	0.807a	0.368b	0.374b
ozone	22.6c	22.2c	212.3d	176.3b*	786f	962c*	0.750b	0.602c*	0.635c	0.375b*

F_o fluorescence origin, F_m fluorescence maximum, F_v:F_m ratio of variable to maximum fluorescence, NPQ non-photochemical quenching. Lower case letters indicate mean separation within column and species at $P \leq 0.01$. * Indicates significant difference between 2 day and 4 day means of each parameter at $P \leq 0.01$.

65Ozone did not change the NPQ level of centipedegrass at either the two or four day exposure indicating that the ozone treatment did not cause photoinhibition. The level of NPQ in liriope and St. Augustinegrass were not significantly different from each other before ozone exposure. After two days of ozone exposure St. Augustinegrass was the only species to have significantly higher level of NPQ. After four days of exposure liriope was the only species to have significantly higher level of NPQ.

St. Augustinegrass appeared to be the most sensitive species in this study with a significant decrease in Fv:Fm and appearance. Centipedegrass, the most tolerant species in the study, exhibited no change in Fv:Fm or appearance. This species also exhibited a very significant decrease in F_o indicating an increase in electron transport rate. Liriope was intermediate to these species with a significant decrease in the Fv:Fm and a significant increase in NPQ after four days of ozone exposure.

HPLC Carotenoid Analysis. Centipedegrass had no significant changes in β-carotene (Table 3.3). Centipedegrass did, however, have a higher endogenous level of β-carotene. Levels of β-carotene were nearly 60% and 40% higher in centipedegrass than in St. Augustinegrass and Liriope, respectively. St. Augustinegrass and liriope had significantly decreased levels of β-carotene after two days of exposure to 200 ppb ozone but after four days the levels were significantly increased bringing their β-carotene levels back to the control values. Zeaxanthin was increased in centipedegrass at both 2 and 4 days after ozone fumigation. Violaxanthin was only reduced at 4 days. Because zeaxanthin is formed by the de-epoxidation of violaxanthin it would appear that there was an increase in the biosynthesis of violaxanthin. St. Augustinegrass levels of violaxanthin were significantly decreased after two and four days of ozone exposure but zeaxanthin was only increased at two days. This may be due to the oxidation

Table 3.3 Carotenoid composition of *Eremochloa ophiuroides* (centipedegrass), *Stenotaphrum secundatum* (St. Augustinegrass), *Liriope muscari* 'Big Blue' subjected to 2 and 4 days (200 ppb for 8 h) of ozone and filtered air determined by HPLC analysis and expressed as μg g^{-1} fresh weight.

	β-carotene		Violaxanthin		Zeaxanthin		Total Carotenoids	
	2 day	4 day	2 day	4 day	2 day	4 day	2 day	4 day
Centipedegrass								
control	3895a	4274a	170a	166a	1.8a	1.6a	4066.8a	4441.6a
ozone	3776a	3994a	163a	138b*	2.6b	2.8b	3941.6a	4134.8a
Liriope								
control	2834b	2960b	169a	184a	2.2a	1.8a	3005.2b	3145.8b
ozone	1890c	2983b*	156a	127b*	2.2a	3.1b*	2048.2c	3113.1b*
St. Augustinegrass								
control	2413b	2844b	175a	174a	1.7a	1.2a	2589.7b	3019.2b
ozone	1370c	2546b*	146b	123b*	1.6a	3.3a*	1517.6c	2672.3b*

F_o fluorescence origin, F_m fluorescence maximum, F_v:F_m ratio of variable to maximum fluorescence, NPQ non-photochemical quenching. Lower case letters indicate mean separation within column and species at $P \leq 0.01$. * Indicates significant difference between 2 day and 4 day means of each parameter at $P \leq 0.01$.

of violaxanthin, which is converted to ABA (Li and Walton, 1990). This would indicate that after two days of ozone fumigation St. Augustinegrass was using violaxanthin to close the stomata and not to engage the xanthophyll cycle. After two days of ozone fumigation the violaxanthin and zeaxanthin levels were not altered in liriope. Liriope only used the antioxidant β-carotene at two days after ozone fumigation for the protection of the PSII reaction centers. After four days however, both St. Augustinegrass and liriope had significantly lower levels of violaxanthin and increased zeaxanthin indicating the engagement of the xanthophyll cycle.

3.4 Discussion

Correlation coefficients of chlorophyll measured by the SPAD meter indicated that the visual damage to St. Augustinegrass was negatively correlated to the levels of chlorophyll in the leaf. Chlorophyll levels after exposure to elevated ozone as measured by the SPAD meter appears to be a good indicator of species sensitivity and tolerance to ozone. The meter may be viable as a quantitative measure of tolerance to increased ozone levels due to an increase in chlorophyll content.

Both St. Augustinegrass and centipedegrass are C4 plants. Intuitively it would be expected that both C4 plants would be more tolerant to ozone due to their ability to concentrate CO_2 at Rubisco allowing for a higher level of photochemistry at lower stomatal conductance levels and a lack of photorespiration competing for assimilates. This was not the case however, since centipedegrass was tolerant to ozone and St. Augustinegrass was very sensitive to ozone. A possible explanation may be the differences in relative growth rates between the two species. Centipedegrass is a very slow growing species and St. Augustinegrass is a fast growing species. In fact, both

species found to be ozone tolerant in the first study are slow growing plants. Studies indicate that faster growing species are more susceptible to ozone than slower growing species (Reiling and Davison, 1992; Karlsson et al., 1997; Bortier et al., 2000).

The idea that slow growing species are more tolerant to ozone due to lower gas exchange and metabolic rates was first postulated by Harkov and Brennan (1982). It would seem logical that species with a fast growth rate would encounter higher doses of ozone and as a result show more sensitivity than slower growing species. Support for this theory is found in the meta-analysis of Hayes et al. (2007). Species with large, thin leaves have also have higher sensitivity to increased ozone due to the higher internal air volume in the stomatal cavity causing higher concentrations of ozone to reach the apoplast (Sellden et al., 1995). This study supports these theories. St. Augustinegrass is not only a fast growing species it also has large thin leaves. It may also explain why liriope with thick fibrous leaves was sensitive to ozone but had no visual injury to the leaves.

Interestingly, certain compounds with plant growth regulator properties are known to protect sensitive plant species from visible damage. It has long been known that systemic fungicides can protect sensitive species from visible damage (Manning et al., 1974). Triazole derivatives are described as sterol biosynthesis inhibitors or anti-gibberellins and are used as either fungicides or plant growth regulators (Burden et al., 1987). Fungicides, such as Bayleton, and growth regulators, such as Bonzi, exhibit both fungicidal and plant growth regulator properties (Fletcher et al., 1986). A common effect of plant growth regulators is increased chlorophyll biosynthesis. A recent study on the effects of plant growth regulators by chlorophyll fluorescence found the minimal fluorescence of plants with systemic fungicides applied was decreased (Gliozeis et al.,

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Ozone is an environmental stress factor that can cause severe damage to plants. Further work to characterize the relationship between plant hormones, such as ABA and IAA, and ozone tolerance of fast and slow growing species are needed. Short-term

studies are also warranted regarding the apparent differences in the speed in which protective mechanisms of slow and fast growing species are initiated. The levels of other antioxidants that may play a role in plant protection against increased levels of ozone need to be investigated.

