USE OF ETHYLENEDIUREA (EDU) TO ASSESS OZONE EFFECTS ON NATIVE VEGETATION

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USE OF ETHYLENEDIUREA (EDU) TO ASSESS OZONE EFFECTS ON

NATIVE VEGETATION

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Zoltan Szantoi, son of Zoltan Szantoi and Erzsebet Pap, was born May 1, 1977 in Szentes, Hungary. He and his brother, Robert Szantoi were reared on a small tomato farm in Csanytelek, Hungary. In June, 1995, he graduated from Janos Bartha Horticultural High School. He attended Istvan Barsony Environmental Technician School in Csongrad Hungary and graduated in 1997. He then entered Samuel Tessedik College, Szarvas, Hungary in September, 1997 and graduated with a Bachelor of Science degree in Environmental Agricultural Engineering in June 2001. During 2001-2002, he worked as an intern at the Heritage Seedling Inc. in Salem Oregon. Working in the industry for one year after the internship he entered the Graduate School, Auburn University, in January 2003.

THESIS ABSTRACT

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Ground-level (tropospheric) ozone (O_3) is the most significant phytotoxic gaseous pollutant in the eastern United States. Plants are subjected to acute and chronic exposures of tropospheric O_3 that can cause foliar injury on sensitive plants as well as negative effects on a number of plant processes, including photosynthesis, rate of senescence, water use efficiency, dry matter production, pollen tube extension, flowering and yield.

Most of our knowledge about the effects of O_3 on natural vegetation has come from studies conducted in controlled field experiments with open top chambers although this method has inherent technical problems and limitations that affect the applicability of results to ambient conditions. An alternative method is the use of protective chemicals such as ethylenediurea N-[-2-(2-oxo-1-imidizolidinyl) ethyl]-N'-phenylurea (EDU). EDU has been widely used to suppress acute and chronic O_3 injury on agricultural crops and has been used to detect plant injuries, but comparatively little research has been conducted on native vegetation. The overall goal of this study was to assess visible injury, cell wall composition as related to nutritive quality, and biomass yield on native plants. It is hypothesized that EDU protects vegetation from ambient O_3 concentrations, and therefore can be utilized as a diagnostic tool to assess damage to plant communities in natural environments. To achieve this goal, studies with cutleaf coneflower (*Rudbeckia laciniata* L.) and with purple coneflower (*Echinacea purpurea)* were constructed under controlled field conditions. They were exposed to different levels of O_3 and treated by EDU. The results indicated that both plant species were sensitive to elevated ozone: significant changes occurred in biomass yield and cell wall constituents. Response to EDU alone was inconsistent. Higher concentrations of EDU appear to alleviate negative O_3 effects on nutritive quality for purple coneflower. Increasing levels of EDU were observed to decrease the root and total biomass of cutleaf coneflower, indicating possible toxicity, however, higher concentrations of EDU did alleviate visible symptoms. Further testing is needed to determine if EDU is a useful tool for investigating ambient O_3 effects under field conditions (no chambers).

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CHAPTER I.

INTRODUCTION AND LITERATURE REVIEW

Industrial development has led to an increase in trophospheric (i.e. ground-level) ozone (O_3) concentrations. Analyses of historical measurements suggest that surface O_3 concentrations at mid-to high latitudes have more than doubled during the last century (Marenco et al., 1994). Elevated concentrations of O_3 are found in urban areas, but also occur in rural and remote regions due to transport (Agrawal and Agrawal, 2000). In the eastern United States, O_3 concentrations have also increased: Atlanta, Georgia ranks sixth among the most O_3-polluted cities in the US, and Birmingham, Alabama and Macon, Georgia were among the 25 most O_3-polluted cities in 2002 (ALA, 2002). Shelby, Madison, Jefferson, Baldwin and Morgan Counties in Alabama have all experienced increased numbers of high O_3 days during the summer months. The O_3 concentration can reach 124 parts per billion (ppb) in these counties, which is an unhealthy level for human beings (ALA, 2002).

Increases in ozone concentrations

Ozone concentrations vary considerably because of meteorological conditions (sunlight, winds and temperature) and variations in nitrogen oxide (NOx) emissions. Ozone concentrations at the earth's surface in central Europe 100 years ago were about 10 parts per billion (ppb) and exhibited a seasonal cycle with a maxima during the spring months. Ozone concentrations detected in the eastern US on summer afternoons during the 1980-1995 period ranged from 50 – 80 ppb, with concentrations frequently in excess of 100 ppb (Fiore et al., 1998). Ozone has increased between 1 and 2 % per year during the last two decades (Hough and Derwent, 1990). Recent models show that O_3 concentrations will continue to increase between 0.3% and 1.0% per year on a global basis for the next 50 years (Vingarzan, 2004).

Ozone effects in terrestrial vegetation

Detrimental effects on vegetation from O_3 include decreased agricultural and commercial forest yields, reduced growth and increased predisposition to other abiotic and biotic stresses (Chappelka and Chevone, 1992, Chappelka and Samuelson, 1998). Due to transport of O_3 pollution, effects on plant communities and ecosystems have been observed in many remote locations, far away from urban areas (cities, power plants) (Finkelstein et al., 2004, Skelly et al., 1999, Mauzerall and Wang, 2001). Foliar O_3 symptoms on native plant species have been found in various parts of the world (Chappelka and Samuelson, 1998, Skelly et al., 1999, VanderHeyden et al., 2000). For example, in the Great Smoky Mountains National Park (GRSM) about 25-30 native species were observed with distinctive symptoms during July and August (Neufeld et al., 1992, Chappelka et al., 1997, Chappelka et al., 2003).

The effects of acute O_3 exposures may result in direct foliar injury and harm plant tissue; however, longer-term, chronic O_3 effects may reduce growth, and yield of agricultural crops, plus alter community composition of forest and herbaceous plants (Bell and Treshow, 2002). Ozone has been shown to cause negative effects on a number of plant processes, including photosynthesis, water use efficiency, rate of senescence, dry matter production, flowering, pollen tube extension, and yield (Krupa, 1984). The reduced yield of agricultural crops and forest trees will lead to economic losses. Olszyk et al., (1988) estimated yield reductions due to O_3 in

California to be in the range of 20 –25 % for cotton, bean and onions. In another study, Garcia et al., (1986) showed that profits on Illinois farms growing agricultural crops were negative related to estimated 7-h mean O_3 concentrations. They estimated that a 10 % increase in seasonal mean O_3 concentrations would cause a 6% decrease in profits.

It has proven to be difficult to verify whether or not ambient O_3 concentrations significantly affect wild (native) plants in the field, because of the ever-present nature of O_3 and the reality that herbaceous plants response is altered by many other factors such as moisture and nutrition (Chappelka and Samuelson, 1998). Davison and Barnes, (1998) stated that many herbaceous wild plant species are more sensitive to O_3 than an agricultural crops. However, research concerning ambient O_3 effects on native plants is limited. In a foliar injury survey on black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) in the GRSM during summer 1992 Chappelka et al., (1997) observed O_3 injury but effects on ecosystem structure and function were not defined. They found that the selected sensitive plants, black cherry and tall milkweed, were exhibiting visible symptoms due to O_3 and approximately 50% of the plants had some visible injury.

Forest ecosystems are a key portion of the land cover in the southern U.S., encompassing nearly 60% of the land area (USDA Forest Service 1992). Native herbaceous plants are represented in large numbers in these regions, and these species are usually more sensitive to O_3 than are woody plants (Chappelka et al., 2003, Chappelka and Samuelson, 1998). Moreover, to outline and classify air quality standards for all types of vegetation, research is needed not just with agricultural crops and forest trees, but also with native plant communities. The effects of O_3 on plant community structure and function are shown in Fig. 1.

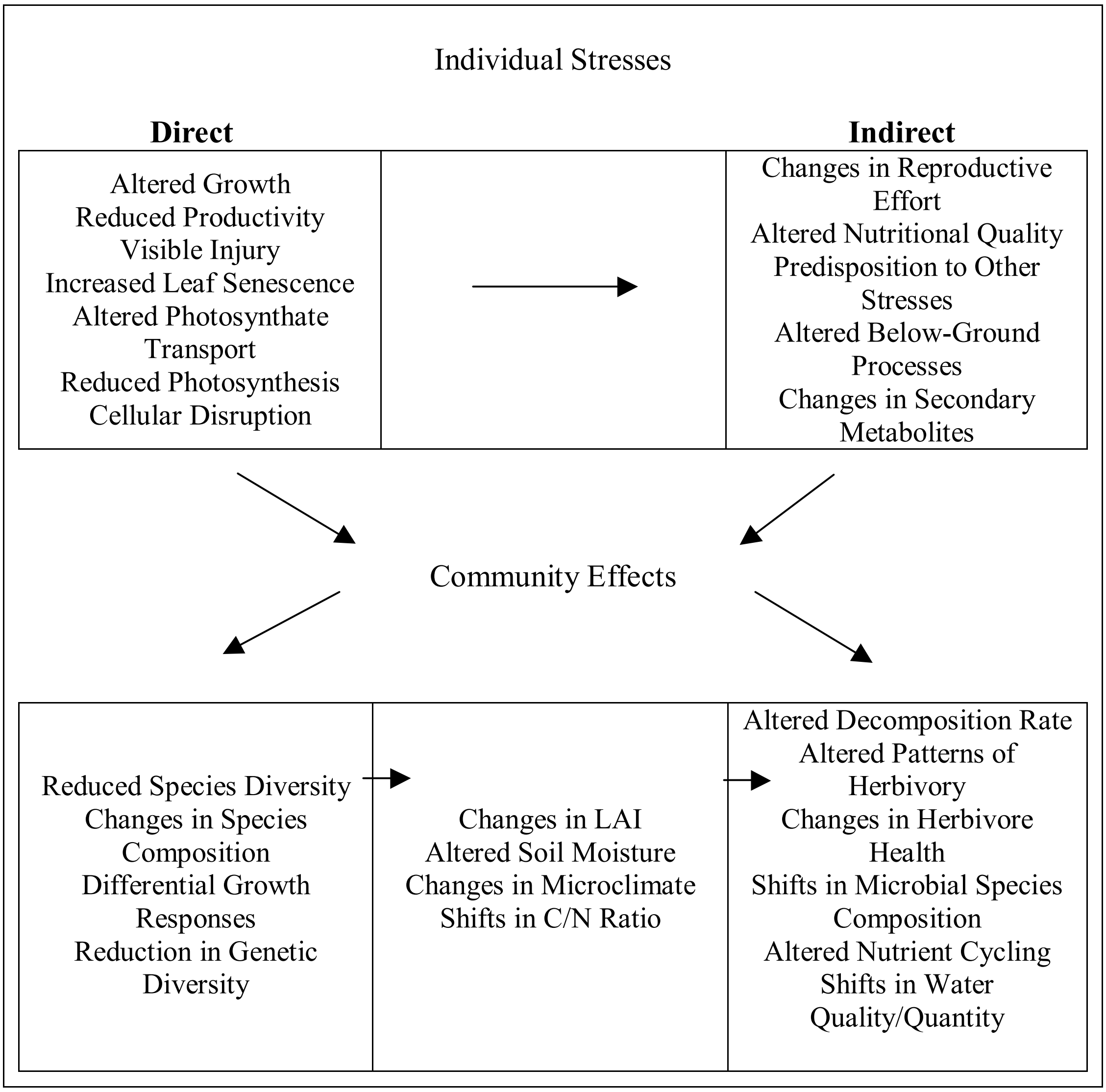

Fig. 1. The effects of O_3 on plant community structure and function (Modified from Krupa et al., 2004)

Open-top chamber studies

Much of the understanding of O_3 effects on plants result from field chamber exposure or controlled environment studies. Heagle et al., (1973) developed, cylindrical open-top plastic-covered field chambers (OTC). Since then, open-top chamber has become the most widely used apparatus for studying O_3 effects on plants in the field. One of the advantages of OTCs is that O_3 concentrations can be manipulated independently of ambient conditions, data from OTC exposure-response studies have been used to develop models to predict future changes and effect elevated ambient O_3 concentrations will have on plant yield. However, Manning and Krupa (1992) reported problems with the chamber environment. (Table 1) Resulting from increases in temperatures (+2.0 – 3.7°C higher), and decreases in light (12-20% less than in relative ambient environment), especially with dirty plastic and a 5 – 10% decrease of humidity. Also plants in dust filtered environment may yield less than in ambient air, but they are often taller than in the ambient air (Manning and Krupa, 1992). In addition, OTCs are comparatively costly to assemble and maintain, and they require an electrical source. The study site at Auburn University used in this study costs about $300,000 to assemble, requires a full-time technician to manage and maintain it, and the electricity bill was approximately $75-100 per chamber per month when operational (Chappelka, personal communication).

Although chamber experiments have the advantage of providing basic understanding of cause and effects, the outcome from such studies cannot be directly extrapolated to the chamberless ambient environment, or unmanaged ecosystems. (Krupa and Legge, 2000).

Chamberless systems

Vegetation in the ambient environment does not naturally grow in chambers. Therefore, alternatives of open-top chambers that more closely replicate natural conditions would have obvious advantages. Over 30 years, investigators have evaluated a diverse group of chemical compounds, like pesticides, growth retardants, growth regulators and antiozonants (Manning and Krupa, 1992). Researchers have attempted to determine which of these compounds would protect plants from O_3 injury. Many studies have been conducted with the systemic fungicide Benomyl® (methyl – 1 – butyl – carbamyl – 2 – benzimidazole) (Manning et al., 1974) and with the antiozonant ethylenediurea (EDU) (Tonneijck, van Dijk, 1997).

The most successful and most commonly used protective chemical is ethylene diurea (N - [2 - (2 – oxo – 1 – imidazolidinyl) ethyl] - N' - phenylurea) (Carnahan et al., 1978). Ethylenediurea (EDU) has been widely used to suppress both acute and chronic O_3 injury on a range of plants under ambient conditions (Kosta-Rick and Manning, 1993, Tonneijk and van Dijk, 1997). Previous studies used EDU as an antiozonant mostly for agronomic crops (Manning, 1992) but rarely for trees, (Kuehler and Flagler, 1999, Ainsworth and Ashmore, 1992) or herbaceous native vegetation (Bergweiler and Manning, 1999).

Despite the effort of researchers, EDU's manufacturer, Dupont Chemical Company, stopped production of EDU for commercial use in agricultural crops due to expensive production costs. However, the continued interest in research during the 1990s encouraged Dupont Co. to produce a considerable quantity of EDU, for future O_3 research (Manning, 1992). Since then, investigators have renewed their interest in using EDU as a research tool. Many studies were conducted world-wide, but still mostly with agricultural crops: example include radish *(Raphanus sativus)* in Egypt

(Farag et al., 1993*, Trifolium repens* in Finland and Italy (Ball et al., 1998)*,* Bel – W3 tobacco *(Nicotiana tabacum)* in the USA (Godzik and Manning, 1998) and *Glycine max* in Pakistan (Wahid et al., 2001). These studies have indicated that EDU has a potential as a research tool for O_3 injury survey work and plant response assessment. Moreover, EDU can be used in remote areas where electricity and funding are limited, such as national parks, national preserves or rural areas. For example, EDU can be used to conduct O_3 research within GRSM, where the O_3 concentrations are higher than most other national parks in the US (Ayers, 2000).

To properly assess the effects of EDU on vegetation and its ability to protect against O_3 injury it is necessary to carefully examine the chemical before using it. Factors such as the appropriate time of use and application rates to avoid any potential toxic effects of the chemical should be well conceptualized (Manning, 2003). Also, the selection of the proper plant species is very important, because some species may not react to EDU or O_3, or EDU may exhibit toxic effects.

