The Pennsylvania State University
The Graduate School
Intercollege Graduate Degree Program in Ecology

THE RESPONSE OF BLACK CHERRY AND HYBRID POPLAR TO OZONE UNDER VARYING ENVIRONMETAL CONDITIONS

A Thesis in
Ecology

by
Teodora Orendovici

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy
August 2005

The thesis of Teodora Orendovici has been reviewed and approved* by the following:

Donald D. Davis
Professor of Plant Pathology
Thesis Co-Advisor
Co-Chair of the Committee

John M. Skelly
Professor of Plant Pathology
Thesis Co-Advisor
Co-Chair of the Committee

Dennis Decoteau
Professor of Horticulture and
Plant Ecosystem Health

Dennis Lamb
Professor of Meteorology

Stephen Rathbun
Associate Professor of Statistics

David A. Mortensen
Professor of Crop and Soil sciences
Chair of the Intercollege Graduate Degree Program in Ecology

---

* Signatures are on file in the Graduate School.

ABSTRACT

Ozone concentrations were monitored from the end of May to the beginning of September for 3 consecutive years (2002-2004) in remote, forested areas of north-central Pennsylvania using Ogawa Passive ozone monitors. Ambient ozone concentrations were measured on a weekly basis at 20 monitoring sites to explore spatial and temporal patterns of ozone distribution. Passive samplers were sited at low-to-high elevations with three high-elevation sites being co-located with real-time ozone analyzers; resulting data were used for cross-correlation and for synthetically generating hourly frequency distributions of ambient ozone. The efficiency of passive samplers was highly dependent upon climatic factors; i.e. temperature, wind speed and relative humidity. Ambient ozone concentrations were greater in years with greater temperatures and lower relative humidity. During all three monitoring seasons, ambient ozone levels were positively correlated to elevation. Ozone concentrations were greater in 2002 and moderate to low in 2003 and 2004. In addition, spatial models of ozone distribution revealed that the central part of north-central Pennsylvania exhibits lower ozone concentrations than the surrounding areas.

A linear model was developed to estimate the influence of local environmental conditions on stomatal conductance of two ozone sensitive tree species. One year-old seedlings of black cherry (R-12) (*Prunus serotina*) and ramets of hybrid poplar clone NE388 (*Populus maximiwizii* x *trichocarpa)* were established in the early spring of the 2003 within open plots at the 20 ozone monitoring locations.

Gas exchange measurements were performed at 12 sites (6 sites at high elevation and 6 sites at low elevation), under field conditions, on seedlings and cuttings of the two tree species. The measurements were performed three times a day, under favorable weather conditions (clear sky) during 2003 and 2004 study seasons. Gas exchange data from the 12 sites were used to empirically model stomatal conductance and to model and map ozone flux for the two tree species throughout the study area.

Throughout both seasons variances were observed in measured stomatal conductance (g_s) and photosynthetic rates (P_n) on a daily basis. The most significant environmental factors explaining stomatal variance were: tree species, air temperature, elevation, and

measurement time. The most significant factors in limiting g_s were vapor pressure deficit (Vpdl) and air temperature. Stomatal conductance rates were higher for hybrid poplar than for black cherry during both years. Stomatal conductance rates were greater at noon and in the early afternoon at high-elevated sites during 2003. During 2004 g_s rates were higher at low-elevation sites. Photosynthetic rates were greater at high-elevation sites in the morning and at noon during both study seasons. Apparently greater air temperature at the low-elevation sites inhibited g_s in 2003, reducing ozone uptake. Also lower than normal air temperatures at the high elevation sites might have inhibited g_s rates in 2004. Since environmental factors explained less than 30% of the variation in stomatal conductance Vpdl and P_n were used to increase the accuracy of the g_s model.