3.5 Literature Cited

Antonielli, M., S. Pasqualini, L. Ederli, P. Batini, S. Moscatello, and F. Loreto. 1997. Physiological characteristics of tobacco cultivars with contrasting sensitivity to ozone. Environmental and Experimental Botany 38: 271-277.

Arbaugh, M.J., P. R. Miller, J. J. Carroll, B. Takemoto, T. Procter. 1998. Relationships of ozone exposure to pine injury in the Sierra Nevada and San Bernardino Mountains of California, USA. Environmental Pollution 101: 291-301.

Bjorkman, O. and B. Demming. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence at 77K among vascular plants of diverse origins. Planta 170: 489-504.

Bleasdale, J. K. A. 1952. Atmospheric pollution and plant growth. Nature 169: 376-377.

Bleasdale, J. K. A. 1973. Effects of coal smoke pollution gases on the growth of ryegrass (*Lolium perenne* L.). Environmental Pollution 5: 275-285.

Bortier, K., L. De Temmerman, R. Ceulemans. 2000. Effects of ozone exposure in open-top chambers on poplar (*Populous nigra*) and beech (*Fagus sylvatica*): a comparison. Environmental Pollution 109: 509-516.

Brennan, E, and P. M. Halisky. 1970. Response of turfgrass cultivars to ozone and sulfur dioxide in the atmosphere. Phytopathology 60: 1544-1546.

Burden, R. S., G. A. Carter, T. Clark, D. T. Cooke, S. J. Croker, A. H. B. Deas, P. Hedden, C. S. James, and J. R. Lenton. 1987. Comparative activity of the enantiomers of tiadimenol and paclobutrazol as inhibitors of fungal growth and plant sterol and gibberellin biosynthesis. Pesticide Science 21**:** 253-267.

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

Davies, B. H. and H. P. Kost. 1988. Chromagraphic methods for the separation of carotenoids. In: H. P. Kost, G. Zweig, and J. Sherma (Eds.) CRC handbook of chromagraphy, plant pigments. Vol. 1 Fat soluble pigments. CRC Press, Inc. Boca Raton, Florida.

Davis, D. D. and J. B Coppolino. 1974. Relative Ozone Susceptibility of Selected Woody Ornamentals. Hortscience 9(6): 537-539.

Delgado, E. J. Azon-Bieto, X. Palazon, and H. Medrano. 1992. Leaf photosynthesis and respiration of high CO_2-grown tobacco plants selected for survival under CO_2 compensation point conditions. Plant Physiology 98: 949-954.

Demmigs-Adams, B. and W. W. Adams III. 1992. Photoprotection and other responses of plants to high light stress. Annual Review of Plant Physiology and Plant Molecular Biology 43:599-626.

Demmigs-Adams, B. and W. W. Adams III. 1996. The role of the xanthophylls cycle carotenoids in the protection of photosynthesis. Trends in Plant Science 1: 21-26.

Evans, L. S., J. H. Adamski II, and J. R. Renfro. 1995. Relationships between cellular injury of leaves, and ozone exposure levels for several dicotyledonous plant species at Great Smoky Mountains National Park. Environmental and Experimental Botany 35(2): 229-237.

Ferdinand, J. A., T. S. Fredericksen, K. B. Kouterick, and J. M. Skelly. 1999. Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. Environmental Pollution 108: 297-302.

Fletcher, R. A., G Hofstra, and G. Jian-guo. 1986. Comparative fungitoxic and plant growth regulating properties of triazole derivatives. Plant Cell Physiology 27: 367-371.

Genty, B., J.-M.Briantais, and N. R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990: 87-92.

Gliozeris, S., A. Tamosiunas, and L. Stuopyte. 2007. Effect of some growth regulators on chlorophyll fluorescence in *Viola x wittrockiana* 'Wesel Ice'. Biologija 53(2): 24-27.

Griffin, J. J., T. C. Ranney, and D. M. Pharr. 2004. Photosynthesis, chlorophyll fluorescence, and carbohydrate content of *Illicium* taxa grown under varied irradiance. Journal of the American Society of Horticultural Science 129(1): 46-53.

Harkov, R. S. and E. Brennan. 1982. An ecophysiological analysis of the responses of woody and herbaceous plants to oxidant injury. Journal of Environmental Management 15: 251-261.

Hayes, F., M. L. Jones, G. Mills, and M. Ashmore. 2007. Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. Environmental Pollution 146: 754-762.

Heath, R. L., 1975. Ozone and Responses of Plants to Air Pollution. Academic Press, London, N.Y., pp. 23-25.

Heath, R. L. 1994. Possible mechanisms for the inhibition of photosynthesis by ozone. Photosynthetic Research 39: 439-451.

Karlsson, P. E., E. L. Medin, G. Wallin, G. Selldén, L. Skärby. 1997. Effects of ozone and drought stress on the physiology and growth of two clones of Norway spruce (*Picea abies*). New Phytologist 136: 265-275.

Kats, G., D. M. Olszyk, and C. R. Thompson. 1985. Open-top experimental chambers for trees. Journal of the Air Pollution Control Association 12: 1298-1301.

Kimura, M and D. R. Rodriguez-Amaya. 1999. Sources of error in the quantitative analysis of food carotenoids by HPLC. Archivos Latinoamericanos de nutricioin 49: 58s-66s.

Lawson, T., J. Craigon , A. M. Tulloch, C. R. Black, J. J. Colls, G. Landon. 2001. Photosynthetic responses to elevated CO2 and ozone in field-grown potato (*Solanum tuberosum*) Journal of Plant Physiology 158:309-323.

Li, Y. and D. C. Walton. 1990. Violaxanthin is an abscisic acid precursor in water-stressed dark-grown bean leaves. Plant Physiology 92: 551-559.

Lucas, P. 1985. Hemispherical Domes for Fumigation of Plants. In: W. E. Hogsett, D. Olszyk, D. P. Ormrod, G. E. Taylor, and D. T. Tingey (Eds.), Air Pollution Exposure Systems and Experimental Protocols: Volume 2: Description of Facilities.. EPA-600/3-87/037b, U. S. Environmental Protection Agency, Environmental Research Laboratory Office of Research and Development, Corvallis, Oregon.

Madison, J. H. and A. H. Anderson. 1963. A chlorophyll index to measure turfgrass response. Agronomy Journal 55: 461-464.