Failure to verify these suppositions has cast doubt on some results and it is used by critics to discredit the use of EDU (Manning, 1992). Some advantages and disadvantages of the use of protective chemicals are shown in Table 1.

EDU has been used recently in several investigations. For example, Bortier et al., (2000) conducted an experiment with *Populus nigra*, injected with EDU. After one growing season, stem diameter increase was significantly higher (16%) for the EDU-treated saplings compared with control. Biomass increased also (9%), and O_3 foliar injury was slightly less for the EDU-treated seedlings.

Studies with *Trifolium subterraneum* (3 growing seasons) and *Phaseolus vulgaris* (1 growing season) to ambient O_3 in the Netherlands using EDU-treated and non-treated plants were conducted at different sites. Visible injury was different by site and year, but the usage of EDU reduced injury to nearly zero. In this case the investigators did not find relationship between biomass and EDU treatments (Tonneijck and van Dijk, 2002 a-b).

Basic toxicology studies with EDU, with and without exposure to O_3, need to be conducted to find the proper rates and number of applications for each plant species to provide protection against O_3 without adversely affecting plant growth. Open-top chambers may be a useful tool for conducting there toxicology studies. However, as previously mentioned OTCs have their limitations and possible effects on plants. Therefore it is important to also conduct studies under ambient field condition, also.

Open-air field plots, with no interfering chambers or apparatus, are the best place to examine the effects of tropospheric O_3 on plant growth and yields. It would be ideal to compare the growth and yield of plants in an area with high O_3 concentrations with that of those in a nearby area with low O_3 concentrations (Manning and Krupa, 1992).

Hypothesis and objectives

It is hypothesized that EDU protects vegetation from ambient O_3 concentrations, and therefore can be utilized as a diagnostic tool to assess damage to plant communities in natural environments. The overall goal of this study was to assess visible O_3 injury, cell wall composition as related to nutritive quality, and biomass yield on native plants, and to determine if EDU alleviates these O_3 effects. To achieve this goal, a one-year study for cutleaf coneflower (*Rudbeckia laciniata* L.) and a two-year study for purple coneflower (*Echinacea purpurea)* were conducted. Specific hypotheses and objectives are below:

H_{0i}: Ozone has no effect on visible injury, biomass yield and nutritive quality in purple and cutleaf coneflower.

H_{0ii}: Ethylenediurea has no effect on visible injury, biomass yield and nutritive quality in purple and cutleaf coneflower.

H_{0iii}: Ethylenediurea x O_3 interactions have no effect on visible injury, biomass yield and nutritive quality in purple and cutleaf coneflower.

Specific objectives were: (1) perform exposure-response, toxicology studies using different EDU rates under controlled (OTC) field conditions, with different O_3 concentrations and (2) quantitatively assess the degree of O_3 effects on foliar symptom expression, growth and cell wall composition as related to nutritive quality.

Table 1
Advantages and disadvantages of the use of open-top-chambers and protective chemicals

Advantages	**Disadvantages**
	Open-top-chambers
widely used	limited space in the chambers
plants can be grown to maturity in the field	microclimate effects may affect results
dose-response studies at concentrations above ambient can be made by ozone additions	expensive and labor intensive
cost effective, durable	may increase plant growth in cool season and winter
	need electricity
	Protective chemicals
No chambers required, ambient conditions	Ozone studies require addition of other exposure methods
Microclimate effects eliminate	Ambient conditions must be measured
Plants and plots can be varied easily	Repeat of chemical application needed
High degree of replication	Plant toxicology studies needed before start of experiments
Relatively in expensive	Results are subject to change from year to year (different conditions)
Less equipment needs as OTCs	

Modified from Manning and Krupa, 1992

LITERATURE CITED

Agrawal, S.B., Agrawal, M., 2000. Environmental Pollution and Plant Responses. Lewis Publishers ISBN 1-56670-341-7

Ainsworth, N., Ashmore, M.R., (1992) Assessment of ozone effects on beech by injection of a protectant chemical. *Forest Ecology and Management* 51: 129-136.

American Lung Association, 2002. *State of the Air 2002.* Online at: h*ttp://www.lungusa.org/air2002/index.html*

Ayers, H., 2000. Second Annual Report of Polluted Parks in Peril: The Five Most Air Polluted National Parks in the United States, 2000. *http://www.npca.org/across_the_nation/visitor_experience/code_red/codered.pdf*

Ball G.R., Benton, J., Palmer-Brown, D., Fuhrer, J., Skarby, L., Gimeno B.S., Mills G., 1998. Identifying factors which modify the effects of ambient ozone on white clover (*Trifolium repens*) in Europe. *Environmental Pollution* 103: 7-16.

Bell, J.N.B., Treshow, M., (eds.) 2002. Air Pollution and Plant Life 2. Edition 2002. XIV, 466 Pages, ISBN 0-471-49091-1 - John Wiley and Sons

Bergweiler, C.J., Manning, W.J., (1999). Inhibition of flowering and reproductive success in spreading dogbane (*Apocynum androsaemifolium*) by exposure to ambient ozone. Environmental Pollution 105:333-339.

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

Carnahan J.E, Jenner E.L. and Wat E.K.W., 1978. Prevention of ozone injury to plants by a new protectant chemical. *Phytopathology* 68 pp. 1225–1229.

Chappelka A.H., Neufeld H.S., Davison A.W., Somers G.L., Renfro J.R., 2003. Ozone injury on cutleaf coneflower (Rudbeckia laciniata) and crown-beard (Verbesina occidentalis) in Great Smoky Mountains National Park. Environ Pollut. 2003;125(1):53-9.

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

Chappelka, A.H., Renfro, J., Somers, G., Nash. B., 1997. Evaluation of ozone injury on foliage of black cherry (Prunus serotina) and tall milkweed (Asclepias exaltata) in Great Smoky Mountains National Park. Environ. Pollut. 95:13-18.

Chappelka, A.H., Chevone, B.I., 1992. Tree responses to ozone. In: Surface Level Ozone Exposures and Their Effects on Vegetation (ed. Lefohn, AS), pp. 271 324. Lewis Publishers, Inc., Chelsea, MI.

Davison, A.W., Barnes, J., 1998. Effects of ozone on wild plants. New Phytologist 139, 135151.

Farag, S.A., Rizk, H., El-Bahnasaway, R., Meleigy, M.I., 1993. The effect of pesticides on surface ozone concentrations. *International Journal of Environmental Education and Information 12: 217-224.*

Finkelstein, J., Hitchcock, L., 2004. Midwest, Mid-Atlantic, and California Cities Top List for Worst Dangerous Soot Pollution; DC Ranks 22nd for Smog *http://www.ems.org/nws/2004/09/23/midwest_mid_atla*

Fiore, A.M., Jacob, D.J., Logan, J.A., Yin, J.H., 1998. Long-term trends in ground level ozone over the contiguous United States, 1980–1995. *J. Geophys. Res.* 103:1471–80

Garcia, P., Dixon, B.L., Mjelde, J.W., Adams, R.M., 1986. `Measuring the Benefits of Environmental Change Using a Duality Approach: The Case of Ozone and Illinois Cash Grain Farms'. *Journal of Environmental Economics and Management*, 13: 69-80.

Godzik, B., Manning, W.J., 1998. Relative effectiveness of ethylenediurea, and constituent amounts of urea and phenylurea in ethylenediurea, in prevention of ozone injury to tobacco. *Environmental Pollution* 103: 1-6.

Heagle, A.S., Body, D.E., Heck, W.W., 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual., 1:365-368.

Hough, A.M., Derwent, R.G., 1990. Changes in the Global Concentration of Tropospheric Ozone due to Human Activities. Nature 344(12): 645-648.

Kostka-Rick, R., Manning, W.J., 1993a. Dynamics of growth and biomass partitioning in fieldgrown bush bean (Phaseolus vulgaris L.) treated with the antiozonant ethylenediurea (EDU). Agriculture, Ecosystems and Environment 47: 195-214.

Kostka-Rick, R., Manning, W.J., 1993b. Dose-response studies with ethylenediurea (EDU) and radish. Environmental Pollution 79: 249-260.

Kostka-Rick, R., Manning, W.J., 1993c Dose-response studies with the antiozonant ethylenediurea (EDU), applied as a soil drench to two growth substrates, on greenhousegrown varieties of Phaseolus vulgaris L. Environmental Pollution 82: 63-72. 1993.

Krupa, S.V., Muntifering, R., Chappelka, A.H., 2004. Effects of ozone on plant nutritive quality characteristics for ruminant animals. The Botanica 54, 129-140.

Krupa, S. V., Legge, A.H., 2000. Passive sampling of ambient, gaseous air pollutants: an assessment from an ecological perspective. Environ. Pollution, 107, 31-45.

Krupa, S. V., 1984. Field exposure methodology for assessing the effects of photochemical oxidants on crops. In Proc. 77th Annual Meetings. Air Pollution Control Association, Paper #84-104.2, pp.1-13.

Kuehler, E.A., Flagler, R.B., 1999. The effects of sodium erythorbate and ethylenediurea on photosynthetic function of ozone-exposed loblolly pine seedlings. Environmental Pollution 105: 25-35.

Manning, W.J., 2003. Assessing plant responses to ambient ozone: Growth of ozone-sensitive loblolly pine seedlings treated with ethylenediurea (EDU) and sodium erythorbate (NaE). Environ. Pollut. (In Press).

Manning, W. J., 1993. Bioindicator Plants for Assessment of Air Quality: General Considerations and Plant Responses to Ambient Ozone. A AND WMA ANNUAL MEETING. CONF 86; VOL 11//A, pages 93-WA-80.01 Publisher AIR & WASTE MANAGEMENT ASSOCIATION

Manning, W.J., 1992. Assessing the effects of ozone on plants: Use and misuse of ethylenediurea (EDU). Proceedings of the 85th Annual Meeting and Exhibition on Air and Waste Management Association 11 pp.

Manning, W. J., Krupa, S.V., 1992. Experimental methodology for studying the effects of ozone on crops and trees. In Surface Level Ozone Exposures and Their Effects on Vegetation. Edited by A. S. Lefohn. Lewis Publishers, Inc. Chelsea, MI. pp. 93-156.

Manning, W.J., Feder, W.A., Vardaro, P.M., 1974. Suppression of oxidant injury by benomyl: Effects on yields of bean cultivars in the field. Journal of Environmental Quality 3(1): 1-3.
Marenco, A., Gouget, H., Nédeélec, P., Karcher, F., 1994. Evidence of long-term increase in tropospheric ozone from Pic du Midi data series. Consequences: Positive radiative forcing. J. Geophys. Res., 99(D8), 16:16, 616-16, 632.
Mauzerall, D. L., Wang, X. P., 2001. Protecting agricultural crops from the effects of tropospheric ozone exposure: Reconciling science and standard setting in the United States, Europe, and Asia, Ann.Rev. Energy and Environ., 26, 237–268.
Neufeld, H.S., Renfro, J.R., Hacker, W.D., Silsbee, D., 1992. Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. In: R.D. Berglund, Editor, Tropospheric Ozone and the Environment II, Air and Waste Manage. Assoc., Pittsburgh, PA, pp. 594–617.
Olszyk, D. M., Maas, E.V., Kats, G., Francois, L.E., 1988. Soil salinity and ambient ozone: lack of stress interaction for field-grown alfalfa. J. Environ. Qual., 17:299-304.
Skelly, J., Davis, D., Steiner, K., Zhang, J., Schaub, M., Ferdinand, J., Savage, J., Stevenson, R., 1999. Impact of ambient ozone on physiological., visual, and growth responses of sensitive Eastern hardwood tree species under natural and varying site conditions. Final report to the National Center for Environmental Research NCER, Office of Research and Development of the U.S. Environmental Protection Agency EPA.
Skelly, J.M., Innes, J.L., Savage, J.E., Snyder, K.R., VanderHeyden, D.J., Zhang, J., Sanz, M.J., 1999. Observation and confirmation of foliar symptoms of native plant species of southern Switzerland and southern Spain. Water, Air, and Soil Pollution 116, 227–234.
Tonneijck, A.E.G., van Dijk, C.J. (2002): Injury and growth response of subterranean clover to ambient ozone as assessed by using ethylenediurea (EDU): Three years of plant monitoring at four sites in the Netherlands. Environmental and Experimental Botany 48: 33-41.
Tonneijck, A.E.G., van Dijk, C.J., 2002. Assessing effects of ambient ozone on injury and yield of bean with ethylenediurea (EDU): Three years of plant monitoring at four sites in The Netherlands. Environmental Monitoring and Assessment 77: 1-10.
Tonneijck, A.E.G., van Dijk, C.J., 1997. Effects of ambient ozone on injury of Phaseolus vulgaris at four rural sites in the Netherlands as assessed by using ethylenediurea (EDU). New Phytologist 135. pp. 93–100
Treshow, M., 1984. Air Pollution and Plant Life. John Wiley and Sons Inc, March, 486 Pages
United States Department of Environmental Protection (US EPA). 1996. Air Quality Criteria for Ozone and Other Photochemical Oxidants, Vol. II. EPA/600/P-93/004bF, Research Triangle Park, NC.
United States Department of Agriculture Forest Service. 1992. General Technical Report W0-59 August
VanderHeyden, D.J., Skelly, J., Innes, J., Hug, C., Zhang, J., Landolt, W., Bleuler, P., 2000. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environmental Pollution 109, 473–478.

Wahid, A., Milne, E., Shamsi, S.R.A., Ashmore, M.R., Marshall, F.M., 2001. Effects of oxidants on soybean growth and yield in the Pakistan Punjab. Environmental Pollution 113: 271-280
Vingarzan, R., 2004. A review of surface ozone background levels and trends. Atmospheric Environment 38, 34313442.

CHAPTER II.

USE OF ETHYLENEDIUREA (EDU) TO ASSESS OZONE EFFECTS ON PURPLE CONEFLOWER (*Echinacea purpurea*)

ABSTRACT

Purple coneflower (*Echinacea purpurea*) plants were placed into open-top chambers (OTC) for 6 and 12 weeks in 2003 and 2004, respectively, and exposed to charcoal-filtered air (CF) - representative of clean air, and twice-ambient levels (2X) of ozone both years, plus non-filtered (NF), ambient air in 2004. Plants were treated with ethylenediurea (EDU) weekly as a foliar spray at 0 (control) 100, 200 and 300 ppm in 2003, and 0, 200, 400 and 600 ppm in 2004. Plants were evaluated for foliar injury periodically each year. At the end of each growing season plants were harvested for dry weights, and nutritive quality was assessed. Ozone (O_3) injury symptoms were observed on foliage in the 2X chambers (95%) in both years. However, no symptoms were observed in the CF and NF chambers either year. Above-ground biomass was not affected by elevated O_3 in 2003, but root and total weights were decreased in 2004. Relative food value (RFV) was lower in plants exposed to elevated O_3 in 2003 and 2004 (25% and 17%, respectively). Neutral detergent fiber (NDF), lignin and nitrogen (N) concentrations were higher in plants in the 2X chambers in 2004; plants in CF and NF chambers had similar concentrations of cell wall constituents in 2004. EDU had effects on above-ground biomass in 2003, and on N concentrations in 2004. Significant EDU×O_3 interactions for NDF, ADF and lignin concentrations in 2003 indicated EDU (100, 200 and 300 ppm) ameliorated O_3 effects on nutritive quality compared with the control treatment. Interactions although present in 2004, were inconsistent regarding decreases in O_3 injury.