The influences of ozone on foliar symptoms and relative growth parameters of the two ozone-sensitive species were also investigated. One main objective was to determine whether foliar injury expressed by genetically stable ozone-sensitive plants can provide quantitative information about ozone exposures. Visible ozone-induced foliar injury was assessed weekly. Above-ground relative growth was measured at the end of the experiment as relative height (H) and basal diameter (D). During both years of investigation ambient ozone and environmental conditions were conducive to development of typical ozone-induced visible symptoms on both tree species. Percentage of total leaf area affected (expressed as percent injury %INJ) was positively correlated with cumulative ozone exposures. Foliar injury was better correlated with a flux-based approach than for an exposure-based approach. The best correlations in terms of exposure-based approach were obtained when the SUM40 (ozone over the threshold of 40 ppb) ozone exposure index was used. More severe foliar injuries were observed on plants growing on plots located at higher elevation sites. The onset of foliar injury for both tree species was first observed at sites located at higher elevation during both years. The year-to year inconsistent plant response to ozone exposures was likely due to the strong influence of environmental factors and possible to the plant defense mechanisms. We found that more study is required before making quantitative estimations of ozone exposures based on plant foliar injury.

TABLE OF CONTENTS

LIST OF TABELS

LIST OF FIGURES

AKNOWLEDGEMENTS

This project would have not been possible without the hard work and support of many people including:

Dr John M. Skelly, whose excitement, encouragement and advice were my guiding force for my projects and also for my professional development.

Dr. Don D. Davis a dedicated teacher and researcher for his advice and help throughout this project.

Dr. Dennis Decoteau, Dr. Dennis Lamb, and Dr. Stephen Rathbun for their involvement as members of my committee and for their time in reading this thesis.

Bureau of Air Quality, Pennsylvania Department of Environmental Protection for financing this project.

Jim Savage, the Pennsylvania State University for managing many details of this project.

John Ferdinand, the Pennsylvania State University for continued help in collection of data.

Pennsylvania Bureau of Forestry and various landowners from north-central Pennsylvania for allowing us to install the research plots on their land.

and to fellow students and colleagues: Mary-Beth, Lee Kline, David Parr, a special tanks for support and help throughout this project.

Finally I would like to tank my son Razvan Orendovici for his support, help, and patience throughout my studies.

CHAPTER I

Literature review

1.1. Ozone in the troposphere

Because of continuing effects on human health and the environment the prevalence of tropospheric photochemical oxidants is of major international concern (US EPA, 1996). The most important of the tropospheric photo-oxidants is ozone. Ozone (O_3) enters the troposphere from the stratosphere and is destroyed by heterogeneous chemical reactions; it is also generated naturally in the troposphere by electrical discharges during thunderstorms and by the action of sunlight on nitrogen dioxide (NO_2) (Junge, 1962; Fishman *et al.*, 1979). Natural or background concentrations of ozone in the troposphere are generally considered to be 10-40 ppb (Singh *et al.*, 1978). The U.S. Environmental Protection Agency (EPA) presently uses a 40 ppb background ozone level as baseline in its ozone risk assessments. Some researchers consider 10-20 ppb to be the typical background (Altshuller, 1987) and others believe that natural sources of ozone are sufficient to provide 50-60 ppb at remote northern USA sites in the spring (Lefohn *et al.*, 2001). Fiore *et al.*, (2003) define background concentration of ozone as a function of season, altitude, and total surface ozone concentration, with natural levels typically of 10-25 ppb and never exceeding 40 ppb.

There is strong evidence to suggest that average tropospheric ozone concentrations have been rising during this century as a result of increased input of the ozone precursors, i.e. carbon monoxides (CO), nitrogen oxides (NO_X) and volatile organic compounds (VOC_S) into the atmosphere (US EPA, 1996). Ozone concentrations are known to follow a seasonal cycle, with peaks occurring in the summer months, maximum concentrations occurring between March and August (Mueller and Hidy, 1983; Shaw and Paur, 1983; Pratt *et al.*, 1983), and considerable differences recorded on a year-to-year basis. Ozone concentrations also vary daily; in a typical diurnal pattern ozone concentrations are usually at a minimum at sunrise and increase through the morning to peak concentrations in the early afternoon, and then decrease toward the evening (Martinez and Singh, 1979).