Manning, W. J., W. A. Feder, P. M. Vardaro. 1974. Suppression of oxidant injury by benomyl: effects on yields of bean cultivars in the field. Journal of Environmental Quality 3: 1-3.

Maquard, R. D. and J. L. Tifton. 1987. Relationship between extractable chlorophyll and an *in situ* method to estimate leaf greenness. Hortscience 22: 1327.

Markwell JP, N. R. Baker, M. Bradbury, and J. P. Thornber. 1995. Use of zinc ions to study thylakoid protein phosphorylation and the state 1-state 2 transitions *in vitro*. Plant Physiology 74: 348–354.

McElroy, J. S., D.A. Kopsell, J. C. Sorochan, and C. E. Sams. 2006. Response of Creeping Bentgrass Carotenoid Composition to High and Low Irradiance. Crop Science 46: 2606-2612.

Miller, A. and A. M. Armitage. 2002. Temperature, irradiance, photoperiod, and growth retardants influence production of *Angelonia angustifolia* benth. Angel Mist series. HortScience 37: 319-320.

Netto, A. T., E. Camppostrial, J. G. Oliveira, and O. K. Yamanishi. 2002. Portable chlorophyll meter for the quantification of photosynthetic pigments, nitrogen, and the possible use for assessment of the photochemical process in *Carica papaya* L. Brazil Journal of Plant Physiology 14(3): 203-210.

National Turfgrass Research Initiative. 2003. National Turfgrass Federation.

North Carolina Turfgrass Industry. 1999. North Carolina Department of Agriculture.

Ommen O. E., A. Donnelly, S. Vanhoutvin, M. van Oijen, R. Manderscheid. 1999. Chlorophyll content of spring wheat flag leaves grown under elevated CO2 concentrations and other environmental stresses within the 'ESPACE-wheat' project European Journal of Agronomy 10:197-203.

Pell, E. J., C. D. E. Schlagnahfer, and R. N. Arteca. 1997. Ozone-induced oxidative stress; mechanisms of action and reaction. Plant Physiology 100: 264-273.

Ranieri, A.,D. Giuntini. F. Ferraro, C. Nali, B. Baldan. 2001. Chronic ozone exposure induces alterations in thylakoid functionality and composition in two poplar clones. Plant Physiology and Biochemistry 39: 999-108.

Ranieri, A., D. Giuntini, F. Ferraro, C. Nali, B. Baldan, G. Lorenzini, and G. F. Soldatini. 2003. Chronic ozone induces alterations in thylakoid functionality and composition in two poplar clones. Plant Physiology and Biochemistry 39: 999-1008.

Reiling, K. and A. W. Davison. 1992. The response of native, herbaceous species to ozone; growth and fluorescence screening. New Phytologist 120, 29–37.

Saitanis, C. J., A. N. Riga-Karandinos, and M. G. Karandinos. 2001. Effects of ozone on chlorophyll and quantum yield of tobacco (*Nicotinoa tabacum* L.) varieties. Chemosphere 42: 945-953.

Samdur M. Y., A. L. Singh, R. K. Mathur, P. Manivel, B. M. Chikani BM, M. A. Khan. 2000. Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron-deficiency chlorosis. Current Science Bangalore 79:211-214.

Schreiber, U., B. Bilger, and C. Neubauer. 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. In: Ecophysiology of photosynthesis.

Sellden, G. and H. Pleijel. 1995. Photochemical oxidant effects on vegetation-response in relation to plant strategy. Water, Air, and Soil Pollution 85: 111-122.

Siefermann-Harms, D. 1987. The light harvesting and protective functions of carotenoids in photosynthetic membranes. Physiologia Plantarum 69: 561-568.

Staszak, J., N. E. Grulke, and W. Prus-Glowacki. 2004. Genetic differences of *Pinus ponderosa* trees tolerant and sensitive to ozone. Water, Air, and Soil Pollution 153: 3-14.

Steinke, K. and J. C. Stier. 2003. Nitrogen selection and growth regulator applications for improving shaded turf performance. Crop Science 43: 1399-1406.

Tingey, D. T. and W. E. Hogsett. 1985. Water stress reduces ozone injury via stomatal mechanism. Plant Physiology 77: 944-947.

CHAPTER 4. CONCLUSIONS

The results of these studies showed that there are differential responses in warm-season turfgrasses and ornamental monocots to increased levels of ozone. The first study gave evidence of differential responses of the species to ozone with only one showing visual injury at 200 ppb for two 8-hour days of fumigation. Significant differences were observed on visual appearance and the Chl *a* fluorescence parameter. On the basis of these results it possible to distinguish between sensitive and tolerant species to acute ozone treatment. St. Augustinegrass is extremely sensitive to ozone, showing visual damage before the end of the treatment and also a significant reduction in the Fv:Fm ratio. The decrease in the Fv:Fm ratio indicates impaired PSII electron transport and reduced photochemical efficiency. Zoysiagrass and centipedegrass proved to be tolerant as they not only had no visual damage but also had no reduction in the Fv:Fm ratio. The other species proved to be affected by ozone but were not as sensitive or tolerant as the other three species.

Correlation coefficients indicated that after two and four days of ozone exposure visual damage to St. Augustinegrass was highly correlated to the levels of chlorophyll in the leaf and to the species as measured by the SPAD meter. The chlorophyll content determined by the SPAD chlorophyll meter revealed differences among the three species. After two days of 200 ppb ozone exposure St. Augustinegrass and liriope had a decrease in chlorophyll content of 42.6% and 5%, respectively. After four days of ozone exposure further decreases in chlorophyll of 9% and 5%, respectively, were found. An increase of 18% and 30% in chlorophyll after two and four days, respectively, of ozone exposure was found in centipedegrass. Therefore, the meter may not only be viable as an objective

measure of injury but it may also be an indicator of tolerance to increased ozone levels due to increased chlorophyll content.

Correlation coefficients of chlorophyll measured by the SPAD meter indicated that the visual damage to St. Augustinegrass was negatively correlated to the levels of chlorophyll in the leaf. Chlorophyll levels after exposure to elevated ozone as measured by the SPAD meter appears to be a good indicator of species sensitivity and tolerance to ozone. The meter may be viable as a quantitative measure of tolerance to increased ozone levels due to an increase in chlorophyll content.

Both St. Augustinegrass and centipedegrass are C4 plants. Intuitively it would be expected that both C4 plants would be more tolerant to ozone due to their ability to concentrate CO_2 at Rubisco allowing for a higher level of photochemistry at lower stomatal conductance levels and a lack of photorespiration competing for assimilates. This was not the case however, since centipedegrass was tolerant to ozone and St. Augustinegrass was very sensitive to ozone. A possible explanation may be the differences in relative growth rates between the two species. Centipedegrass is a very slow growing species and St. Augustinegrass is a fast growing species. In fact, both species found to be ozone tolerant in the first study are slow growing plants. Studies indicate that faster growing species are more susceptible to ozone than slower growing species (Reiling and Davison, 1992; Karlsson et al., 1997; Bortier et al., 2000).