INTRODUCTION

Ozone (O_3) in the lowest layer of the atmosphere (troposphere) is recognized as an air pollutant due to its deleterious effects on human health, vegetation and materials (Nebel and Wright, 1998), and it is considered the most significant phytotoxic air pollutant in the eastern United States (United States Environmental Protection Agency, 1996). Tropospheric O_3 is formed in the presence of sunlight through the chemical interaction of reactive volatiles and oxides of nitrogen (NO_x) that result from high-temperature combustion of fossil fuels. From 1990-1999, urban O_3 formation has fluctuated from year to year, and the average number of unhealthy air-quality days in the final year of the decade (1999) was approximately 10% greater than in 1990 (EPA, 2001). More importantly, O_3 can be transported great distances from urban to rural areas (Chameides et al., 1994).

A recent study (Vingarzan, 2004) indicates that O_3 concentrations will continue to increase between 0.5% and 2.0% per year in the Northern Hemisphere during the next several decades, reaching average global surface O_3 concentrations of 38–71 ppb by 2060. The US EPA now uses an 8-hour air quality standard (US EPA 2001) and, based on the standard a slight decrease in O_3 concentrations in the past two decades (1980-2000) has been observed nationwide. However, O_3 concentrations in the eastern US have increased slightly and remained high due to elevated temperatures and urbanization (Chameides and Cowling, 1995). In its 2005 report, the American Lung Association (ALA) ranked Atlanta, GA No. 9 on the list of cities with the highest year-round particulate matter pollution (ALA, 2005), and Jefferson, AL and Fulton, GA are among the 25 most O_3-polluted counties in 2001-2003 (ALA, 2005).

Ozone can directly affect plant tissue and disrupt normal patterns of resource acquisition and allocation ultimately reducing crop yield (Krupa and Manning, 1988), with losses estimated in the hundreds of millions of dollars (US EPA, 2001). Plant reproduction can be adversely affected by O_3 without injury symptoms on the leaves (Black et al., 2000). Research on how O_3 exposure affects herbaceous plants and their nutritive quality is limited (Fuhrer and Booker, 2003). Davison and Barnes (1998) reported that many herbaceous wild plant species may be more sensitive to O_3 than agricultural crop species.

Krupa et al., (2004) stated that O_3 effects on nutritive quality of vegetation due to changes in cell wall constituents (cellulose, hemicellulose and lignin) do not appear to be uniform across all plant species. However, exposure to elevated O_3 concentrations caused a 14% decrease in nutritive quality or relative food value (RFV; Rohweder et al., 1978) of eastern gamagrass (*Tripsacum dactyloides*) (Lewis et al., in press) by the end of the growing season, and a 7 % loss in sericea lespedeza (*Lespedeza cuneata*) (Powell et al., 2003).

Production of organic food, natural medicines and crops for animals is increasing the concern about sustainable agriculture. Incorporation of information on biomass yield with nutritive quality assessment is essential to characterize possible O_3 impacts on the consumable food value of the ecosystem (Krupa et al., 2004), understanding air pollution effects such as O_3 exposure on natural ecosystems is a vital question that needs to be addressed.

The protective antiozonant ethylenediurea, N-{2-(2-oxo-l-imidazolidinyl) ethyl}-N'-phenylurea (Carnahan et al., 1978) has been the predominantly used method for assessing effects of O_3 on crop yield (Kostka-Rick and Manning, 1993a-b, Tonneijk and van Dijk, 1997). The majority of previous studies that have used EDU as an antiozonant were mostly for agronomic crops (Manning, 1992), but just a few studies have been conducted with native vegetation (Bergweiler and Manning, 1999).

Echinacea purpurea (purple coneflower) has been selected for use in this study because it is one of the most important herbaceous species in the US due to its medicinal use for humans (biomedicine), as well as a forage crop for ruminant animals (Barrett, 2003, Stubbendieck et al, 1989). It also has a distinct purple flower and is planted as a horticultural crop. Purple coneflower is a rough, pubescent, perennial plant belonging to the Aster family (Asteraceae), and reaches heights up to 60 cm. (Cox, 1978). This plant has a history of medicinal use by Native Americans, who chewed the ground roots to alleviate coughs and sore throats (Barrett, 2003). Lately in North America, powdered purple coneflower has been used to treat wounds, snake bites, headache and the common cold. Annual sales of products from purple coneflower have been estimated at $300 million in the US (Brevoort, 1998). Moreover, it is a significant forage resource for grazing livestock and wildlife in the Great Plains region of the central US, as its nutritive quality is typically very good until plants reach maturity, after which it is largely avoided (Stubbendieck et al., 1989).

It is hypothesized that EDU protects vegetation from ambient O_3 concentrations, and therefore can be utilized as a diagnostic tool to assess damage to plant communities in natural environments. The overall goal of this study was to assess visible O_3 injury, cell wall composition as related to nutritive quality, and biomass yield of purple coneflower, and to determine if EDU alleviates these O_3 effects. To achieve this goal, a two-year study (2003-2004) was conducted. Specific objectives were: (1) perform exposure-response, toxicology studies using different EDU rates under controlled open-top chamber (OTC) conditions, with different O_3

concentrations and (2) quantitatively assess the degree of O_3 effects on foliar symptom expression, growth and nutritive quality.

MATERIALS AND METHODS

Study site

The site was located on the Auburn University campus (32° 36′ N, 85° 30′ W). Initially, it had been a previously forested area with loblolly (*Pinus taeda*) and longleaf (*Pinus palustris*) pine trees. Clearing was conducted in 1986. The size of the area was approximately 1.5 hectares. The range of topography was from level to a 1-3% slope. Soil type is a Cowarts (Typic Kanhapladult) soil series. The 30-yr average annual precipitation for this area is 1370 mm. The area had been seeded with bahiagrass (*Paspalum notatum*) in 1987. Within the chambers, the soil was sprayed with a pre-emergence herbicide (Roundup®) approximately one month before the research was initiated, then covered with straw to minimize invasion of weeds.

The O_3 exposure system consisted of 9 large (4.8 m height, 4.5 m diameter) open-top chambers (OTC), each consisting of an aluminum frame bounded by clear plastic (Heagle et al., 1989). Incoming air ventilated by large fans inflates the double wall of the bottom of the chamber and moves outward through perforations in the inner wall, moving under and over the plants inside and then upward and out of the chamber.

Plant materials

Purple coneflower seeds (purchased from Prairie Nursery, Inc. Westfield, WI) were germinated and placed into trays in a greenhouse 6 weeks prior to placement of plants into chambers. In the third week post-germination, each plant was replanted into a

3.78-L pot filled with a 1:1 mixture of peat moss and Norfolk sandy loam soil. Plants were watered daily and fertilized (4 g of 15:16:17 of N: P_2O_5: K_2O) once weekly. Potted purple coneflower seedlings were placed into chambers on Sept 4, and May 7, respectively in 2003 and 2004. Plants received one week acclimatization before O_3 treatments were initiated in 2003, and 4 days in 2004. Five pots of each EDU treatment were placed into each chamber and arranged into lines. Plants were watered three times daily (1000, 1400 and 1800 h) for 15 minutes and fertilized once every fourth week with 4 g of fertilizer (14-14-14 of N: P_2O_5: K_2O). Study duration was 6 weeks in 2003 and 12 weeks in 2004.

Ozone exposures

During 2003, two O_3 exposures replicated two times (blocks) were applied in the OTCs: CF, carbon filtered air (approx. 0.5 × ambient air) ~ 14-16 ppb, representing a clean environment (Lefohn et al., 1990); and 2X (2 × ambient air), which is representative of concentrations found in the vicinity of large metropolitan areas such as Birmingham, AL (Chameides and Cowling, 1995). Blocking was applied to the chambers due to the different light exposures at the site. Ambient-air O_3 concentrations (AA) were monitored in open chamberless plots during the duration of the experiment.

Ozone was generated by passing pure oxygen through a high-intensity electrical discharge source (Griffin Inc., Lodi, NY, USA) and added to the chambers from 0900-2100 h (12 h d^{-1}, 7 d wk^{-1}). Chamber fans were operated 15 h d^{-1}, 7 d wk^{-1} between 0700-2200 h. The fans were turned off from 2200-0700 h to allow dew formation. Ozone treatments were initiated on Sept 11, 2003 and ended on Nov. 9 (6 weeks of fumigation). Ozone concentrations were continuously monitored in each chamber twice per hour. All O_3 monitoring equipment was calibrated and audited according to US EPA procedures (Chappelka, 2003).

In 2004, three O_3 treatments replicated three times were applied in the OTCs: CF, NF, (non-filtered, ambient air) and 2X. The O_3 generation and exposure methods were the same as in 2003. Ozone treatments were initiated on May 11, 2004 and ended on August 3, 2004 (12 weeks of fumigation). A weather station was located in the Auburn/Opelika area, from which temperature and moisture data were collected (Alabama Weather Information System (AWIS) Inc, Auburn, AL, 2005).

EDU treatments

Ethylenediurea (EDU) was obtained from Dr. W.J. Manning, University of Massachusetts (Manning personal communication). Three EDU and one control treatment (tap water) were applied in 2003. The concentrations used were 0, 100, 200 and 300 ppm active ingredient. Based on the results of 2003, different EDU rates were assigned in 2004, which consisted of 0, 200, 400 and 600 ppm active ingredient.

EDU treatments were initiated after the fourth day following placement of the purple coneflower under different O_3 levels in the OTCs. The entire foliage of each plant was sprayed until saturation. EDU was applied weekly as a foliar spray, during exposure periods in 2003 and 2004.

Plant measurements

2003

Three biweekly measurements were conducted (Sept 11– November 9) for ocular evaluation of incidence (% of plants injured), and % of leaves injured. A modified Horsfall-Barratt rating scale (Horsfall and Barratt, 1945) was used to quantify the relative % leaves and leaf area injured / injured plant (classes: 1= 0%, 2= 1-6%, 3= 7-25%, 4= 26-50%, 5= 51-75% and 6= 76-100%). Total numbers of flowers were counted before harvest. Harvested plants were oven-dried at 50°C to a constant weight, and total above-ground biomass was recorded. Pooled leaves for each experimental unit (5 plants) were ground in a Wiley mill to pass a 1-mm screen prior to analysis for nutritive quality.

Plant cell wall constituents were sequentially fractionated into neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and lignin according to procedures of Van Soest et al. (1991) using an ANKOM fiber analyzer (ANKOM Technology Corporation, Fairport, NY). Relative food value (RFV), a presumptive estimate of nutritive quality (Rohweder et al., 1978), was calculated from leaf concentrations of NDF and ADF using the Linn and Martin (1989) prediction equation. Nitrogen (N) concentration was determined by the Kjeldahl method according to Association of Official Analytical Chemists (AOAC, 1995).

2004

Every third week (May 11 – August 3), leaf greenness was measured with a SPAD 502 Minolta Chlorophyll Meter (Spectrum Tech. Inc, Plainfield, IL), as was observation of visible injury (same as 2003) and % of leaf area injured. Three times during the growing season (June 2 and 30 and July 30), numbers of leaves and flowers were counted. In addition, at harvest each plants were separated by anatomical components (root, stem, foliage and flowers), dried to a constant weight as in 2003, and biomass determined. Nutritive quality assessment was conducted similar to 2003.

Experimental design and statistical analysis

The overall design was a completely randomized block (CRB), split-plot for which the units of analysis were purple coneflower plants, placed randomly in each chamber, 5 plants per EDU treatment. Two blocks (2 OTCs in each block) were used in 2003, and three blocks (3 OTCs in each block) in 2004. Whole-plot treatments (O_3) were CF and 2X in 2003 and CF, NF and 2X in 2004. EDU rates were sub-plot treatments, and were 0, 100, 200 and 300 ppm, and 0, 200, 400 and 600 ppm for 2003 and 2004, respectively. Data were analyzed using analysis of variance (ANOVA) in both years and repeated measures analysis in 2004 [(MANOVA) JMP IN 5.1 statistical software package from the Statistical Analysis Institute 1989]. ANOVA was used for biomass, nutritive quality characteristics and number of flowers (one final dataset at the end of the experiment), and MANOVA was applied to assess differences in incidence (% of plants), % of leaf area of visible foliar injury per plant injured, % injured leaves, leaf greenness (2004 only) and leaf number over time.

RESULTS

Weather data

Average temperature for November 2003 was higher than the 30-year (1971-2000) mean (+3.1°C), monthly precipitation values in September and October were below the 30-year averages (- 3.7 cm and - 4.4 cm, respectively), and November precipitation was slightly above (+ 1.5 cm) the 30-year average. In 2004, the average temperatures for May through August were within 2.1°C of the 30-year (1971-2000) average. Monthly rainfall values for May-July were below the 30-year average; however, rainfall was 3.9 cm above the 30-year average in August (Table 1).

Table 1
Average monthly air temperatures and rainfall amounts for selected months in 2003 and 2004, and 30-year averages for the Auburn, AL area

Month	Air temperature (°C)		Precipitation (cm)	
	2003	30-year average	2003	30-year average
September	23.5	23.7	5.9	9.6
October	18.5	18.0	3.0	7.4
November	15.6	12.5	10.9	9.4
	2004	30-year average	2004	30-year average
May	23.3	21.2	9.4	9.7
June	25.6	24.9	8.5	10.3
July	27.2	26.2	8.7	14.9
August	25.7	26.1	13.1	9.2

(Alabama Weather Information System (AWIS) Inc, Auburn, AL, 2005)

Ozone exposures

Mean 12-h (0900-2100 h) O_3 exposures over the treatment period were similar to the target values for each treatment in 2003 and 2004 (Table 2). In 2003, the peak 1-h ambient O_3 concentration was 73, which occurred in October, with mean 12-h O_3 concentrations averaging 35 ppb. The maximum O_3 concentration was 167 ppb in October for the 2X treatment. Across all months, mean 12-h O_3 concentration for the

2X treatment was 82 ppb. Mean 12-h O_3 concentration for the CF treatment was approximately 55 % lower than that of AA in 2003 (Table 2).

During 2004, the peak 1-h O_3 concentration in July (83 ppb) was the highest recorded for the NF treatment. Mean daily O_3 concentrations for the NF treatment averaged 33 ppb for the entire experiment. The peak value for the 2X treatment was 177 ppb (July). Average daily O_3 concentrations for the 2X treatment over the experiment were 73 ppb. Mean 12-h O_3 concentration for the CF treatment was about 36 % lower than that of the NF treatment in 2004 (Table 2).

Table 2
Average 12-h O_3 concentrations in 2003 and 2004.

2003	Average 12-h O_3 concentration (ppb)		
Air treatment	CF	AA	2X
September (mean)	17	37	79
(min-max)	2-47	5-67	5-144
October (mean)	16	35	86
(min-max)	3-37	5-73	6-167
November (mean)	13	29	70
(min-max)	2-26	3-60	7-131
2004	CF	NF	2X
May (mean)	21	32	69
(min-max)	8-49	12-57	12-107
June (mean)	19	29	70
(min-max)	3-42	3-59	3-122
July (mean)	25	34	80
(min-max)	5-54	6-83	9-177
August (mean)	21	38	77
(min-max)	6-37	11-65	12-125

O_3 exposures (12h) were from 0900h-2100 d^{-1}.

Plant measurements – 2003

Various effects were observed, and these are indicative of differences in plant condition within and between O_3 and EDU treatments. P-values from ANOVA are shown in Table 3, exclusive of dependent variables for which there was no significant effects ($p<0.10$) of O_3 or EDU.