1.2. Ozone as an air pollutant within forests of Pennsylvania

The spatial and temporal distribution of tropospheric ozone plays an essential role in estimating its potential risk to forested areas of northeastern USA. Numerous studies have examined the synoptic and local scale transport of ozone into more rural and forested areas (Gilliam and Turrill, 1995; Winner *et al.*, 1989; Comrie, 1994, 1994a). Forested areas within remote and mountainous parts of the northeastern USA have been exposed from moderate to high ambient ozone concentrations throughout the growing season, with occasional episodes of very high ozone associated with stagnant high-pressure systems (Hayes and Skelly, 1977; Comrie, 1994; Gilliam and Turrill, 1995; Hildebrand *et al.*, 1996). In the forested areas of north-central Pennsylvania, distribution of ozone has been previously studied, and on a spatial and temporal scale considerable differences in ambient ozone exposures have been demonstrated (Simini *et al.*, 1992; Skelly *et al.*, 1994a; Yuska *et al.*, 2002). Skelly *et al.* (2001) noted a trend of lower ozone concentrations in the north-central region of Pennsylvania surrounded by areas that have higher ozone concentrations. Jagodzinski (2000) also noted an ozone gradient across Pennsylvania; that is, lower ozone concentrations in the central region of the state surrounded by the more ozone-laden air.

Ozone concentrations depend upon site-specific characteristics and atmospheric conditions that affect local photochemistry and transport processes of pollutants from the source. Several authors have reported a significant correlation between ozone and elevation, with average concentrations increasing with elevation (Winner *et al.*, 1989; Skelly *et al.*, 2001; Yuska *et al.*, 2003). However other gradients of ozone concentrations as monitored in the eastern USA have shown no ozone increases with elevation (Lefohn, 1992). He also pointed out that the relationship between ozone concentrations and elevation might depend upon time of day. During the evening, night, and early morning hours, sites located below the nocturnal boundary layer usually experience a decrease in ozone concentration caused by chemical consumption and deposition, while those sites at elevations above the boundary layer do not experience such a depletion of ozone due to the absence of mixing. In such conditions higher ozone levels are found at higher elevation sites. Stull (1988) defines the atmospheric boundary layer as "the part of the troposphere that is directly influenced by the presence of the earths surface", responding to such forcings as solar heating, and evapo-transpiration. Each of these forcings generates turbulence of various-sized eddies, which can be as deep as the boundary layer itself, lying on top of one other. During high pressure conditions over land, the solar heating causes thermal plumes to rise thus transporting moisture, heat, and aerosols. The plumes rise and expand adiabatically until a thermodynamic equilibrium is reached at the top of the atmospheric boundary layer. Drier air from the free troposphere penetrates down, replacing rising air parcels. During the day, high temperature created by solar heating, along with wind patterns increase the vertical mixing and thus differences of ozone concentrations, depending on elevation, tend to decrease (Aneja *et al.*, 1994; 1994b). Yuska *et al.*, 2003 found a high correlation between ozone concentrations and elevation with just a few outliers not supporting this conclusion; they suggested that the lack of correlation between elevation and ozone concentrations observed in a few cases in the central area of PA might be due to the air mass trajectory pattern which originates in northwest PA.

1.3. Passive sampling

During the last decade there has been a growing interest in the use of passive samplers for quantifying ambient ozone concentrations, particularly in more remote, forested, and wilderness areas. Recent case studies using passive samplers to determine forest exposure to gaseous pollutants indicate potential for the development of meso-scale spatial models and the verification of atmospheric transport models (Bytnerowicz *et al.*, 2002; Cox, 2003).

Passive samplers allow the quantification of cumulative ozone exposures, with exposures reported as average concentrations over a given sampling duration. There are a number of advantages and limitations associated with this approach. The use of passive samplers has been attractive because they are inexpensive and easy to deploy. In addition, monitoring of ozone using passive samplers is not affected by temperature or humidity, and under ambient conditions the interference of potential co-pollutants is negligible (Koutrakis *et al.*, 1993). However, there are still some uncertainties that need consideration. Therefore availability of sites for co-location with electronic air-quality monitors is considered necessary for providing estimates of accuracy and reduce the uncertainties (Cox, 2003).