The idea that slow growing species are more tolerant to ozone due to lower gas exchange and metabolic rates was first postulated by Harkov and Brennan (1982). It would seem logical that species with a fast growth rate would encounter higher doses of ozone and as a result show more sensitivity than slower growing species. Support for this

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Ozone is an environmental stress factor that can cause severe damage to plants. Further work to characterize the relationship between plant hormones, such as ABA and IAA, and ozone tolerance of fast and slow growing species are needed. Short-term studies are also warranted regarding the apparent differences in the speed in which protective mechanisms of slow and fast growing species are initiated. The levels of other antioxidants that may play a role in plant protection against increased levels of ozone need to be investigated.

4.1 Literature Cited

Antonielli, M., S. Pasqualini, L. Ederli, P. Batini, S. Moscatello, and F. Loreto. 1997. Physiological characteristics of tobacco cultivars with contrasting sensitivity to ozone. Environmental and Experimental Botany 38: 271-277.

Bortier, K., L. De Temmerman, R. Ceulemans. 2000. Effects of ozone exposure in open-top chambers on poplar (*Populous nigra*) and beech (*Fagus sylvatica*): a comparison. Environmental Pollution 109: 509-516.

Burden, R. S., G. A. Carter, T. Clark, D. T. Cooke, S. J. Croker, A. H. B. Deas, P. Hedden, C. S. James, and J. R. Lenton. 1987. Comparative activity of the enantiomers of tiadimenol and paclobutrazol as inhibitors of fungal growth and plant sterol and gibberellin biosynthesis. Pesticide Science 21**:** 253-267.

Fletcher, R. A., G Hofstra, and G. Jian-guo. 1986. Comparative fungitoxic and plant growth regulating properties of triazole derivatives. Plant Cell Physiology 27: 367-371.

Gliozeris, S., A. Tamosiunas, and L. Stuopyte. 2007. Effect of some growth regulators on chlorophyll fluorescence in *Viola x wittrockiana* 'Wesel Ice'. Biologija 53(2): 24-27.

Harkov, R. S. and E. Brennan. 1982. An ecophysiological analysis of the responses of woody and herbaceous plants to oxidant injury. Journal of Environmental Management 15: 251-261.

Hayes, F., M. L. Jones, G. Mills, and M. Ashmore. 2007. Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. Environmental Pollution 146: 754-762.

Karlsson, P. E., E. L. Medin, G. Wallin, G. Selldén, L. Skärby. 1997. Effects of ozone and drought stress on the physiology and growth of two clones of Norway spruce (*Picea abies*). New Phytologist 136: 265-275.

Manning, W. J., W. A. Feder, P. M. Vardaro. 1974. Suppression of oxidant injury by benomyl: effects on yields of bean cultivars in the field. Journal of Environmental Quality 3: 1-3.

Reiling, K. and A. W. Davison. 1992. The response of native, herbaceous species to ozone; growth and fluorescence screening. New Phytologist 120, 29–37.

Sellden, G. and H. Pleijel. 1995. Photochemical oxidant effects on vegetation-response in relation to plant strategy. Water, Air, and Soil Pollution 85: 111-122.

Siefermann-Harms, D. 1987. The light harvesting and protective functions of carotenoids in photosynthetic membranes. Physiologia Plantarum 69: 561-568.

REFERENCES

Adedipe, N., R. E. Barrett, and D. P. Ormrod. 1972. Phytotoxicity and growth responses of ornamental bedding plants to ozone and sulfur dioxide. Journal of the American Society of Horticultural Science 97: 341-345.

Antonielli, M., S. Pasqualini, L. Ederli, P. Batini, S. Moscatello, and F. Loreto. 1997. Physiological characteristics of tobacco cultivars with contrasting sensitivity to ozone. Environmental and Experimental Botany 38: 271-277.

Arbaugh, M.J., P. R. Miller, J. J. Carroll, B. Takemoto, T. Procter. 1998. Relationships of ozone exposure to pine injury in the Sierra Nevada and San Bernardino Mountains of California, USA. Environmental Pollution 101: 291-301.

Armond, P. A.O. Bjorkman, and L. A. Staehelin. 1980. Dissociation of Supramolecular Complexes in Chloroplast Membranes- a Manifestation of Heat Damage to the Photosynthetic apparatus. Biochimica et Biophysica Acta 601: 433-442.

Ashmore, M. R. and J. N. B. Bell. 1991. The role of ozone in global change. Annals of Botany 67: 39-48.

Baird, C. 1995. Environmental Chemistry. Freeman Company, New York, New York, p. 484.

Becker, K., M. Saurer, A. Egger, J. Fuhrer. 1989. Sensitivity of white clover to ambient ozone in Switzerland. New Phytologist 112: 235-243.

Berrang, P., D. F. Karnosky, R. A. Mickler, and J. P. Bennett. 1986. Natural selection for ozone response in *Populus tremuloides*. Canadian Journal of Forestry Research 16: 1214-1216.

Bestwick, C. S., I. R. Brown, and J W. Mansfield. 1998. Localized changes in peroxidase activity accompanying hydrogen peroxide generation during the development of a nonhost hypersensitive reaction in lettuce. Plant Physiology 118: 1067-1078.

Bjorkman, O. and B. Demming. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence at 77K among vascular plants of diverse origins. Planta 170: 489-504.

Black, V. J., C. R. Black, J. A. Roberts, and C. A. Stewart. 2000. Impact of ozone on the reproductive development of plants. New Phytologist 147: 271-447.

Bleasdale, J. K. A. 1952. Atmospheric pollution and plant growth. Nature 169: 376-377.

Bleasdale, J. K. A. 1973. Effects of coal smoke pollution gases on the growth of ryegrass (*Lolium perenne* L.). Environmental Pollution 5: 275-285.

Blum, U. and W. W. Heck. 1980. Effects of acute ozone exposures on snap bean at various stages of its life cycle. Environmental and Experimental Botany 20: 73-85.

Bortier, K., L. De Temmerman, R. Ceulemans. 2000. Effects of ozone exposure in open-top chambers on poplar (*Populous nigra*) and beech (*Fagus sylvatica*): a comparison. Environmental Pollution 109: 509-516.

Brennan, E, and P. M. Halisky. 1970. Response of turfgrass cultivars to ozone and sulfur dioxide in the atmosphere. Phytopathology 60: 1544-1546.

Brimblecombe, P. 1976. Attitudes and responses towards air pollution in medieval England. Journal of the Air Pollution Control Association 26: 941-945.

Burden, R. S., G. A. Carter, T. Clark, D. T. Cooke, S. J. Croker, A. H. B. Deas, P. Hedden, C. S. James, and J. R. Lenton. 1987. Comparative activity of the enantiomers of tiadimenol and paclobutrazol as inhibitors of fungal growth and plant sterol and gibberellin biosynthesis. Pesticide Science 21**:** 253-267.