Table 3
Significance levels for split-plot analysis of foliar measurements and nutritive quality characteristics of purple coneflower exposed to O_3 and treated with ethylenediurea (EDU) in 2003.

	d.f.	Plant injured	% Leaves injured	Above ground Biomass	NDF	ADF	RFV	Lignin
O_3	1	0.017*	0.060†	0.519	0.032*	0.004*	0.022*	0.115
EDU	3	0.843	0.386	0.039*	0.063†	0.137	0.166	0.009*
Linear trend	1	-	-	0.435	0.021*	-	-	0.002*
Quadratic trend	1	-	-	0.008*	0.441	-	-	0.075
Cubic trend	1	-	-	0.517	0.170	-	-	0.335
EDU × O_3	3	0.455	0.399	0.286	0.073†	0.057†	0.182	0.011*

Significance at the * $p<0.05$, and † $p<0.10$ level.
NDF = neutral detergent fiber; ADF = acid detergent fiber; RFV = relative food value.

Percent plants and leaves injured were measured at 3 times during the study, but only the final values (recorded before harvest) were analyzed because there was very little injury during the first two measurements. Significant differences were observed between O_3 treatments for % plants and leaves injured (Table 4). Ninety-five percent of the plants were injured under elevated O_3 conditions, and 25 % leaves were injured. Foliar injury was minimal (1 plant with slight injury observed) in the charcoal-filtered chambers. Concentrations of NDF and ADF were increased by 31 % and 25 %, respectively, due to the 2X O_3 exposure (Tables 3 and 4). Additionally, RFV was decreased by 25% due to exposure to elevated O_3 concentrations (Tables 3 and 4).

Table 4
Effects of O_3 on nutritive quality and selected morphological characteristics in 2003.

O_3 levels	% Plant injured	% Leaves injured	NDF (%)	ADF (%)	RFV
CF	2.5	0.08	19.22	12.66	384.69
2X	95	25.15	25.21	15.83	286.19

NDF = neutral detergent fiber; ADF = acid detergent fiber; RFV = relative food value.

There were significant differences among EDU treatments for total biomass production. The 100- and 200-ppm EDU-treated plants produced more biomass above ground than those in the other two EDU treatments (Tables 3 and 5), as indicated by a significant quadratic contrast (p=0.008).

Table 5
The effects of EDU on biomass, and concentrations of NDF (neutral detergent fiber) and lignin during 2003.

EDU (ppm)	Above ground Biomass (g)	NDF (%)	Lignin (%)
0	63.50	24.34	4.23
100	70.00	21.64	2.99
200	71.25	22.17	2.80
300	60.25	20.71	2.57

Significant differences were found among EDU levels for % NDF and lignin (Tables 3 and 5). There was a decreasing linear response (p=0.02) of % NDF to EDU. The 300-ppm EDU treatment reduced NDF concentration by 16 % compared with the control-treated plants. There was also a significant linear decrease (Table 3 and 5) in lignin concentration with increasing level of EDU.

There was a significant interaction between O_3 treatment and application rate of EDU for concentrations of NDF, ADF and lignin (Fig. 1). For NDF, the interaction was due to a significant difference among EDU rates within the 2X treatment, but not in the CF treatment. There was greater attenuation of the increase in NDF concentration due to elevated O_3 observed for the 100-, 200- and 300-ppm compared with 0-ppm EDU treatments. At the highest level of EDU (300 ppm), NDF concentration was decreased by 22% compared with the control treatment.

The pattern of the ADF interaction was similar to that observed for NDF. There was a significant linear trend for EDU rates within the 2X treatment (p=0.009), but it was not significant in the CF treatment (Fig. 1).

The interaction of EDUxO_3 lignin concentration was due to a significant difference among EDU rates within the 2X treatment, but not in the CF. Plants treated with 0 ppm EDU (control) had significantly higher % lignin than purple coneflower treated with any higher level of EDU (p=0.001) under the 2X-O_3 treatments (Fig. 1). Percent lignin was decreased by 51 % for the 300-ppm EDU treatment compared with the 0 ppm EDU treatment under elevated O_3 (2X). The plants in the CF treatment did not exhibit significant differences in lignin concentration among EDU levels (p=0.800).

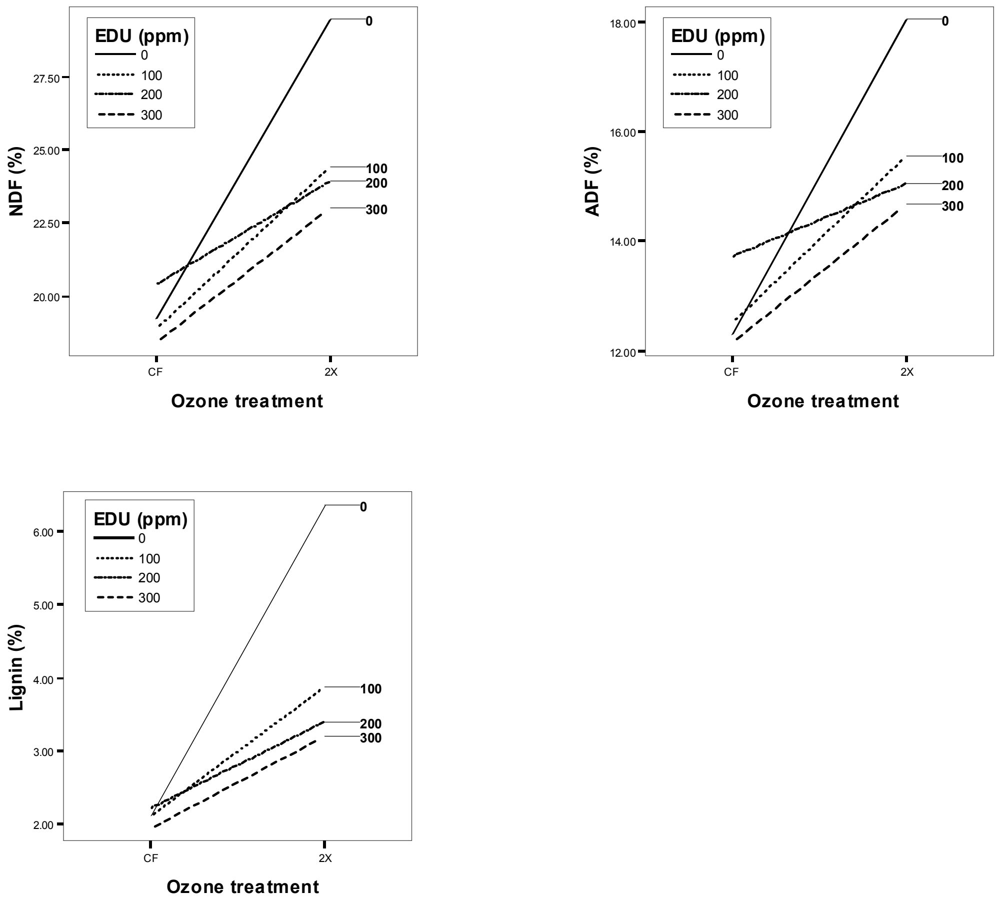

Fig. 1. EDU and nutritive quality characteristics at different O_3 treatments on purple coneflower in 2003.

Plant measurements – 2004

Leaf greenness analysis revealed insignificant variations; therefore it was excluded from the results. Analysis of average number of flowers excluded the first sampling date (Julian date 185) because of the high number of non-flowering plants. Numbers of flowers were effected by EDU (Table 6) and followed a quadratic trend (p=0.001). The 0- and 600-ppm EDU treatments had similar average number of flowers (4.17

and 3.86, respectively), and the 200- and 400-ppm treatments had similar values: 2.99 and 2.69, respectively (Fig. 2).

There was a significant interaction of O_3 by EDU for average number of flowers (Table 6). EDU levels elevated different responses within the CF and NF treatments, and no significant differences were found among EDU levels for the 2X-O_3 treatment ($p=0.018$) (Fig. 2).

Table 6
Significance levels (p-values) for repeated measure analysis of foliar measurements for purple coneflower exposed to O_3 and treated with EDU in 2004.

	Number of Flowers	Number of Leaves
Average Across Time		
O_3	0.191	0.581
EDU	0.011*	0.138
EDU×O_3	0.045*	0.822
Average over Time (Wilks' Lambda test)		
Time×O_3	0.287	0.628
Time×EDU	0.408	0.042*
Time×EDU×O_3	0.883	0.763

Significance at the * $p<0.05$ level.

There was a significant change across time due to EDU for number of leaves (Table 6). The increase in number of leaves over time was significantly greater for the 600-ppm EDU treatment due to the large increase in leaves in the last time period ($p=0.011$) (Fig. 2).

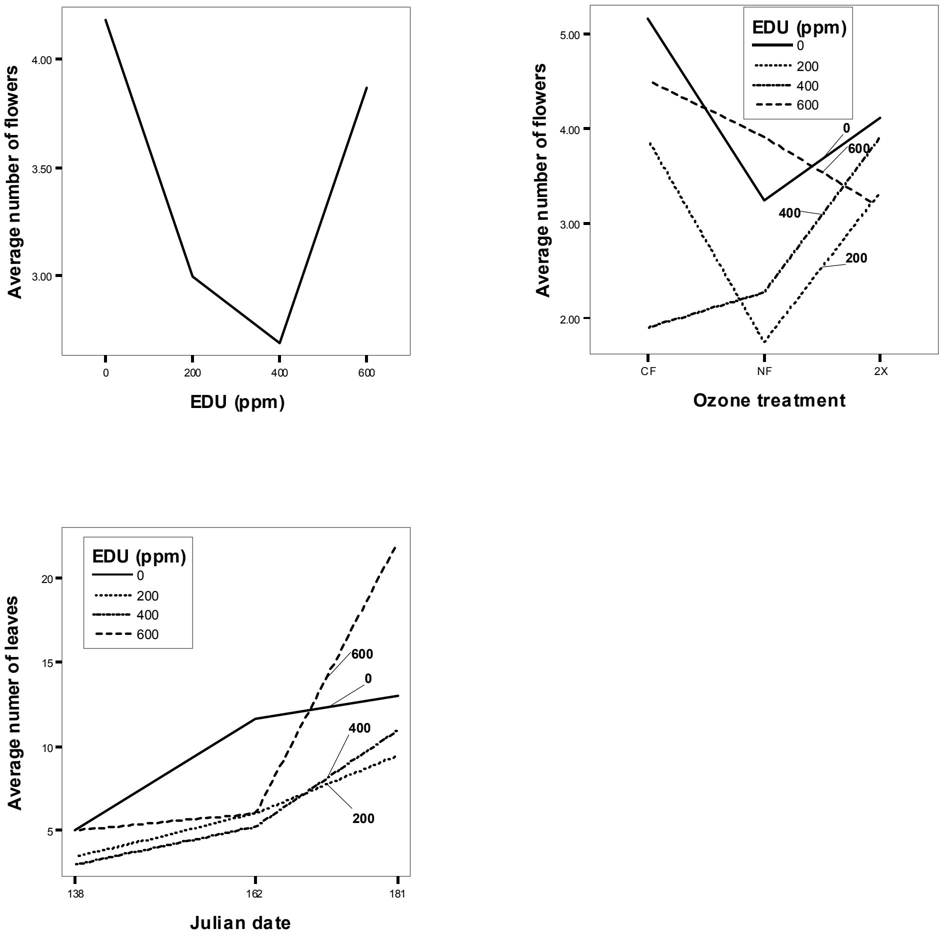

Fig. 2. EDU effects on average number of flowers and number of leaves in purple coneflower in 2004.

Regarding visible foliar injury, the CF and NF treatments were excluded from analysis because no visible O_3 injury was observed (Table 7). Therefore, only the 2X treatment was used to test for EDU and time effects. The percentage of leaf area injured decreased when averaged across all the dates examined (Table 7). The control (0 ppm EDU) treatment had significantly more injury than did the other levels of EDU (p=0.009) (Fig. 3).

Table 7
P-values from repeated measures analysis for foliar measurements for purple coneflower exposed to elevated O_3 (2X) and treated with EDU.

	d.f.	Incidence	% Leaves injured	% of leaf area injured
Average Across Time				
EDU	3	0.624	0.335	0.030*
Average Over Time (Wilks' Lambda test)				
Time×EDU	9	0.148	0.921	0.953

Significance at the * p<0.05 level.

There was a quadratic trend across time (p=0.017) illustrating that the 200- and 400-ppm EDU rates reduced the extent of leaf area injury more than did the 600-ppm rate (Fig. 3). Foliar injury was significantly less for the 200-ppm EDU treatment compared with the other three levels of EDU (p=0.019).

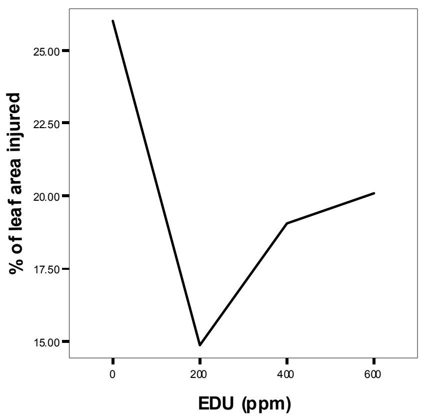

Fig. 3. Changes in percentage of leaf area injured due to different EDU rates for purple coneflower in 2004.

Table 8
Significant levels for tests of biomass and nutritive quality for purple coneflower exposed to O_3 and treated with EDU in 2004.

	d.f.	Flower weight	Root weight	Total biomass	NDF	ADF	RFV	Lignin	N
O_3	2	0.425	0.034*	0.101†	0.019*	0.263	0.024*	0.001*	0.018*
EDU	3	0.033*	0.480	0.521	0.141	0.050*	0.114	0.466	0.056†
Linear t.		0.993	-	-	-	0.086†	-	-	0.215
Quad t.		0.005*	-	-	-	0.251	-	-	0.015*
Cubic t.		0.422	-	-	-	0.043*	-	-	0.643
EDU × O_3	6	0.149	0.914	0.737	0.220	0.072†	0.126	0.416	0.686

Significance at the * $p<0.05$, and † $p<0.10$ level. Variables stem and foliage biomasses were excluded from the table because of their insignificant values.
NDF = neutral detergent fiber; ADF = acid detergent fiber; RFV = relative food value; N = nitrogen.

The results of the ANOVA for plant anatomical, chemical composition and nutritive quality characteristics are shown in Table 8. Plants exposed to 2X-O_3 were significantly lower than were CF and NF plants (p=0.013) for root weight (Tables 8 and 9). Total biomass was also significantly lower for 2X-treated purple coneflower than for CF and NF (p=0.043) (Table 8 and Table 9). There was a significant (p=0.005; quadratic) EDU effect on flower weight, meaning that 200- and 400-ppm EDU levels were significantly lower than 0 and 600 ppm EDU levels (Tables 8 and 10).

Table 9
Ozone effects on root- and total-biomass, NDF, lignin, RFV and nitrogen in 2004.

O_3 levels	Root weight (g)	Total Biomass (g)	NDF (%)	Lignin (%)	RFV	Nitrogen (mg/g)
CF	10.67	38.25	21.47	1.89	336.70	14.28
NF	10.77	32.73	22.41	1.97	319.64	13.76
2X	5.40	25.06	26.13	2.81	271.40	16.56

NDF = neutral detergent fiber; RFV = relative food value.

The 2X treatment was significantly different than the CF and NF O_3 treatments for all nutritive quality characteristics except ADF concentration (Tables 8 and 9). Concentration of NDF was increased by 19% due to elevated O_3, and RFV was decreased by 17%. Nitrogen concentration was 15% greater and percentage of lignin was increased by 43% (Table 9). There were no differences in ADF concentration due to O_3 levels; however, ADF concentration exhibited linear and cubic responses among the EDU rates such that the 0 and 200 ppm EDU rates had significantly lower concentrations of ADF than did 400- and 600-ppm levels (p=0.018). Nitrogen concentration exhibited a quadratic response among the EDU rates such that the 0 and 600 ppm EDU had significantly higher N concentration than the 200- and 400-ppm.