1.4. Ozone effects on vegetation

Ozone at ambient concentrations has been demonstrated to cause a range of effects including visible leaf injury, growth and yield reduction and altered sensitivity to biotic and abiotic stressors (Skelly *et al.*, 1987; Chappelka *et al.*, 1992; Fredericksen *et al.*, 1995). Increasing ozone exposures have been correlated with increasing foliar injury on sensitive species, with ozone-induced injury progressing as upper leaf surface pigmentation, i.e., stippling to premature leaf senescence. Adaxial leaf surface stipple has been described as the classic symptom of ozone injury on broadleaf species Richards *et al.*, 1968; Karnosky, 1976; Davis and Coppolino, 1974; Skelly, 2000). The upper leaf surface may exhibit minute tan, brown, red, purple, or black coloration that appears uniformly over the leaf surface. The coloration of stippling is usually characteristic of a species but can vary with environment and/or physiological conditions. Stipple is restricted to certain areas of the leaf surface with veins and small veinlets not usually involved **(**Krupa *et al.*, 1998; Skelly, 2000). Also, leaves towards the base of stems (older leaves) exhibit an increasing presence of the stipple due to their season-long exposure to ozone. Premature leaf drop of the symptomatic leaves for individual species may occur as early as mid to late July depending upon ozone concentrations and local environmental conditions. Apart from the direct effect of ozone on vegetation a major area of interest was to correlate the impact of cumulative ozone exposures on individual plants, native species, and regional ecosystems. A strong correlation between increasing ozone exposures and symptom expression of sensitive plant species such as black cherry (*Prunus serotina* Ehrh.), poplar species (*Populus* spp.) yellow-poplar (*Liriodendron tulipifera* L.), and white ash (*Fraxinus americana*, L), has been well documented under controlled and natural forest conditions within the eastern USA (Davis *et al.*, 1981; Davis and Skelly, 1992; Simini *et al.*, 1992; Davis *et al.* 1993; Fredericksen *et al.*, 1995;

Hildebrand *et al.*, 1996; Karnosky *et al.*, 1996; Chappelka *et al.*, 1997). A serie of studies in the Shenandoah National Park in Virginia and The Great Smoky Mountains National Park in North Carolina has shown the occurrence of visible foliar injury on black cherry and yellow-poplar under ambient ozone exposures (Chappelka *et al.*, 1992; Neufeld *et al.*, 1995; Skelly at al., 1997; Hildebrand *et al.*, 1996). Hildebrand *et al.* (1996) found that there was an exponential increase in foliar ozone symptoms with cumulative ambient ozone concentrations for black cherry. These results demonstrated that foliar injury was positively correlated to cumulative ozone exposures, and that local site factors (e.g., soil moisture, light conditions) played a decisive role in determining plant response to ozone exposures.

Most of the studies on the effects of ozone on plant species have been conducted under controlled ozone exposures within Continuously Stirred Tank Reactor (CSTR) chambers, and within open-top chambers (Skelly at al., 1997; Neufeld *et al.*, 1992; Lee *et al.* 1999; Schaub *et al.*, 2003). These studies have been conducted under conditions of favorable available resources (temperature, light, soil moisture, relative humidity), all of them favoring the expression of foliar injury. Data from these and other studies suggest that natural vegetation exposed to ozone under natural forest condition most likely responds differently than when exposed under more uniform and controlled conditions. In fact, field studies have demonstrated that a relatively low percentage of any given population of ozone-sensitive plants will show visible injury response to elevated ozone concentrations under natural conditions and ambient ozone exposures (Skelly *et al.*, 1987, Davis and Orendovici, 2005). Therefore to extrapolate results from experiments conducted under controlled environment to natural forests is not possible, and investigations as to whether there is any similarity between plant responses within CSTR chamber system exposures and natural forests, could be of interest.

1.5. Bio-indicators

In order to examine the impact of pollutants on forest ecosystems, the USDA-Forest Service (USDA-FS) Forest Health Monitoring programs in the northeast USA have developed a series of observational surveys on native vegetation (Smith and Manning 1990). A large part of the activity of these surveys has involved the study of the effects of ozone by means of field observations of visible foliar symptoms on native forest species. These surveys involve the use of tree and other native plant species that have been identified as sensitive to ozone exposures based upon controlled-chamber studies. Some particularly sensitive native plant species used as bio-indicators of ozone injury include black cherry, blackberry (*Rubus* sp.), common milkweed (*Asclepias syriaca* L), and yellow-poplar (Chappelka *et al.*, 1986; Davis and Skelly, 1992; Neufeld *et al.*, 1992, 1995).