Bungener, P., S. Nussbaum, A. Grub, and J. Fuhrer. 1999. Leaf injury characteristics of grassland species exposed to ozone in relation to soil moisture condition and vapor pressure deficit. New Phytologist 142: 271-282.

Chang, Y.-S. and M. R. Yu. 2001. Correlation between ozone resistance and relative chlorophyll fluorescence or relative stomatal conductance of bedding plants. Botanical Bulletin of Academia Sinica 42: 265-272.

Chapman, S. 1930. A theory of upper-atmospheric ozone. Royal Meteorological Society 3: 103-125.

Chappelka, A. H. 2002. Reproductive development of blackberry (*Rubus cuneifolius*) as influenced by ozone. New Phytologist155: 249-255.

Chappelka, A. H. and L. J. Samuelson. 1998. Ambient ozone effect on forest trees of the eastern United States: a review. New Phytologist 139: 91-108.

Chimiklis, P. E. and R. L. Heath. 1975. Ozone-induced loss of intracellular potassium ion from *Chlorella sorokiniana*. Plant Physiology 56: 723-727.

Civerolo, K. and R. R. Dickerson. 1998. Nitric oxide soil emissions from tilled and untilled cornfields. Agriculture and Forest Meteorology 90: 307-311.

Clark, A. J., W. Landolt, J. B. Bucher, and R. J. Strasser. 2000. Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence index. Environmental Pollution 109: 501-507.

Colvile, R. N. 2002. Emissions, dispersion and atmospheric transformation. In: J. N. B. Bell and M. Treshow (Eds.), Air Pollution and Plant Life. John Wiley and Sons, England.

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

Criegee, Rudolf. 1975. Angewandte Chemie International Edition 14(11): 745-752.

Croce, R., S. Weise, and R. Bassi. 1999. Carotenoid binding sites of the major light-harvesting complex II of higher plants. Journal of Biological Chemistry 224: 29613-29623.

Crutzen, P. J. 1995. On the Role of Ozone in Atmospheric Chemistry. In: A. R. Bandy (Ed.), The Chemistry of the Atmosphere- Oxidants and Oxidation in the Earth's Atmosphere. The Royal Society of Chemistry, Cambridge, United Kingdom.

Dat, J. F., R. Pellinen, T. Beckman, B. Van De Cotte, C. Langebartels, J. Kangasjarvi, D. Inze, and f. Van Breusegem. 2003. Changes in hydrogen peroxide homeostasis trigger an active cell death process in tobacco. Plant Journal 33: 621-632.

Davies, B. H. and H. P. Kost. 1988. Chromagraphic methods for the separation of carotenoids. In: H. P. Kost, G. Zweig, and J. Sherma (Eds.) CRC handbook of chromagraphy, plant pigments. Vol. 1 Fat soluble pigments. CRC Press, Inc. Boca Raton, Florida.

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

Davis, D. D. and J. B Coppolino. 1974. Relative Ozone Susceptibility of Selected Woody Ornamentals. Hortscience 9(6): 537-539.

Delgado, E. J. Azon-Bieto, X. Palazon, and H. Medrano. 1992. Leaf photosynthesis and respiration of high CO_2-grown tobacco plants selected for survival under CO_2 compensation point conditions. Plant Physiology 98: 949-954.

Demmig-Adams, B. 2003. Linking the xanthophyll cycle with thermal energy dissipation. Photosynthesis Research 76: 73-80.

Demmigs-Adams, B. and W. W. Adams III. 1992. Photoprotection and other responses of plants to high light stress. Annual Review of Plant Physiology and Plant Molecular Biology 43:599-626.

Demmigs-Adams, B. and W. W. Adams III. 1996. The role of the xanthophylls cycle carotenoids in the protection of photosynthesis. Trends in Plant Science 1: 21-26.

Dominy, P. J. and R. L. Heath, 1985. Inhibition of the K^+-stimulated ATPase of the plasmalemma of pinto bean leaves by ozone. Plant Physiology 77: 43-45.

Edwards, G. E. and N. R. Baker. 1993. Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosynthesis Research 37: 89-102.

Elagoz, V. and W. J. Manning. 2002. Ozone and bean plants: morphology matters. Environmental Pollution 120: 521-524.

Evans, L. S., J. H. Adamski II, and J. R. Renfro. 1995. Relationships between cellular injury of leaves, and ozone exposure levels for several dicotyledonous plant species at Great Smoky Mountains National Park. Environmental and Experimental Botany 35(2): 229-237.

Farage, P. K. and S. P. Long. 1999. The effects of O_3 fumigation during leaf development on photosynthesis of wheat and pea: An *in vivo* analysis. Photosynthesis Research 59: 1-7.

Federal Register. 1979. National primary and secondary ambient air quality standards; revisions to the national ambient air quality standards for ozone. 44FR:8202-8237.

Ferdinand, J. A., T. S. Fredericksen, K. B. Kouterick, and J. M. Skelly. 1999. Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. Environmental Pollution 108: 297-302.

Finlayson-Pitts, B. J. and J. N. Pitts. 1986. Atmospheric Chemistry. Fundamental and Experimental Techniques. Wiley, New York.

Finlayson-Pitts, B .J. and J. N. Pitts. 2000. Chemistry of the Upper and Lower Atmosphere - Theory, Experiments, and Applications. Academic Press, San Diego, CA.

Fisherman, J., V. G. Brackett, and K. Fakhruzaman. 1979. Distribution of tropospheric ozone from satellite and ozone sonde measurements. Journal of Atmospheric and Terrestrial Physics 54: 589-597.

Fletcher, R. A., N. O. Adedipe, and D. P. Ormrod. 1972. Abscisic acid protects bean leaves from ozone-induced phytotoxicity. Canadian Journal of Botany. 50: 2389-2391.

Fletcher, R. A., G Hofstra, and G. Jian-guo. 1986. Comparative fungitoxic and plant growth regulating properties of triazole derivatives. Plant Cell Physiology 27: 367-371.

Fracheboud, Y., P. Haldimann, J. Leipner, and P. Stamp. 1999. Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). Journal of Experimental Botany 50: 1533-1540.

Franzaring, J., A. E. G. Tonneijck, A. W. N. Kooijman, and T. A. Deuck. 2000. Growth responses to ozone in plant species from wetlands. Environmental and Experimental Botany 44: 39-48.

Fusco, A. C. and J. A. Logan. 2003. Analysis of 1970-1995 trends in tropospheric ozone at Northern Hemisphere midlatitudes with the GEOS-CHEM model. Journal of Geophysical Research 108: 10.1029/2002JD002742.

Genty, B., J.-M.Briantais, and N. R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990: 87-92.

Gliozeris, S., A. Tamosiunas, and L. Stuopyte. 2007. Effect of some growth regulators on chlorophyll fluorescence in *Viola x wittrockiana* 'Wesel Ice'. Biologija 53(2): 24-27.