Table 10
Ethylenediurea effects on significant variables in 2004.

EDU (ppm)	Flower weight (g)	ADF (%)	Nitrogen (mg/g)
0	5.36	15.60	15.92
200	3.69	15.62	14.50
400	4.16	16.96	13.89
600	5.20	16.13	15.16

ADF = acid detergent fiber.

Finally, a significant EDU×O_3 interaction was observed for ADF concentration (Table 8). Percent ADF was significantly higher for the 400-ppm EDU level within CF-treated plants compared with the other EDU treatments (p=0.004) (Fig. 4), but there were no differences among EDU levels for the NF and 2X O_3 treatments (p=0.943).

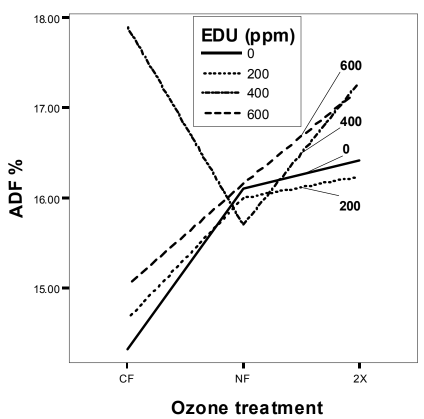

Fig. 4. Ethylenediurea and O_3 interaction of acid detergent fiber (ADF) in purple coneflower in 2004.

DISCUSSION

Results from this experiment demonstrate that O_3 had an overall effect on growth and nutritive quality of purple coneflower. The antiozonant EDU alone had effects on total above-ground biomass and concentrations of NDF and lignin in 2003, and on flower weight and number and concentrations of ADF and nitrogen in 2004. There were some interactions observed regarding primarily visible injury and nutritive quality. Response to EDU levels was different within different O_3 treatments for % NDF, ADF and lignin in 2003, and for number of flowers averaged across time and ADF concentrations in 2004.

Foliar injury and flower production

In this study, visible O_3 symptoms were only observed with the 2X-treated plants. There were no effects of EDU in 2003, but EDU decreased the percentage of leaf area injured in the 2X-treated plants at all concentrations (200, 400 and 600 ppm) in 2004. Previous studies have shown that EDU could protect herbaceous plants from foliar injury (Braunschon-Harti et al., 1995; Manning, 2000; Agrawal et al., 2003b). Godzik and Manning (1998) reported that 300 ppm of EDU protected Bel-W3 tobacco from foliar O_3 injury and did not have an adverse affect on plants exposed to CF air.

To assess the reproductive ability of purple coneflower, number of flowers was counted in both years; however elevated O_3 did not reduce flower quantity in this study. Findley et al. (1997) found that elevated O_3 concentration reduced the number of floral buds and inflorescences formed in buddleia (*Buddleia davidii*) by 29 to 41 %. Flower production was not affected by EDU in 2003; however, EDU played an important role in 2004 in which plants treated with 200 and 400 ppm EDU yielded fewer flowers than did plants treated with the 0 or 600 ppm EDU levels. Gimeno et al., (2004) observed that elevated O_3 significantly reduced the flower biomass production in three clover species. When plants were protected from ambient or elevated O_3 for 45 days, a beneficial effect on flower production was observed.

Biomass values

Elevated O_3 concentrations did not affect total above-ground biomass in 2003; however, root and total biomass were decreased in 2004. Exposure to elevated O_3 can harm plant tissues and interrupt normal patterns of resource acquisition and distribution such that chronic exposure over a growing season ultimately reduces plant biomass yield (Krupa et al., 2004). Ozone did not alter total above-ground biomass in 2003, due in part to the shorter duration of the project compared with 2004, or the other environmental variables such as temperature. Environmental factors (such as temperature) that control stomatal conductance essentially vary the sensitivity of vegetation to O_3 exposure (Barbel, 2002). Mean overall temperature was 6 C° lower in 2003 than in 2004.

There were also different impacts due to the applied EDU rates. The 100- and 200-ppm EDU treatments increased total biomass values compared with the control and 300 ppm EDU by 14% in 2003. There were no EDU effects on root and total biomass values of purple coneflower plants in 2004, which may have been due to the higher EDU levels employed compared with 2003. Similarly, EDU negatively affected flower weight at the 200- and 400-ppm levels in 2004. Elevated O_3 is known to decrease photosynthesis and cause changes in photosynthate allocation in sensitive plants (Cooley and Manning, 1987). Ozone did not affect leaf number in 2004; however, EDU decreased foliage production compared with the control treatment during the middle of the growing period. Exposure of purple coneflower to elevated O_3 resulted in a decrease in root and total biomass of 50% and 35%, respectively, in 2004. Krupa et al., (2004) reported that root biomass in graminaceous crops was reduced more than shoot biomass due to O_3. Karlsson et al. (2003) found that the root biomass was reduced by 30% after the first growing season in a study with birch (*Betula pendula*), and root mass reduced by 35% by the termination of the experiment. Additionally, in an experiment over 5 years with soil-grown birch in an open-air O_3 fumigation system, root biomass was reduced by 33.8% while no significant reduction was detected in above-ground biomass (Oksanen, 2001). Szantoi et al., (in review) found O_3 effects on foliage, stem, root and total biomass values on cutleaf coneflower and EDU effects on root and total biomass in 2004.

There were no interactions found between O_3 concentration and EDU application on biomass, Szantoi et al., (in review) found similar results from experiment with cutleaf coneflower in which EDU and O_3 interaction was not detected for any biomass value; however, Braunschon-Harti et al., (1995) observed interaction between EDU and O_3 levels for root biomass in their study with common bean.

Nutritive quality

Exposure to elevated O_3 increased concentrations of cell-wall constituents that are negatively associated with nutritive quality for ruminant herbivores (Van Soest, 1994). Lewis et al. (2004) observed in eastern gamagrass (*Tripsacum dactyloides*) and big bluestem (*Andropogon gerardii*) that nutritive quality can be negatively affected by O_3 without a concomitant decline in biomass. While nutritive quality of purple coneflower was decreased due to elevated O_3, EDU attenuated the increase in concentrations of cell-wall constituents in 2003 such that the nutritive quality was not as adversely impacted by O_3.

In 2003, O_3 decreased the nutritive quality (increased concentration of NDF by 31% and concentration of ADF by 25%, and decreased RFV by 25%), but EDU attenuated the nutritive quality (NDF and lignin) in plants exposed to elevated O_3. During 2004, exposure to elevated O_3 decreased nutritive quality of purple coneflower; increased the NDF concentration by 19%, lignin by 43% and decreased the RFV value by 16 %; and EDU alone attenuated the concentrations of ADF and N. Forage concentrations of ADF and NDF are inversely related to digestibility and intake, respectively (Van Soest, 1994), which means that higher NDF and ADF concentrations are negatively related to RFV. Reductions in nutritive quality by O_3 have been reported for several plant species. Pleijel and Danielsson (1997) stated that elevated O_3 exposure decreased biomass and nutritive quality in O_3-sensitive plants. Decreased nutritive quality (increased NDF, ADF and lignin concentrations) of bahiagrass and sericea lespedeza exposed to O_3 was sufficient to have short-term nutritional implications to their utilization by agriculturally important ruminant herbivores (Muntifering et al., 2000 and Powell et al., 2003). Szantoi et al. (in review) found that exposure to elevated O_3 increased concentrations of cell wall constituents of cutleaf coneflower along with biomass decrease. Lignin concentrations were unchanged in 2003, but were increased by 43% under elevated O_3 in 2004. Bender et al., (2006) reported increased concentrations of lignin in *Poa pratensis* by 25%, Sanz et al., (2005) by 366% in a study with subterranean clover (*Trifolium subterraneum*) and Szantoi et al., (in review) by 83% in cutleaf coneflower under elevated O_3 levels. As has been reported, lignin synthesis increase as a general response of plants under environmental stresses (Hock and Wolf, 2005; Sanz et al., 2005). Nitrogen concentration was significantly higher in plants that were exposed to elevated O_3 (by 15%). Fenn et al. (2003) stated that higher nitrogen concentration may enhance plant growth, but excess N may increase plant susceptibility to biotic and abiotic factors. In a recent study, Sanz et al. (2005) found that nitrogen fertilization intensified O_3 effects on concentrations of ADF in subterranean clover. Bender et al., (2006) found no O_3 effects on nitrogen concentration in *Poa pratensis*, while Szantoi et al., (in review) observed a 58% increase under elevated O_3 in cutleaf coneflower plants.

There has been little research conducted on EDU effects to nutritive quality. In this present study, there was a decreasing linear trend in NDF and lignin concentrations as the level of EDU was increased in 2003; the effect was most pronounced for the control treatment compared with 300-ppm EDU. In 2004, EDU was not effective; higher EDU levels (400 and 600 ppm) increased ADF concentrations of the plants. Response to EDU was different under elevated O_3 levels. In 2003, higher EDU successfully decreased the NDF, ADF and lignin concentrations, whereas EDU had no effect in charcoal-filtered or non-filtered treatments. During 2004, the 400-ppm EDU increased ADF concentrations under charcoal-filtered air. Szantoi et al., (in review) observed interactions of EDU and elevated levels of O_3

where higher levels of EDU effectively decreased NDF and ADF concentrations, and increased RFV of cutleaf coneflower plants. The RFV for purple coneflower plants in ambient and charcoal-filtered air was more than 3 times, and under elevated O_3 it was 2.7 times higher than a full bloomed alfalfa (*Medicago sativa*) plant, which is considered as standard forage plant (RFV=100) (Linn and Martin, 1989).

CONCLUSIONS

Changes in growth and nutritive quality could lead to significant modifications of ecosystem structure and functioning with implications to system productivity and sustainability (Powell et al., 1999). Results from this experiment demonstrate that O_3 had an overall effect on growth and nutritive quality of purple coneflower. However, responses to EDU were inconsistent. In 2003, it affected the number of flowers, total above-ground biomass, and foliage concentrations of NDF and lignin, and in 2004 it affected flower weight and number, and concentrations of ADF and N. Ozone response was ameliorated at higher rates of EDU regarding nutritive quality. This is an important finding and requires further investigation.

This is the first report of O_3-induced effects on *Echinacea purpurea* and the first to report EDU usage on a species important for medical purposes. Before field testing it is imperative to conduct toxicological studies under controlled conditions (OTCs) to determine the rate of EDU to be used and if the species of interest exhibits a toxic response to EDU (Manning, 1992). In addition, it is important to determine if the species is responsive to O_3 and EDU treatments. Purple coneflower showed O_3 effects during the study period; however ambient O_3 mean 12-hr concentrations during both seasons were below 40 ppb, these lower values are regarded as not injurious to O_3-sensitive plants (Kärenlampi and Skärby, 1996).

This study with purple coneflower supports the concept that it is not necessary to have a yield loss or visible injury in order to exhibit decreased nutritive quality (Krupa et al., 2004). However, antiozonant EDU attenuated the elevated O_3 impact on some nutritive characteristics. It appears that purple coneflower can be used for future research, since we didn't find a phytotoxic effect at any rates of EDU; also it may be useful as a tool to test purple coneflower in the field under ambient O_3 around areas with higher O_3 levels.

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LITERATURE CITED

Agrawal, M., Rajput, R.K., Singh, 2003b. Use of ethylenediurea to assess the effects of ambient ozone on Vigna radiata, International Journal of Biotronics 32, pp. 35–48.

American Lung Association., 2002. State of the Air 2002. Online at: http://www.lungusa.org/air2002/index.html

AOAC., 1995. Official Methods of Analysis 16th edn. Arlington, Virginia: Association of official Analytical Chemists.

Barbel, Z., 2002. Relations between crown condition and ozone and its dependence on environmental factors, Environmental Pollution, Volume 119, Issue 1, 55-68.

Barrett, B., 2003. Medicinal properties of Echinacea: a critical review, Phytomedicine 10 66–86.

Bender, J., Muntifering, R.B., Lin, J.C., Weigel, H.J., 2006. Growth and nutritive quality of Poa pratensis as influenced by ozone and competition, Environmental Pollution, In Press, Corrected Proof,

Bergweiler, C.J. and Manning, W.J. 1999. Inhibition of flowering and reproductive success in spreading dogbane (Apocynum androsaemifolium) by exposure to ambient ozone. Environmental Pollution 105:333-339.

Black, V.J., Black, C.R., Roberts, J.A., Stewart, C.A., 2000. Tansley Review No. 115. Impact of ozone on the reproductive development of plants, New Phytologist 147 pp. 421–447.

Brevoort, P., 1998. The blooming U.S. botanical market: a new overview. Herbalgram 44: 33-46

Brunschon-Harti S., Fangmeier A and Jager H.J., 1995.Influence of ozone and ethylenediurea on the antiozonant systems in beans (Phaseolus vulgaris), Environmental Pollution 90, pp. 89–94. Brevoort, 1998

Carnahan, J.E., Jenner, E.L., Wat, E.K.W., 1978. Prevention of ozone injury to plants by a new protectant chemical. Phytopathology 68 pp. 1225–1229.

Chameides, W.L., Kasibhatla, P.S., Yienger, J., Levy, I.I.H., 1994. Growth of continental-scale metro-agro-plexes, regional ozone pollution and world food production. Science 264, 7477.

Chameides, W.J., Cowling, E.B., 1995. The State of the Southern Oxidant Study (SOS): Policy-Relevant Findings in Ozone Pollution Research 1988–1994. In: SOS, Coll. For. Res., NC St. Univ., Raleigh, NC , p. 94.

Chappelka, A.H., Neufeld, H.S., Davison, A.W., Somers, G.L., Renfro, J.R., 2003. Ozone injury on cutleaf coneflower (Rudbeckia laciniata) and crown-beard (Verbesina occidentalis) in Great Smoky Mountains National Park. Environ Pollut. 2003;125(1):53-9.

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

Cox, J., 1978. Purple coneflower. Organic Gardening, May/Jun98, Vol. 45 Issue 5, p52, 2p, 2c

Davison, A.W., Barnes, J., 1998. Effects of ozone on wild plants. New Phytologist 139, 135151.

Fenn, M.E., Poth, M., Bytnerowicz, A., Sickman, J.O., Takemoto, B.K., 2003. Effects of ozone, nitrogen deposition and other stressors on montane ecosystmes in the Sierra Nevada. In: Ozone Air Pollution in the Sierra Nevada: Distribution and Effects on Forests. Bytnerowicz, A., Arbaugh.

Findley D.A., Keever G.J., Chappelka A.H., Eakes D.J., Gilliam C.H., 1997. Differential response of buddleia (Buddleia davidii Franch.) to ozone, Environmental Pollution, Volume 98, Issue 1, 105-111.

Fuhrer, J., Booker, F., 2003. Ecological issues related to ozone: agricultural issues. Environment International 29, 141154.

Gimeno, B.S., Bermejo, V., Sanz, J., de la Torre, D., Gil, J.M., 2004. Assessment of the effects of ozone exposure and plant competition on the reproductive ability of three therophytic clover species from Iberian pastures. Atmospheric Environment 38: 2295–2303

Godzik, B., Manning, W.J., 1998. Relative effectiveness of ethylenediurea, and constituent amounts of urea and phenylurea in ethylenediurea, in prevention of ozone injury to tobacco. Environmental Pollution 103: 1-6.