Griffin, J. J., T. C. Ranney, and D. M. Pharr. 2004. Photosynthesis, chlorophyll fluorescence, and carbohydrate content of *Illicium* taxa grown under varied irradiance. Journal of the American Society of Horticultural Science 129(1): 46-53.

Grime, J. P. and R. Hunt. 1975. Relative growth-rate: its range and adaptive significance in a local flora. Journal of Ecology 63: 393-422.

Guderian, R. 1977. Air pollution. Phytotoxicity of acidic gases and its significance in air pollution control. Ecological Studies 22: 127-136.

Guderian, R., D. T. Tingey, and R. Rabe. 1985. Effects of photochemical oxidants on plants. In: Air Pollution by Photochemical Oxidants. Springer-Verlag, Berlin. Pp. 127-333.

Guidi, L., C. Nali, S. Ciompi, G. Lorenzini, and G. F. Soldatini. 1997. The use of chlorophyll fluorescence and leaf gas exchange as methods for studying the different responses to ozone of two bean cultivars. Journal of Experimental Botany 48:173-179.

Guidi, L., C. Nali, G. Lorenzini, F. Filippi, and G. F. Soldatini. 2001. Effect of chronic ozone fumigation on the photosynthetic process of poplar clones showing different sensitivity. Environmental Pollution 113: 245-254.

Haagen-Smit, A. J. 1952. Chemistry and Physiology of Los Angeles Smog. Industrial Engineering Chemistry 44: 1342-1346.

Haagen-Smit, A. J. and M. M. Fox. 1954. Photochemical ozone formation with hydrocarbons and automobile exhaust. Journal of the Air Pollution Control Association 4: 105.

Harkov, R. S. and E. Brennan. 1982. An ecophysiological analysis of the responses of woody and herbaceous plants to oxidant injury. Journal of Environmental Management 15: 251-261.

Hayes, F., M. L. Jones, G. Mills, and M. Ashmore. 2007. Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. Environmental Pollution 146: 754-762.

Heath, R. L., 1975. Ozone and Responses of Plants to Air Pollution. Academic Press, London, N.Y., pp. 23-25.

Heath, R. L. 1994. Alterations of plant metabolism by ozone exposure. In: Alscher, R. G. and Wellburn, A. R. (Eds.). Plant Responses to the Gaseous Environment: molecular, metabolic, and physiological aspects. Chapman and Hall, London, United Kingdom.

Heath, R. L. 1994. Possible mechanisms for the inhibition of photosynthesis by ozone. Photosynthetic Research 39: 439-451.

Heck, W. W., O. C. Taylor, and D. T. Tingey. 1988. Assessment of Crop Loss from Air Pollutants. Elsevier, London.

Heggestad, H. E. and J. T. Middleton. 1959. Ozone in high concentrations as cause of tobacco leaf injury. Science 129: 208-210.

Hogsett, W. E., D. Olszyk, D. P. Ormrod, G. E. Taylor, and D. T. Tingey (). 1987. Air Pollution Exposure Systems and Experimental Protocols: Volume 1: A Review and Evaluation of Performance. EPA-600/3-87/037a, U. S. Environmental Protection Agency, Environmental Research Laboratory Office of Research and Development, Corvallis, Oregon.

Hough, A. M. and R. G. Derwent. 1990. Changes in the global concentration of tropospheric ozone due to human activities. Nature 344: 645-648.

Jacobson, J. S. and A. C. Hill. 1970. Recognition of air pollution injury to vegetation: a pictorial atlas. Air Pollution Control Association 8: 136-139.

Jeong, J. H., H. Nakamura, and J. Ota. 1980. Physiological studies on photochemical oxidant injury in rice plants: Varietal difference of abscisic acid content and its relations to the resistance to ozone. Japanese Journal of Crop Science 49: 456-460.

Kangasjarvi, J., J. Talvinen, M. Utriainen, and R. Karjalainen. 1994. Plant defense systems induced by ozone. Plant Cell and Environment 17: 783-794.

Kangasjarvi, J., F. Jaspers, and H. Kollist. 2005. Signaling and cell death in ozone-exposed plants. Plant Cell and Environment 28: 1021-1036.

Karlsson, P. E., E. L. Medin, G. Wallin, G. Selldén, L. Skärby. 1997. Effects of ozone and drought stress on the physiology and growth of two clones of Norway spruce (*Picea abies*). New Phytologist 136: 265-275.

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

Kats, G. D. M Olszyk, and C. R. Thompson. 1985. Open-top experimental chambers for trees. Journal of Air pollution Control Association 12: 1298-1301.

Kerstiens, G. and K. J. Lendzian.1989. Interactions between ozone and plant cuticles. 1. Ozone deposition and permeability. New Phytologist 112: 13-19.

Kimura, M and D. R. Rodriguez-Amaya. 1999. Sources of error in the quantitative analysis of food carotenoids by HPLC. Archivos Latinoamericanos de nutricioin 49: 58s-66s.

Klass, J. van Wijk. 2004. Plastid proteomics. Plant Physiology and Biochemistry 42(12): 963-977.

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

Laisk, A., O. Kull, and H. Moldau.1989. Ozone concentration in leaf intercellular air spaces is close to zero. Plant Physiology 90: 1163-1167.

Lawson, T., J. Craigon , A. M. Tulloch, C. R. Black, J. J. Colls, G. Landon. 2001. Photosynthetic responses to elevated CO2 and ozone in field-grown potato (*Solanum tuberosum*) Journal of Plant Physiology 158:309-323.

Lefohn, A. S. 1992. Ozone Standards and Their Relevance for Protecting Vegetation. In: A. S. Lefohn (Ed.), Surface Level Ozone Exposures and Their Effects on Vegetation. Lewis Publishers, Chelsea, MI, p. 325.

Lefohn, A. S. and J. K. Foley. 1992. NCLAN results and their application to the standard-setting process: Protecting vegetation from surface ozone exposures. Journal of Air and Waste Management Association 42: 1046-1052.

Lehnerr, B., A. Grandjean, F. Machler, and J. Fuhrer. 1987. The effect of ozone in ambient air on ribulosebisphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. Journal of Plant Physiology 130: 189-200.

Lesley, J. W. and O. C. Taylor. 1973. Temperature and air pollution effects on early fruit production of F_2 tomato hybrids. California agriculture 1973-1972: 13-14.

Li, Y. and D. C. Walton. 1990. Violaxanthin is an abscisic acid precursor in water-stressed dark-grown bean leaves. Plant Physiology 92: 551-559.

Lucas, P. 1985. Hemispherical Domes for Fumigation of Plants. In: W. E. Hogsett, D. Olszyk, D. P. Ormrod, G. E. Taylor, and D. T. Tingey (Eds.). Air Pollution Exposure Systems and Experimental Protocols: Volume 2: Description of Facilities. EPA-600/3-87/037b, U. S. Environmental Protection Agency, Environmental Research Laboratory Office of Research and Development, Corvallis, Oregon.

Madison, J. H. and A. H. Anderson. 1963. A chlorophyll index to measure turfgrass response. Agronomy Journal 55: 461-464.

Manning, W. J., W. A. Feder, P. M. Vardaro. 1974. Suppression of oxidant injury by benomyl: effects on yields of bean cultivars in the field. Journal of Environmental Quality 3: 1-3.

Mansfield, T. A. and P. H. Freer-Smith. 1984. The role of stamata in resistant mechanisms. In: M. J. Koziol and F. R. Whatley (Eds.), Gaseous Air Pollutants and Plant Metabolism. Butterworths, London, pp. 131-146.

Maquard, R. D. and J. L. Tifton. 1987. Relationship between extractable chlorophyll and an *in situ* method to estimate leaf greenness. Hortscience 22: 1327.

Marenco, A., P. Gouget, P. Nedelec, J.-P. Pages, and F. Karcher. 1994. Evidence of long-term increase in tropospheric ozone from Pic du Midi data series: Consequences: Positive radiative forcing. Journal of Geophysical Research 99: 16617-16632.

Markwell JP, N. R. Baker, M. Bradbury, and J. P. Thornber. 1995. Use of zinc ions to study thylakoid protein phosphorylation and the state 1-state 2 transitions *in vitro*. Plant Physiology 74: 348–354.

Maxwell, K. and G. N. Johnson. 2000. Chlorophyll fluorescence- a practical guide. Journal of Experimental Botany 51: 659-668.

McElroy, J. S., D.A. Kopsell, J. C. Sorochan, and C. E. Sams. 2006. Response of Creeping Bentgrass Carotenoid Composition to High and Low Irradiance. Crop Science 46: 2606-2612.

Melhorn, H. 1990. Ethylene-promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone, and paraquat. Plant, Cell, and Environment 13: 971-976.

Melis, A. 1999. Photosystem II damage and repair cycle in chloroplasts: What modulates the rate of photodamage *in vivo*? Trends in plant Science 4: 130-135.

Middleton, J. T., A. S. Crafts, R. F. Brewer and O. C. Taylor. 1956. Plant damage by air pollution. California Agriculture, June, pp. 9-12.

Millecan, A. A. 1971. A survey and assessment of air pollution damage to California vegetation in 1970. APTD-0694, Air Pollution Control Office. United States Environmental Protection Agency. Research Triangle Park, North Carolina.

Miller, A. and A. M. Armitage. 2002. Temperature, irradiance, photoperiod, and growth retardants influence production of *Angelonia angustifolia* benth. Angel Mist series. HortScience 37: 319-320.

Neiburger, M., J. G. Edinger, and W. D. Bonner. 1982. Understanding our Atmospheric Environment. W.H. Freeman & Company, San Francisco, CA.

Netto, A. T., E. Camppostrial, J. G. Oliveira, and O. K. Yamanishi. 2002. Portable chlorophyll meter for the quantification of photosynthetic pigments, nitrogen, and the possible use for assessment of the photochemical process in *Carica papaya* L. Brazil Journal of Plant Physiology 14(3): 203-210.

Niyogi, K. 1999. Photoprotection revisited: genetic and molecular approaches. Annual Review of Plant Physiology and Plant Molecular Biology 50: 333-359.
National Turfgrass Research Initiative. 2003. National Turfgrass Federation.

North Carolina Turfgrass Industry. 1999. North Carolina Department of Agriculture.

Ommen O. E., A. Donnelly, S. Vanhoutvin, M. van Oijen, R. Manderscheid. 1999. Chlorophyll content of spring wheat flag leaves grown under elevated CO2 concentrations and other environmental stresses within the 'ESPACE-wheat' project European Journal of Agronomy 10:197-203.

Ormrod, D. P., O. Adedipe, and G. Hofstra. 1971. Responses of cucumber, onion, ad potato cultivar to ozone. Canadian Journal of Plant Science 51: 263-288.

Ormrod, D. P. and B. A. Hale. 1995. Physiological responses of plants and crops to ozone stress. In: M. Pessarakli (ed.), Handbook of Plant and Crop Physiology, Marcel Dekker Inc., New York, pp. 735-760.

Otto, H. W. and R. H. Daines. 1969. Plant injury by air pollutants: Influence of humidity on stomatal apertures and plant response to ozone. Science 163 (3872): 1209-1210.

Pell, E. J., C. D. E. Schlagnahfer, and R. N. Arteca. 1997. Ozone-induced oxidative stress; mechanisms of action and reaction. Plant Physiology 100: 264-273.

Pellinen, R., T. Palva, and J. Kangasjarvi. 1999. Subcellular localization of ozone-induced hydrogen peroxide production in birch (*Betula pendula*) leaf cells. Plant Journal 20: 349-356.

Pogson, B. J., K. K. Niyogi, O. Bjorkman, and D. DellaPenna. 1998. Altered xanthophyll compositions aversely effect chlorophyll accumulation and nonphotochemical quenching in *Arabiopsis* mutants. Proceedings of the National Academy of Science 95: 1332-13329.

Poorter, H. 1998. Do slow growing species and nutrient stressed plants respond relatively strongly to elevated CO_2? Global Change Biology 4:693-697.

Prather, M, D. Ehhalt, F. Dentener, R. Derwent, E. Dlugokencky, E. Holland, L. Isaksen, J. Katima, V. Kirchhoff, P. Matson, P. Midgley, and M. Wang. 2001. Atmospheric chemistry and greenhouse gases. In: Climate Change 2001: J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell, and C. A. Johnson (Eds)., The Scientific Basis, Cambridge University Press, pp.183-235.

Pritchard, S. G. and J. S. Amthor. 2005. Crops and Environmental Change. Food Products Press, New York, p. 17.

Pye, J. M. 1988 impact of ozone on the growth and yield of trees: a review. Journal of Environmental Quality 17: 347-360.

Ranieri, A.,D. Giuntini. F. Ferraro, C. Nali, B. Baldan. 2001. Chronic ozone exposure induces alterations in thylakoid functionality and composition in two poplar clones. Plant Physiology and Biochemistry 39: 999-108

Ranieri, A., D. Giuntini, F. Ferraro, C. Nali, B. Baldan, G. Lorenzini, and G. F. Soldatini. 2003. Chronic ozone induces alterations in thylakoid functionality and composition in two poplar clones. Plant Physiology and Biochemistry 39: 999-1008.

Rao, M. V. and K. R. Davis. 1999. Ozone-induced cell death occurs via two distinct mechanisms in Arapidopsis: the role of salicylic acid. Plant J. 17: 603-614.