Heagle, A. S., Body, D. E., Heck, W.W., 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual., 1:365-368.

Heagle, A.S., Philbeck, R.B., Ferrell, R.E., Heck, W.W., 1989. Design and performance of a large, field exposure chamber to measure effects or air quality on plants. J Environ Qual 18:361–368

Hock, B., Wolf, N.M., 2005. Charasteristics of plant life: hazards from pollutants. In: Hock, B., Elstner, E.F. (Eds.), Plant Toxicology. Marcel Dekker, New York, pp. 1-85

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

Kärenlampi, L., Skärby, L., (Eds.) 1996: Critical Levels for Ozone in Europe: Testing and Finalizing the Concepts. UN ECE Workshop Report.

Karlsson, P.E., Uddling, J., Skarby, L., Wallin, G., Sellden, G., 2003. Impact of ozone on the growth of birch (Betula pendula) saplings Environmental Pollution 124 485–495

Kostka-Rick, R., Manning, W.J., 1993a. Dynamics of growth and biomass partitioning in fieldgrown bush bean (Phaseolus vulgaris L.) treated with the antiozonant ethylenediurea (EDU). Agriculture, Ecosystems and Environment 47: 195-214.

Kostka-Rick, R., Manning, W.J., 1993b. Dose-response studies with ethylenediurea (EDU) and radish. Environmental Pollution 79: 249-260.

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

Krupa, S.V., Muntifering, R., Chappelka, A., 2004. Effects of ozone on plant nutritive quality characteristics for ruminant animals. The Botanica 54, 129-140.

Lefohn, A.S., Krupa S.V., Winstanley, D., 1990. Surface Ozone Exposures Measured at Clean Locations Around the World. Environmental Pollution. 63(3):189-224.

Lewis, J. S., Ditchkoff S. S., Lin J. C., Muntifering R.B., Chappelka A.H., 2004. Nutritive Quality of Big Bluestem (Andropogon gerardii) and Eastern Gamagras (Tripsacum dactyloides) Exposed to Tropospheric Ozone. Journal of Range Management, in press

Linn, J.G., Martin, N.P., 1989. Forage quality tests and interpretation. St. Paul: Bull. AG-FO-2637. University of Minnesota Extension Service.

Manning, W.J., 2000. Use of protective chemicals to assess the effects of ambient ozone on plants. In: Environmental Pollution and Plant Responses, eds. S.B. Agrawal and M. Agrawal., Lewis Publishers, Boca Raton, FL, pp 247-258.

Manning, WJ., 1992. Assessing the effects of ozone on plants: Use and misuse of ethylenediurea (EDU). Proceedings of the 85th Annual Meeting and Exhibition on Air and Waste Management Association 11 pp.

Muntifering, R.B., Crosby, D.D., Powell, M.C., Chappelka, A.H., 2000. Yield and quality characteristics of bluegrass (Paspalum notatum) exposed to ground-level ozone. Animal Feed Science and Technology 84, 243256.

Nebel, B., Wright, R., 1998. Major Depletion of the Ozone Shield. Environmental Science, 418-426.

Oksanen, E.J., 2001. Increasing tropospheric ozone level reduced birch (Betula pendula) dry mass within a five years period. Water, Air, and Soil Pollution 130, 947–952.

Pleijel, H., Danielsson, H., 1997. Growth of 27 herbs and grasses in relation to ozone exposure and plant strategy. New Phytologist 135, 361367.

Powell, M.C., Crosby, D.D., Muntifering, R.B., Chappelka, A.H., 1999. Biomass yield, nutritive quality and secondary chemistry in select warm-season forages exposed to ground-level ozone. In: Nutritional Ecology of Herbivores. Online poster at http://cnrit.tamu.edu/conf/isnh/post-online/post0018/ Guy Stone, T.D.A Forbes, J.W. Stuth and F.M. Byers (Ed.). April 11-16, 1999. San Antonio, TX.

Powell, M.C., Muntifering, R.B., Lin J.C., Chappelka, A.H., 2003. Yield and nutritive quality of sericea leapeseza (Lespedeza cunceata) and little bluestem (Schizachyrium scoparium) exposed to ground-level ozone. Environmental Pollution 122, 313322.

Rohweder, D.A., Barnes, R.E., Jorgensen, N., 1978. Proposed hay grading standards based on laboratory analysis for evaluating quality. J. Anim. Sci. 47:747-759.

Sanz, J., Muntifering, R.B., Bermejo, V., Gimeno, B.S., Elvira, S., 2005. Ozone and increased nitrogen supply effects on the yield and nutritive quality of Trifolium subterraneum, Atmospheric Environment, Volume 39, Issue 32, 5899-5907.

Szantoi, Z., Chappelka, A.H., Muntifering, R.B., Somers, G.L., Assessing ozone effects with Ethylenediurea (EDU): foliar and cell-wall compositional changes in ozone sensitive Cutleaf coneflower (*Rudbeckia laciniata L.*). New Phytologist (in review)

Stubbendieck, J., Nichols, J.T., Butterfield, C.H., 1989. Nebraska range and pasture forbs and shrubs (including succulent plants). Extension Circular 89-118. Lincoln, NE: University of Nebraska, Nebraska Cooperative Extension. 153 p.

Tonneijck A.E.G. and. van Dijk C.J, 1997. Effects of ambient ozone on injury of Phaseolus vulgaris at four rural sites in the Netherlands as assessed by using ethylenediurea (EDU). New Phytologist 135. pp. 93–100

United States Environmental Protection Agency. 2001. Latest finds on national air quality: 2000 status and trends. OAQPS, US EPA, RTP, NC. Report nr EPA 454/K-01-002.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991.Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, Journal of Dairy Science 74, 3583–3597

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant (second ed.), Comstock, Ithaca, NY.

Vingarzan, R., 2004. A review of surface ozone background levels and trends. Atmospheric Environment 38, 34313442.

CHAPTER III.

ASSESSING OZONE EFFECTS WITH ETHYLENEDIUREA (EDU): FOLIAR AND CELL-WALL COMPOSITIONAL CHANGES IN OZONE SENSITIVE CUTLEAF CONEFLOWER (*Rudbeckia laciniata L.)*

ABSTRACT

Ozone (O_3)-sensitive cutleaf coneflower (*Rudbeckia laciniata*) plants were placed into open-top chambers (OTC) in May 7, 2004. Nine OTCs were fumigated – in 3 blocks - with either charcoal filtered air (CF), non-filtered air (NF) or twice-ambient (2X) O_3 air. EDU was applied to foliage weekly at 0 (control), 200, 400 and 600 ppm levels during May – August 2004. Foliar injury was evaluated every third week, and a destructive harvest was carried out to determine foliage, stem, root and total biomass, and to assess nutritive quality at the end of the study period. Foliar injury was observed (15-20% of plants injured) at ambient O_3 concentrations. Elevated O_3 caused foliar injury, changed biomass and decreased nutritive quality. EDU reduced % of leaves injured and decreased root and total biomass. Cell-wall constituents were not affected by EDU; however EDU x O_3 interactions were observed for NDF and ADF. These results demonstrated that O_3 caused significant changes in physiology and productivity of cutleaf coneflower and EDU was not as successful in alleviating O_3 injury as in previous studies.

INTRODUCTION

There have been numerous reports on agricultural crop and forest tree response to tropospheric O_3, but interest in effects on native herbaceous species is relatively new, and plant responses are still not clear (Davison & Barnes, 1998). Chappelka et al. (1997), in a foliar injury survey in the Great Smoky Mountains National Park (GRSM) during the summer of 1992, found that black cherry (*Prunus serotina*) and tall milkweed (*Asclepias exaltata*) were exhibiting visible symptoms of injury due to O_3. Approximately 50% of the plants were injured across both species. In a more recent study, Chappelka et al. (2003) reported that cutleaf coneflower in the GRSM had more foliar injury symptoms resulting from exposure to ambient O_3 concentrations than did crown-beard (*Verbesina occidentalis* Walt.) during the 2000 – 2001 growing seasons. Incidence and severity of injury was greater for cutleaf coneflower growing near trails, which was probably due to differences in microclimatic (Finkelstein et al., 2004) and/or genetic (Davison et al., 2003) factors. Also, O_3 injury was greatest on the lower (older) leaves in both species (Chappelka et al., 2003).

Tropospheric O_3 is considered the most significant phytotoxic air pollutant across many parts of the USA and world-wide (Krupa et al., 2001). Despite national air quality policies aimed at controlling tropospheric O_3 levels, it continues to be a major concern for agricultural production and native vegetation in the southeastern US (United States Environmental Protection Agency, 1996). Due to urbanization, high temperatures and an adequate source of volatile organic chemicals from vegetation, ambient O_3 concentrations are elevated in the southeastern US (Chameides & Cowling, 1995). During the summer months, O_3 concentrations can exceed 120 ppb (US EPA, 1996) in the southeast US (ALA, 2002). Average concentrations exceed 80 ppb (new standard) in several areas of the Southeast, including areas like GRSM and Shenandoah National Parks. Ozone is not limited to just urbanized areas; it can be transported to rural, remote areas (Krupa & Manning, 1988; Chameides et al., 1994). Vingarzan (2004) indicated that O_3 levels will continue to increase between 0.5% and 2.0% per year in the Northern Hemisphere during the next several decades, reaching average global surface O_3 concentrations of 35-48 ppb (nl l^{-1}) by 2040 and 38–71 ppb by 2060, from 20-45 ppb in 2000. These concentrations are in the range that is known to cause plant injury in sensitive species (Chappelka & Samuelson, 1998).

The effects of O_3 exposures on terrestrial vegetation may include foliar injury, decreases in growth and yield, changes in foliar chemistry, and predisposition to abiotic or biotic stresses (Chappelka & Samuelson, 1998; Davison & Barnes 1998; Muntifering et al., 2000). Moreover, O_3 has the potential to cause damage to agricultural crops, forest trees and herbaceous plants at the community or ecosystem level (Davison & Barnes 1998; Bell & Treshow, 2002).

Open-top field chambers (OTCs) (Heagle et al., 1973) and the protective antiozonant chemical ethylenediurea (EDU), N-{2-(2-oxo-l-imidazolidinyl) ethyl}-N'-phenylurea (Carnahan et al., 1978) have been widely used methodologies for determining O_3 exposure on vegetation. Ethylenediurea is, at present, the best-known systemic antiozonant. Several reports have indicated that EDU can be used to assess injury from exposure to ambient O_3 and, most likely, protect vegetation (Ball et al., 1998; Bergweiler & Manning 1999; white clover (*Trifolium repens*) and spreading dogbane (*Apocynum androsaemifolium*), respectively). However, in many cases, the trials were not well designed and assumptions were made without validation

(Manning et al., 2003, Tiwari et al., 2005); namely, that EDU itself had no adverse effects on plants and that the rate(s) used in pot experiments for assessing O_3 injury were readily applicable in the field. These assumptions are not always valid (Manning, 2005).

Rudbeckia laciniata (cutleaf coneflower) has been identified as a very sensitive O_3-indicator plant (Neufeld et al. 1992; Chappelka et al. 2003). Cutleaf coneflower is a perennial, native plant within the group of the Asteraceae family that occurs throughout North America (Niering et al., 2001). It is usually found growing as a shrub, along the forest edge. The plants are characteristically 1.5 – 2 m high with dense foliage about 20-80 cm from the ground. Flowering normally occurs in August (Niering et al., 2001).

This experiment was conducted to assess the use of EDU as an antiozonant by conducting a toxicological study on cutleaf coneflower in OTCs. Foliar injury, biomass and cell-wall constituents were measured and examined. The specific objectives were to: (1) perform exposure-response toxicology studies using different EDU rates under controlled (OTC) field conditions, with different O_3 concentrations and (2) assess quantitatively the extent of O_3 effects on foliar symptom appearance, yield and cell-wall constituents as they relate to nutritive quality for mammalian herbivores.

MATERIALS AND METHODS

Study site

The 1.5-ha research site was located at Auburn University (32° 36′ N, 85° 30′ W) and was originally forested with loblolly pine (*Pinus taeda*) trees. The range of topography is from level to a 1-3% slope, the soil type was a Cowarts (Typic

Kanhapladult) soil series. Average annual precipitation for the Auburn area is 1370 mm (Alabama Weather Information System Inc, Auburn, AL, 2005). The area was seeded with bahiagrass (*Paspalum* notatum) in 1987. Within the chambers the soil was sprayed with a pre-emergence herbicide (Roundup®) one month before the study was initiated, then covered with straw to minimize invasion of weeds. The O_3 exposure system included 9 large (4.8 m height, 4.5 m diameter) OTC, each consisting of an aluminum frame enclosed by clear plastic (Heagle et al., 1989).

Plant materials

Cutleaf coneflower seeds (purchased from Prairie Nursery, Inc., Westfield, WI) were germinated and placed into trays in a greenhouse 6 weeks prior to placement into the OTCs. In the third week post-germination, each seedling was replanted into a 3.78-L pot filled with a 1:1 mixture of peat moss and Norfolk sandy loam soil. Plants were watered daily and fertilized (4 g of 15:16:17 of N: P_2O_5: K_2O) once weekly. The potted plants were placed into OTCs on May 7, 2004 and were acclimated in the chambers for 4 days prior to initiation of treatments. A total of 20 plants were placed randomly in 4 rows in each chamber with EDU treatment randomly assigned to each row. Plants were irrigated three times daily (1000, 1400, and 1800 h) for 15 minutes and fertilized once every fourth week with 4 g of fertilizer (14-14-14 of N: P_2O_5: K_2O).

Ozone exposures

Three O_3 treatments, replicated three times (blocks) were applied: CF (carbon filtered air, approx. 0.5 × ambient-O_3 air), representing a pristine environment (Lefohn et al., 1990); NF (non-filtered ambient air) and 2 × ambient-O_3 air (2X), representative of concentrations found in the vicinity of large metropolitan areas such as Birmingham, AL and Atlanta, GA (Chameides & Cowling, 1995). Ozone was generated by passing pure oxygen through a high-intensity electrical discharge source (Griffin Inc., Lodi, NY, USA) and applied to the chambers from 0900–2100 h (12 h d^{-1}, 7 d wk^{-1}). Chamber fans were operated 15 h d^{-1}, 7 d wk^{-1} between 0700–2200 h. and were turned off from 2200-0700 h to allow dew formation. Ozone treatments were initiated on May 7, 2004 and ended on August 3, 2004 (12 weeks of fumigation). Ozone concentrations were continuously monitored in each chamber twice per hour. All O_3 monitoring equipment was calibrated and audited according to US EPA procedures (Chappelka, 2002).

EDU treatments

Three EDU and one control (tap water) treatment were applied. The concentrations used were 0 (control), 200, 400, and 600 ppm of active ingredient. Ethylenediurea treatments were initiated after the fourth day following placement of plants in the O_3-treated chambers. The entire foliage of each plant was sprayed until leaves were saturated. EDU was applied weekly as a foliar spray during exposure periods.

Plant measurements

Every third week (May 7 – August 3), leaf greenness was measured with a SPAD 502 Chlorophyll Meter (CANON Inc. Tokyo, Japan), and visible injury was ocularly estimated as the incidence (% of plants injured), the % of leaves injured and the % of leaf area injured / injured plant (severity). A modified Horsfall-Barratt rating scale (Horsfall and Barratt, 1945) was used to quantify the relative % leaves injured and leaf area injured / injured plant (classes: 1= 0%, 2= 1-6%, 3= 7-25%, 4= 26-50%, 5=

51-75% and 6= 76-100%). Three times during the growing season, total numbers of leaves were counted. In addition, each plant was separated at harvest by anatomical components (root, stem and foliage) and dried to a constant weight, and biomass was determined. Harvested plants were oven-dried at 50°C to a constant weight, and biomass was recorded. Pooled foliage for each experimental unit (5 plants) was ground in a Wiley mill to pass a 1-mm screen prior to laboratory analysis of cell-wall constituents.