Rao, M. V., H, Lee, R. A. Creelman, I. Raskin, J. E. Mullet, and K. R. Davis. 2000. Jasmonate perception desensitizes O_3-induced salicylic acid biosynthesis and progamed cell death in *Arabidopsis*. Plant Cell 12: 1633-1646.

Reich, P. B. 1987. Quantifying plant responses to ozone: a unifying theory. Tree Physiology 3: 63-91.

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

Reiling, K. and A. W. Davison. 1992. The response of native, herbaceous species to ozone; growth and fluorescence screening. New Phytologist 120, 29–37.

Roshchina, V. V. and V. D. Roshchina. 2003. Ozone and Plant Cell. Kluwer Academic Publishers, Boston.

Saitanis, C. J., A. N. Riga-Karandinos, and M. G. Karandinos. 2001. Effects of ozone on chlorophyll and quantum yield of tobacco (*Nicotinoa tabacum* L.) varieties. Chemosphere 42: 945-953.

Samdur M. Y., A. L. Singh, R. K. Mathur, P. Manivel, B. M. Chikani BM, M. A. Khan. 2000. Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron-deficiency chlorosis. Current Science Bangalore 79:211-214.

Sandermann, H., D. Ernst, W. Heller, and C. Langebartels. 1998. Ozone: an abiotic elicitor of plant defense reactions. Trends in Plant Science 3: 47-50.

Schraudner, M., W. Moeder, C. Wiese, W. Van Camp, D. Inze, C. Langebartels, and H. Sandermann. 1998. Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. Plant Journal 16: 235-245.

Schreiber, U., B. Bilger, and C. Neubauer. 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. In: Ecophysiology of photosynthesis.

Seiler, W. 1974. The cycle of atmospheric CO. Tellus 26: 117-135.

Seinfeld, J. 1989. Urban air pollution: state of the science. Science 243: 745-752.

Sellden, G. and H. Pleijel. 1995. Photochemical oxidant effects on vegetation-response in relation to plant strategy. Water, Air, and Soil Pollution 85: 111-122.

Sheng, S. and B. I. Chevone. 1988. Gas exchange response of soybean cultivars to short term exposure of sulfur dioxide and ozone. Phytopathology 78: 1513.

Shrestha, A. and D. A. Grantz. 2005. Ozone impacts on tomato and nutsedge: Competition above- and below-ground. Crop Science 45: 1587-1595.

Siefermann-Harms, D. 1987. The light harvesting and protective functions of carotenoids in photosynthetic membranes. Physiologia Plantarum 69: 561-568.

Skelly, J. M., J. L. Innes, J.E. Savage, K. R. Snyder, D. Vanderheyden, J. Zhang, and M. J. Sanz. 1999. Observation and confirmation of foliar ozone symptoms of native species of Switzerland and southern Spain. Water, Air, and Soil Pollution 116: 227-234.

Staszak, J., N. E. Grulke, and W. Prus-Glowacki. 2004. Genetic differences of *Pinus ponderosa* trees tolerant and sensitive to ozone. Water, Air, and Soil Pollution 153: 3-14.

Steinke, K. and J. C. Stier. 2003. Nitrogen selection and growth regulator applications for improving shaded turf performance. Crop Science 43: 1399-1406.

Thompson, C. R. and O. C. Taylor. 1969. Effects of air pollutants on growth, leaf drop, and yield of citrus trees. Environmental Science and Technology 3: 934-340.

Tingey, D. T. and G. E. Taylor. 1982. Variation in plant response to ozone: a conceptual model of physiological events. In: M. H. Unsworth and D. P. Ormrod (Eds.), Effects of Gaseous Air Pollution in Agriculture and Horticulture. Butterworth, London, pp. 111-138.

Tingey, D. T. and W. E. Hogsett. 1985. Water stress reduces ozone injury via stomatal mechanism. Plant Physiology 77: 944-947.

Tomlison, H. and S. Rich. 1969. Relating lipid content and fatty acid synthesis to ozone injury of tobacco leaves. Phytopathology 59: 1284-1286.

Treshow, M. 1984. Diagnostics of the influence of air pollution and similarity of symptoms. In: Air Pollution and Plant Life. Wiley, New York. Pp. 126-143.

U.S. EPA (United States Environmental Protection Agency). 1996. Air quality criteria for ozone and other photochemical oxidants, Vol. II. EPA-600/P-93004, U. S. Environmental Protection Agency, U. S. Environmental Criteria and Assessment Office, Research Triangle Park, N. C.

U.S. EPA (United States Environmental Protection Agency). 2006a. Air quality criteria for ozone and other photochemical oxidants, Vol. I. EPA-600/R-05/004bF, U. S. Environmental Protection Agency, U. S. Environmental Criteria and Assessment Office, Research Triangle Park, N. C.

U.S. EPA (United States Environmental Protection Agency). 2006b. Air quality criteria for ozone and other photochemical oxidants, Vol. II. EPA-600/R-05/004bF, U. S. Environmental Protection Agency, U. S. Environmental Criteria and Assessment Office, Research Triangle Park, N. C.

Volz, A and D. Kley. 1988. Evaluation of the Monsouris series of ozone measurements in the nineteenth century. Nature 332: 240-242.

Winner, W. E., C. Gillespie, W. S. Shen, and H. A. Mooney. 1988. Stomatal responses to SO_2 and O_3. In: S. Schulte-Hostede, N. M. Darrall, L. W. Blank, and A. R. Wellburn (Eds.), Air Pollution and Plant Metabolism. Elsevier, London, England, pp. 255-271.

Wohlgemuth, H., K. Mittelstrass, S. Kschieschan, J. Bender, H.-J. Weigel, K. Overmyer, J. Kangasjarvi, H. Sandermann, and C. Langebartels. 2002. Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. Plant Cell and Environment 25: 717-726.

Zaripheh, S. and J. W. Erdman. 2002. Factors that influence the bioavailability of xanthophylls. Journal of Nutrition 132(3): 531S-534S.

VITA

Lou Ann McKnight was born in Marysville, Ohio. She attended Fairbanks High School in Milford Center, Ohio. She has a younger brother, Richard Picklesimer, and two younger sisters, Roberta Picklesimer and Sherrie Picklesimer. Lou Ann is married to John McKnight and has three children; Katherine, Mary, and Nasser. She also has two grandchildren; Judah and Koenn.

She attended the College of the Sequoias in Visalia, California, receiving an Associate of Science degree in mathematics-science in 1996. Graduating with a Bachelor of Science degree in plant science with a minor in chemistry from California State University-Fresno in 1999, she then entered the Master of Science degree program receiving her degree in 2001. Lou Ann is currently a candidate for the Doctor of Philosophy degree in horticulture at Louisiana State University in Baton Rouge, Louisiana.

While working toward her Bachelor of Science degree, Lou Ann worked for BioResearch in Fresno, was team leader for a research project sponsored by Solutions Center, California State University-Fresno in cooperation with the J.G. Boswell, John Deere, and Supima organizations, and was a McNair Scholar.