Cell wall constituents were sequentially fractionated into neutral detergent (NDF), acid detergent fiber (ADF) and lignin according to procedures of Van Soest et al. (1991) using an ANKOM fiber analyzer (ANKOM Technology Corporation, Fairport, NY). Nitrogen (N) concentration was determined by the Kjeldahl method according to Association of Official Analytical Chemists (AOAC, 1995).

Experimental design and statistical analysis

The overall design of the experiment was a completely randomized split-plot. Three blocks (3 OTCs in each block) were used during the fumigation period because of different light conditions at the site. Whole-plot treatments (O_3) were CF, NF and 2X. EDU rates were sub-plot treatments and were 0, 200, 400 and 600 ppm. Biomass and cell-wall compositional data were analyzed with analysis of variance (ANOVA) using JMP IN 5.1 statistical software package from the Statistical Analysis Institute (SAS, 1989). Repeated measures analysis (MANOVA) was used to assess differences in incidence, % of leaves injured, % of leaf area injured / injured plant, leaf greenness and leaf number over time.

RESULTS

Climatic data

Mean monthly air temperatures for May through August were within 2.1°C of the 30-year (1971-2000) average for the Auburn, AL area. Monthly rainfall values for May-July were below the 30-year average; however, rainfall in August was 3.9 cm above the 30-year average as shown in Table 1.

Table 1 Average monthly air temperatures and rainfall amounts for months of exposure in 2004, and 30-year averages for Auburn, AL.

Month	Air temperature (°C)		Precipitation (cm)	
	2004	30-year average	2004	30-year average
May	23.3	21.2	9.4	9.7
June	25.6	24.9	8.5	10.3
July	27.2	26.2	8.7	14.9
August	25.7	26.1	13.1	9.2

(AWIS, Auburn, AL, 2005)

Ozone exposures

Mean 12-h (0900–2100 h) O_3 exposures over the experimental treatment period were similar to the target values. A peak 1-h O3 concentration in July (83 ppb) was the highest in the NF treatment. Mean daily ambient O_3 concentration averaged 33 ppb for the 12-week period. The maximum concentration for the 2X treatment was 177 ppb (July), and average daily 2X O_3 concentrations over the duration of the experiment were 73 ppb. Ozone concentrations in the CF chambers were reduced by approximately 36 % compared with the NF treatment (Table 2).

Table 2 Average 12-h O_3 concentrations for the study duration in 2004, in Auburn, AL[a]

2004	Average 12-hours O_3 concentration (ppb)		
Air treatment	CF	NF	2X
May (mean)	21	32	69
(min-max)	8-49	12-57	12-107
June (mean)	19	29	70
(min-max)	3-42	3-59	3-122
July (mean)	25	34	80
(min-max)	5-54	6-83	9-177
August (mean)	21	38	77
(min-max)	6-37	11-65	12-125

[a]Duration = May 7, 2004 – Aug. 3, 2004.

Plant measurements

Analysis of leaf greenness revealed insignificant differences; therefore it was excluded from the results. No visible injury was found in CF chambers, and about 25 % of visible injury symptoms were observed under ambient O_3 conditions. These treatments were excluded from the statistical MANOVA analysis since the observed injury (NF) appeared late in the season. MANOVA analysis (2X-treated plants) of cutleaf coneflower indicated a marginally significant EDU effect (p=0.106) across time for percentage of leaves injured (Table 3).

Table 3 P-values from repeated measure analysis of foliar injury on cutleaf coneflower exposed to elevated O_3 (2X) and treated with EDU.

	d.f.	Incidence	% Leaves injured	% of leaf area injured
EDU	3	0.200	0.106	0.581
Linear trend	1	-	0.027*	-
Quadratic trend	1	-	0.362	-
Cubic trend	1	-	0.798	-
Time×EDU	9	0.141	0.625	0.902

Significance at the * p<0.05 level.

There was a decreasing linear response in % leaves injured among the EDU levels (p=0.027) over time (Table 4). The 600-ppm EDU treatment reduced leaf injury the most during the treatment period.

Table 4 Ethylenediurea characteristics for % of leaves injured across time on cutleaf coneflower, exposed to elevated (2X) O_3 concentrations.

EDU (ppm)	% Leaves injured
0	13.92
200	13.17
400	11.08
600	9.19

Increasing O_3 concentrations had significant effects on all variables analyzed after harvest. Cutleaf coneflower foliage biomass was decreased by 57% in the 2X O_3 treatment compared with NF and CF treatments. In the 2X treatment, plant stem dry weight was three and two times higher, respectively, than that in the CF and NF treatments. Root weight at the highest O_3 concentration (2X) was decreased by 40 % compared with CF and NF conditions, and plants in the 2X chambers produced 40 % less biomass than those in the CF and NF chambers (Tables 5 and 6).

Table 5 Significance levels of split-plot analysis for biomass and cell-wall constituents for cutleaf coneflower exposed to O_3 and treated with EDU during 2004.

	d.f.	Foliage	Stem	Root	Total	NDF	ADF	Lignin	N
O_3	2	0.013*	<0.001*	0.026*	0.030*	0.003*	0.003*	0.022*	<0.001*
EDU	3	0.153	0.233	0.046*	0.047*	0.356	0.476	0.205	0.235
Linear t.		-	-	0.019*	0.017*	-	-	-	-
Quad t.		-	-	0.209	0.189	-	-	-	-
Cubic t.		-	-	0.232	0.347	-	-	-	-
EDU × O_3	6	0.604	0.548	0.347	0.467	0.062†	0.019*	0.680	0.303

Significance at the * p<0.05, and † p<0.10 level.

NDF = neutral detergent fiber, ADF = acid detergent fiber, N = nitrogen.

Table 6 Means among O_3 treatments, for final harvest data (dry weight) of cutleaf coneflower.

O_3 levels	Foliage (g)	Stem (g)	Root (g)	Total (g)
CF	30.84a	2.01a	43.48ab	76.34ab
NF	31.65a	3.05a	61.02a	95.74a
2X	13.15b	6.72b	30.59b	50.47b

Means within columns followed by the same letter are not significantly different ($p<0.05$).

Elevated O_3 significantly altered concentrations of cell-wall constituents in cutleaf coneflower (Table 7). Concentrations of NDF and ADF were increased by 35 % and 32 %, respectively, compared with the CF and NF treatments. Lignin concentration was increased by 83 % in 2X-treated plants compared with CF- and NF-treated plants. N concentration for the 2X treatment was increased by 58 % compared with the two other O_3 treatments (CF and NF).

Table 7 Means among O_3 treatments, for cell-wall constituents data of cutleaf coneflower.

O_3 levels	NDF (%)	ADF (%)	Lignin (%)	Nitrogen (mg/g)
CF	21.66a	15.32a	3.06a	11.97a
NF	21.33a	15.77a	2.84a	11.97a
2X	29.03b	20.57b	5.38b	19.13b

Means within columns followed by the same letter are not significantly different ($p<0.05$). NDF = neutral detergent fiber, ADF = acid detergent fiber.

There were differences in root and total dry weight (Table 8). A significant linear trend of decreasing biomass was observed with increasing EDU levels.

Table 8 The effects of EDU levels on biomass values of cutleaf coneflower.

EDU levels	Root (g)	Total (g)
Control	54.03	88.18
200 ppm	48.25	77.65
400 ppm	36.20	62.14
600 ppm	41.64	68.76

The ANOVA for concentrations of cell-wall constituents revealed significant interactions between EDU and O_3 for concentrations of NDF and ADF (Fig. 1). The 400- and 600-ppm EDU rates were effective in decreasing NDF concentration of the foliage (Fig. 1) by an average of 13% compared with the control and 200-ppm EDU levels ($p<0.001$). Under CF and NF conditions, there were no significant differences among EDU levels ($p=0.999$, $p=0.527$, respectively). For CF and NF treated-plants, EDU did not have effect on concentration of ADF ($p=0.341$, $p=0.490$ respectively) (Fig. 1). Within the 2X-O_3 treatment, the 400-ppm and 600-ppm EDU levels significantly decreased concentrations of ADF by an average of 14 % compared with the control and 200 ppm treatments ($p<0.001$).

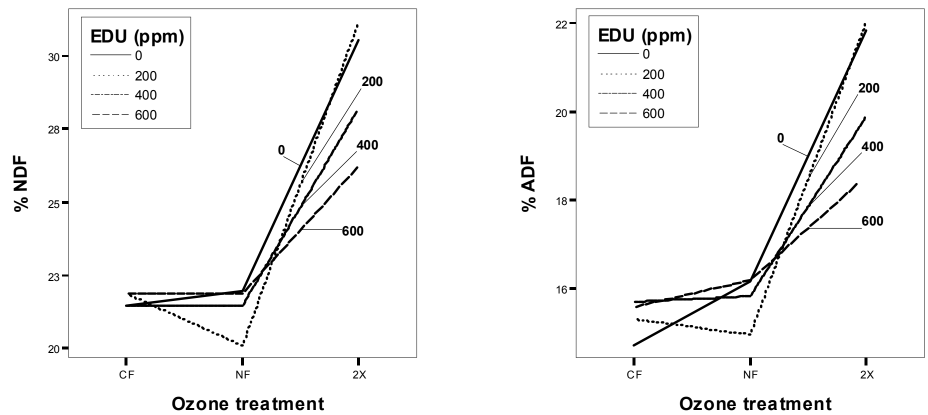

Fig. 1 Ethylenediurea and cell-wall compositional characteristics under different O_3 treatments in 2004.

DISCUSSION

Over the past few years, O_3 effects on visible injury have been investigated on cutleaf coneflower in the Great Smoky Mountains National Park (Chappelka et al., 2003; Davison et al., 2003), where cutleaf coneflower showed high sensitivity for O_3. The results of the present study also revealed that elevated O_3 had an overall effect on foliar injury, yield and concentrations of cell-wall constituents. EDU had effects on visible injury across the duration of the study, and on root and total biomass values. No EDU effects were found for foliar concentrations of cell-wall constituents during the study period. While overall there were no EDU effects on cell-wall constituents, EDU treatments did attenuate increases in concentrations of cell-wall constituents in the elevated (2X) O_3 treatment.

Foliar injury

In the current study, no visible symptoms of O_3 injury were observed for plants in CF chamber (≈21.5 ppb, 12hr mean) and about 15-20% of plants in NF chamber showed visible injury (≈33ppb, 12hr mean). In addition to the low O_3 exposure during the experimental period, the study duration was only 12 weeks; therefore, it is considerable that greater O_3 effects on foliar injury may have occured with a longer-term experiment. The experiment was terminated at this time due to the threat of a tropical storm (Bonnie) that made landfall in the Florida Panhandle on August 9. During our study period ambient O_3 concentrations were below the typical Auburn, AL summer daytime O_3 concentrations (≈50 ppb) (Muntifering et al. 2000). While cutleaf coneflower did show high foliar sensitivity to ambient O_3 levels in the GRSM (Chappelka et al. 2003, Finkelstein et al. 2004), the average ambient O_3 level during the May-August period in GRSM NP was approximately 65 ppb (Chappelka et al. 2003).

Under conditions of elevated O_3 (avg. ≈73 ppb) all plants exhibited visible symptoms. The O_3 concentrations at 2X reported in the current study were comparable to those found in GRSM. This finding is similar to what Chappelka et al. (2003) found *in situ* in the GRSM. At the 600-ppm EDU level the % of leaves injured on O_3-exposed plants significantly decreased over time. EDU has been used widely and successfully as a protective chemical of foliar injury in several studies on herbaceous plants (Kostka-Rick and Manning, 1993b; Brunschon-Harti et al, 1994; Manning, 2000). Lower (100 and 150 ppm) EDU concentrations were applied in most of these studies, in which the protective effects of the chemical on plants exposed to O_3 concentrations in non-filtered treatments, was tested. In earlier studies, Carnahan *et al.* (1978) and Cathey and Heggestad (1982a,b) found that a 500-ppm EDU foliar spray was the optimal rate for protecting bean plants, 4 cultivars of petunia and 44 species of herbaceous plants from O_3; biomass was reduced, however.

Biomass

Krupa et al. (2004) have indicated that chronic exposure of O_3 over a growing season has the potential to reduce plant biomass yield. Elevated O_3 (2X) had highly significant effects on cutleaf coneflower biomass in this experiment. O_3 decreased foliage, root and total biomass production, and a decline in biomass of agronomic and herbaceous plants from exposure to elevated O_3 concentrations has been reported by many researchers (Brunschon-Harti et al., 1994; Findley et al., 1997; Sanz et al., 2005; Szantoi et al., in review). Krupa et al. (2004) stated grasses have reduced root dry weight, more so than for stem biomass, in high-O_3 environments. Results from this current study show that cutleaf coneflower plants under elevated O_3 had significantly increased stem dry weights. In contrast, Brunschon-Harti et al. (1994) found in a study with common bean (*Phaseolus vulgaris* L.), and Findley et al. (1997) with buddleia (*Buddleia davidii Franch*), that exposure to elevated O_3 decreased the shoot biomass. Szantoi et al. (in review) observed that elevated O_3 did not affect stem biomass of purple coneflower significantly. Stem biomass response appears to depend on species sensitivity to O_3. Common bean and buddleia are considered as being sensitive to O_3 (Brunschon-Harti et al., 1994; Tonneijck & van Dijk., 1997; Findley et al., 1997), whereas purple coneflower was somewhat sensitive to O_3 (Szantoi et al., in review). Cutleaf coneflower is considered to be very sensitive to O_3 (Chappelka et al., 2003; Davison et al., 2003).

Results indicated that application of EDU itself had significant effects on root and total biomass, but not on foliage dry weight. Ethylenediurea decreased root and total biomass production compared with the control (0 ppm) treatment. Manning et al. (2003) reported similar findings in a study with loblolly pine seedlings (*Pinus taeda* L.) with 150 ppm, but higher rates (450 ppm) of EDU successfully increased growth parameters over a three-year period. Short-term studies with herbaceous plants have demonstrated that root and total biomass of common beans were increased when treated by EDU itself (Brunschon-Harti et al., 1994). Pihl Karlsson et al. (1995a) found that O_3-sensitive subterranean clover (*Trifolium subterraneum* L.) treated with EDU produced more biomass than the non-EDU treated plants at elevated O_3 levels, whereas the opposite occurred for moderately O_3-sensitive red clover (*T. pratense* L.). Phil Karlsson (1995a) suggested that plant response to EDU depends on the O_3 sensitivity of the species. Tonneijck & van Dijk (1997) observed that EDU did not influence total above-ground biomass of subterranean clover, but significantly enhanced the leaf biomass production in ambient-air plots where O_3 occurred in high concentrations. EDU did not affect root or total biomass in a study with purple coneflower at any rate under elevated O_3 concentrations in a recent study (Szantoi et al., in review). Significant effects of the EDU treatments were not observed for stem dry weight, which is in contrast with results from a study by Brunschon-Harti et al. (1994) on common beans in which EDU treatment significantly increased shoot weight.

There were no interactions between elevated O_3 concentrations and EDU observed for any biomass variable measured, which suggests that different EDU rates were affecting plants similarly under O_3 exposure and EDU was not successful in attenuating the O_3 effect.

Cell-wall constituents

Exposure to elevated O_3 increased concentrations and lignification of cell-wall constituents in cutleaf coneflower, and increased foliar N concentrations. Averaged across O_3 treatments, EDU did not affect any variable measured. However, a significant $O_3 \times$ EDU interaction was observed with NDF and ADF, which is a significant finding because it indicates that EDU can decrease concentrations of NDF and ADF in plants exposed to elevated ozone.

Foliar concentrations of NDF and ADF are used in commercial forage testing procedures to determine forage quality. NDF estimates the variably digestible cell wall constituents, including hemicellulose, and ADF represent the least digestible cell wall constituents; i.e., cellulose and lignin. NDF and ADF concentrations are inversely related to in vivo voluntary intake and digestibility, respectively (Van Soest, 1994), by ruminant herbivores. Concentrations of cell-wall constituents were very similar for plants exposed to charcoal-filtered and non-filtered air, but different from those that were exposed to elevated O_3. Muntifering et al. (2000) reported similar results with early-season-planted bahiagrass (*Paspalum notatum*). Powell et al. (2003) found that concentrations of NDF and ADF were lower in sericea lespedeza (*Lespedeza cuneata*) plants that had been exposed to charcoal-filtered air than in non-filtered air or elevated O_3. NDF and ADF concentrations were increased by 35% and 32%, respectively, in cutleaf coneflower plants exposed to elevated O_3. In a recent study with *Poa pratensis*, Bender et al. (2006) found significant differences in concentrations of NDF, ADF and lignin of plants exposed to background O_3 levels and elevated-O_3 levels. Muntifering et al. (2000), Powell et al. (2003), Sanz et al. (2005) and Szantoi et al. (in review) have detected increased concentrations of NDF and ADF in *Pospalum notatum, Lespedeza cuneata, Trifolium subterraneum* and *Echinacea purpurea*, respectively, that had been exposed to elevated O_3.

Lignin concentration increases as a general response of plants to environmental stresses (Hock & Wolf, 2005; Sanz et al., (2005). Lignin concentrations were increased by 83% due to the exposure to elevated O_3. Sanz et al. (2005) observed increases in lignin concentrations of 200% (non-filtered air) and 366% (elevated O_3 level), respectively, compared with control plants in a study with subterranean clover (*Trifolium subterraneum*). Szantoi et al. (in review) found a 43% increase in lignin concentration of purple coneflower plants exposed to elevated O_3 in 2004; however, similar O_3 levels did not affect lignin concentrations for the same species in 2003.

Nitrogen concentration of cutleaf coneflower plants was increased due to the elevated O_3 treatment by 58% in this study. Higher N may increase plant growth, but excess N may enhance plant susceptibility to other abiotic or biotic factors (Fenn et al., 2003). Literature on N response to O_3 exposure deals mainly with deposition and fertilization effects, and research on N in plants due to elevated O_3 levels is limited. Blum et al. (1982, 1983) found that N was increased in ladino clover (*Trifolium repens*) when exposed to elevated O_3. Scherzer et al. (1998) reported that foliar N in yellow-poplar (*Liriodendron tulipifera* L.) and eastern white pine (*Pinus strobus* L.) seedlings were not affected by elevated O_3 during a 3-year study. More recently, Bender et al. (2006) observed that concentration in *Poa pratensis* was not different between control and elevated O_3; however, in a study with purple coneflower, Szantoi et al. (in review) found increases of Nconcentration by 15%.

Positive effects of EDU are known for protecting a variety of plants from foliar injury in elevated O_3 environments (Kostka-Rick & Manning, 1992; Tonneijk & van Dijk, 1997; Ball et al., 1998; Manning, 2000; and Manning et al., 2003). However, little research has been conducted on EDU and its effects on cell-wall constituents, on lignification and on N. In this study, EDU alone did not have any overall effect on cell-wall constituents on cutleaf coneflower; however, EDU effects differed under different O_3 treatments. Higher rates of EDU decreased the NDF and ADF concentrations of cutleaf coneflower plants exposed to elevated O_3. EDU alone did not have any effect on plants placed in charcoal-filtered or non-filtered air conditions. Szantoi et al. (in review) found that EDU had different effects on NDF and ADF concentrations of purple coneflower plants in 2003 under elevated O_3, where EDU levels of 100, 200 and 300 ppm reduced concentrations of NDF, ADF and lignin in contrast with 0 ppm EDU.

CONCLUSION

Most researchers have focused on crops and tree seedlings to investigate the impacts of O_3 on plants. Recently, there have been studies on native vegetation, most of these have dealt with foliar injury and biomass. Most reports conducted on cutleaf coneflower plants, mainly in its native environment, indicate that this species extremely sensitive to O_3 (Neufeld et al. 1992; Chappelka et al. 2003). In this current study, exposed to charcoal-filtered air did not cause foliar injury or altered biomass or cell-wall constituents, and non-filtered air had modest impact on foliar injury and biomass and did not modify the cell-wall constituents; however, the O_3 concentrations of charcoal-filtered air was 21.5 ppb (12h mean), and for non-filtered air it was 33 ppb (12h mean) in 2004 in the Auburn, AL area. These concentrations are lower than reported in previous studies (Muntifering et al., 2000; Powell et al., 2003). Chronic exposure to elevated O_3 (73 ppb) did cause foliar injury, biomass decline except for stem weight, and increased concentrations of refractory cell-wall constituents that are negatively associated with food value for ruminant herbivores; but increased nitrogen concentration to cutleaf coneflower. EDU was observed to decrease the root and total biomass of the plants. It appears that cutleaf coneflower can be used for future research, however a proper EDU rate is important to be determined because of the potential phytotoxic effect on the plants.

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LITERATURE CITED

AOAC. 1995. Official Methods of Analysis 16th edn. Arlington, Virginia: Association of official Analytical Chemists.

Ball GR, Benton J, Palmer-Brown D, Fuhrer J, Skarby L, Gimeno BS, Mills G. 1998. Identifying factors which modify the effects of ambient ozone on white clover (Trifolium repens) in Europe. Environmental Pollution **103**: 7-16.

Bender J, Muntifering RB, Lin JC, Weigel HJ. 2005. Growth and nutritive quality of Poa pratensis as influenced by ozone and competition, Environmental Pollution, In Press

Bergweiler CJ, Manning WJ. 1999. Inhibition of flowering and reproductive success in spreading dogbane (Apocynum androsaemifolium) by exposure to ambient ozone. Environmental Pollution **105**: 333-339.

Brunschon-Harti S, Fangmeier A, Jeger H. 1995. Influence of ozone and ethylenediurea (EDU) on growth and yield of bean (Phaseolus vulgaris L.) in open-top field chambers, Environmental Pollution, Volume **90**, Issue 1, Pages 89-94.

Chameides WL, Kasibhatla PS, Yienger J, Levy IIH. 1994. Growth of continental-scale metro-agro-plexes, regional ozone pollution and world food production. Science **264**, 74–77.
Chameides WL, Cowling EB. 1995. The State of the Southern Oxidants Study (SOS): policy-relevant findings in ozone-pollution research 1988–94. Raleigh, NC, USA: North Carolina. State University.
Carnahan JE, Jenner EL, Wat EK. 1978. Prevention of ozone injury to plants by a new protectant chemical. Phytopathology **68**: 1225-1229.
Cathey HM, Heggestad HE. 1982a. Ozone and sulfur dioxide sensitivity of petunia: Modification by ethylenediurea. Journal of the American Society of Horticultural Science **107(6)**: 1028.
Cathey HM, Heggestad HE. 1982b. Ozone sensitivity of herbaceous plants: Modification by ethylenediurea. Journal of the American Society of Horticultural Science **107(6)**: 1042.
Chappelka AH, Renfro J, Somers G, Nash B. 1997. Evaluation of ozone injury on foliage of black cherry (Prunus serotina) and tall milkweed (Asclepias exaltata) in Great Smoky Mountains National Park. Environmental Pollution **95**, 13–18.
Chappelka AH, Neufeld HS, Davison AW, Somers GL, Renfro JR. 2003. Ozone injury on cutleaf coneflower (Rudbeckia laciniata) and crown-beard (Verbesina occidentalis) in Great Smoky Mountains National Park. Environ Pollut. **125(1):**53-9.
Chappelka AH, Samuelson LJ. 1998. Ambient ozone effects on forest trees of the eastern United States: a review. New Phytologist **139**, pp. 91–108.
Davison AW, Neufeld HS, Chappelka AH, Wolff K, Finkelstein PL. 2003. Interpreting spatial variation in ozone symptoms shown by cutleaf cone flower, Rudbeckia laciniata L. Environmental Pollution **125**, 61–70.
Davison AW, Barnes JD. 1998. Effects of ozone on wild plants. New Phytologist **139**: 135–151.
Fenn ME, Poth M, Bytnerowicz A, Sickman JO, Takemoto BK. 2003. Effects of ozone, nitrogen deposition and other stressors on montane ecosystmes in the Sierra Nevada. In: Ozone Air Pollution in the Sierra Nevada: Distribution and Effects on Forests. Bytnerowicz, A., Arbaugh.
Findley DA, Keever GJ, Chappelka AH, Eakes DJ, Gilliam CH. 1997. Differential responses of buddleia (Buddleia davidii Franch.) to ozone. Environmental Pollution **98**: 105–111.
Finkelstein J, Hitchcock L. 2004. Midwest, Mid-Atlantic, and California Cities Top List for Worst Dangerous Soot Pollution; DC Ranks 22nd for Smog *http://www.ems.org/nws/2004/09/23/midwest_mid_atla*
Heagle AS, Body DE, Heck WW. 1973. An open-top field chamber to assess theimpact of air pollution on plants. Journal of Environmental Quality **2**, 365–368.
Heagle AS, Philbeck RB, Ferrell RE, Heck WW. 1989. Design and performance of a large, field exposure chamber to measure effects of air quality on plants. Journal of Environmental Quality **18**: 361–368.
Hock B, Wolf NM. 2005. Charasteristics of plant life: hazards from pollutants. In: Hock, B., Elstner, E.F. (Eds.), Plant Toxicology. Marcel Dekker, New York, pp. 1-85

Horsfall JG, Barratt RW. 1945. An improved grading system for measuring plant disease. Phytopathology **35**, p. 655.

Kostka-Rick R, Manning WJ. 1992. Partitioning of biomass and carbohydrates in field-grown radish under ambient concentrations to ozone, and treated with the anti-ozonant ethylene-diurea (EDU), *New Phytologist* **121** , 187–200.

Kostka-Rick R, Manning WJ. 1993b. Dose-response studies with ethylenediurea (EDU) and radish. Environmental Pollution **79**: 249-260.

Krupa SV, Manning WJ. 1988. Atmospheric ozone: formation and effects on vegetation, Environmental Pollution **50**, pp. 101–137.

Krupa SV, Nosal M, Peterson DL. 2001. Use of passive ambient ozone (O3) samplers in vegetation effects assessment, Environmental Pollution, Volume **112**, Issue 3, 303-309.

Krupa SV, Muntifering RB, Chappelka AH. 2004. Effects of ozone on plant nutritive quality characteristics for ruminant animals. The Botanica **54**, 129-140.

Lefohn AS, Krupa SV, Winstanley D. 1990. Surface Ozone Exposures Measured at Clean Locations Around the World. Environmental Pollution. **63**(3):189-224.

Manning WJ. 1992. Assessing the effects of ozone on plants: Use and misuse of ethylenediurea (EDU). Proceedings of the 85th Annual Meeting and Exhibition on Air and Waste Management Association 11 pp.

Manning WJ. 2000. Use of protective chemicals to assess the effects of ambient ozone on plants. In: Environmental Pollution and Plant Responses, eds. S.B. Agrawal and M. Agrawal., Lewis Publishers, Boca Raton, FL, pp 247-258.

Manning WJ, Flagler RB, Frenkel MA. 2003. Assessing plant response to ambient ozone: growth of ozone-sensitive loblolly pine seedlings treated with ethylenediurea or sodium erythorbate, Environmental Pollution, Volume **126**, Issue 1, 73-81.

Manning WJ. 2005. Establishing a cause and effect relationship for ambient ozone exposure and tree growth in the forest: Progress and an experimental approach, Environmental Pollution, Volume **137**, Issue 3, Forests Under Changing Climate, Enhanced UV and Air Pollution, 443-454.

Muntifering RB, Crosby DD, Powell MC, Chappelka AH. 2000. Yield and quality characteristics of bluegrass (Paspalum notatum) exposed to ground-level ozone. Animal Feed Science and Technology **84**, 243–256.

Neufeld HS, Renfro JR, Hacker WD, Silsbee D. 1992. Ozone in Great Smoky Mountains National Park: dynamics and effects on plants. In: Berglund, R.D., Editor, Tropospheric Ozone and the Environment II, Air and Waste Management Association Press, Pittsburgh, PA, pp. 594–617.

Niering WA, Olmstead NC, Thieret JW. 2001. National Audubon Society Field Guide to North American Wildflowers Eastern Region (Revised Edition). New York: Random House Inc.

Pihl Karlsson G, Sellden G, Skarby L, Pleijel H. 1995. Clover as an Indicator Plant for Phytotoxic Ozone Concentrations: Visible Injury in Relation to Species, Leaf age and Exposure Dynamics. New Phytologist, Vol. **129**, No. 2 (Feb., 1995), pp. 355-365

Powell MC, Muntifering RB, Lin JC, Chappelka AH. 2003. Yield and nutritive quality of sericea leapeseza (Lespedeza cunceata) and little bluestem (Schizachyrium scoparium) exposed to ground-level ozone. Environmental Pollution **122**, 313–322.

Rohweder DA, Barnes RE, Jorgensen N. 1978. Proposed hay grading standards based on laboratory analysis for evaluating quality. J. Anim. Sci. **47**:747-759.

SAS Institute Inc. 1989. SAS/STAT users guide, Version 6.03. Cary, NC, USA: SAS Institute Inc.

Sanz J, Muntifering RB, Bermejo V, Gimeno BS, Elvira S. 2005. Ozone and increased nitrogen supply effects on the yield and nutritive quality of Trifolium subterraneum, Atmospheric Environment, Volume **39**, Issue 32, 5899-5907.

Szantoi Z, Chappelka AH, Muntifering RB, Somers GL. In review. Use of Ethylenediurea (EDU) to assess ozone effects on Purple coneflower (*Echinacea purpurea*). Environmental pollution

Scherzer AJ, Rebbeck J, Boerner REJ. 1998. Foliar nitrogen dynamics and decompositionof yellow-poplar and eastern white pine during four seasons of exposureto elevated ozone and carbon dioxide. Forest Ecology and Management **109**, 355–366.

Tiwari S, Agrawal M, Manning WJ. 2005. Assessing the impact of ambient ozone on growth and productivity of two cultivars of wheat in India using three rates of application of ethylenediurea (EDU), Environmental Pollution, Volume **138**, Issue 1, 153-160.

United States Environmental Protection Agency. 1996. Air quality criteria for ozone and other photochemical oxidants. EPA/600/P-93/004 a,b,cF. Research Triangle Park, NC, USA: National Center for Environmental Assessment.

Tonneijck AEG, van Dijk CJ. 1997a. Assessing effects of ambient ozone on injury and growth of Trifolium subterraneam at four rural sites in The Netherlands with ethylenediurea (EDU)", Agriculture, Ecosystems and Environment, Vol. **65** pp.79-88.

Van Soest PJ. 1994. Nutritional Ecology of the Ruminant (second ed.), Comstock, Ithaca, NY.

Vingarzan R. 2004. A review of surface ozone background levels and trends. Atmospheric Environment **38**, 3431–3442.