Certain vascular and non-vascular plants exhibit typical and verifiable symptoms when exposed to specific air pollutants, making them useful as bio-indicators of deteriorating air quality (Manning and Feder, 1980). The foliar symptoms exhibited by the bio-indicators and generally by any sensitive plant species, are an indication of previous exposure of the plants to ozone when the environmental conditions were conductive to ozone uptake and consequently cellular injury (Krupa *et al.*, 1998). Advantages of native plants as bio-indicators are that they can be utilized even in the most remote forests, they have biological relevance, and they provide solid evidence of plant stress. To be a good indicator a plant species should exhibit a distinct, verified response, have a few or no confounding disease or pest problems, and exhibit genetic stability (Manning and Feder, 1980). The last requirement is seldom accomplished when working with native populations. Therefore, Simini *et al.* (1992) have suggested that sensitive black cherry families could be planted for use as perennial bio-indicators of ozone air pollution in northeastern forests, questioning if planted sensitive tree species is a more reliable source for bio-monitoring than already established natural populations. This approach would be more valid than using wild-plants, which may vary in foliar injury symptoms as a result of genetic differences in sensitivity. This fact should be taken into account in field surveys that examine the responses of plant species to ambient ozone exposures (Hildebrand *et al.*, 1996; Skelly *et al.*, 1997).

1.6. **Black cherry (*Prunus serotina* Ehrh.)**

Black cherry is known to be one of the most ozone sensitive tree species within mixed-hardwood forests of the northeastern USA. Its response to ozone exposure has been extensively studied in terms of foliar injury, leaf morphology and whole-plant physiology (Long and Davis, 1991; Bennett *et al.*, 1992; Davis and Skelly 1992; Ferdinand *et al.*, 2000; Schaub *et al.*, 2003, Wei *et al.*, 2004). Black cherry develops very distinct symptoms in the presence of elevated ambient ozone exposures, with very sensitive individuals rapidly developing an adaxial leaf surface stipple followed by reddening and early leaf senescence. Differences in sensitivity to ozone of several open pollinated families of black cherry have been observed and then confirmed by numerous studies (Davis and Skelly, 1992; Skelly *et al.*, 1994a). Results from a study by Lee *et al.* (2002) strongly support the conclusion that wild populations of black cherry exhibit local, genetically based variation in ozone sensitivity. Several studies conducted within the programs of the Pennsylvania State University have shown that open-pollinated seedlings of black cherry differ genetically in sensitivity to ozone (Simini *et al.*, 1991; Skelly *et al.*, 1994b; Lee *et al.*, 1999, 2002). Those differences in O_3 sensitivities among open-pollinated families of black cherry may be due to their varying capabilities to produce antioxidants and/or their specific morphological characteristics (Kouterick, 1995; Ferdinand, 1997). Open-top chamber studies on several black cherry open-pollinated families grown in north-central Pennsylvania determined that the R-12 (Ridgeway 12) black cherry genotype is a most sensitive genotype to ozone based upon visible foliar ozone injury response (Simini *et al.*, 1991; Skelly *et al.*, 1994; Kouterick, 1995; Ferdinand *et al.*, 1997). Later studies by Lee *et al.* (1999) examined the sensitivity of 15 clones of black cherry within a 25-year old orchard derived from bud grafted seedlings using buds from wild ortets growing within the Allegheny National Forest in Pennsylvania and within the Monongahela National Forest in West Virginia; the most striking result of the study was the extreme variation in sensitivity between clones of geographically proximate natural origins, particularly clones of R-12 and R-14. Once again the R-12 clone within the seed orchard proved to be the most symptomatic of the 15 clones, in terms of ozone induced foliar injury that developed during the two-season investigation (1996, 1997) (Lee *et al.*, 1999). All these results suggest that R-12 clone of black cherry might serve as a good bio-indicator of ozone air pollution.

1.7. Hybrid poplar *(Populus* sp.*)*

Poplar species (*Populus* sp.), and hybrid poplars are among the most ozone sensitive tree species of eastern USA (Berrang *et al.*, 1991; Woodbury *et al.*, 1994; Karnosky *et al.*, 1996). Bortier *et al.* (2000) confirmed previous observations that fast growing tree species such as most poplar species and hybrid clones, are more sensitive and responsive to tropospheric ozone than slower growing species such as beech (*Fagus* spp.). Bortier *et al.* (2000) found that ozone exposure caused highly significant reductions in growth rate, stomatal conductance and chlorophyll content on *Populus nigra* L. Also, recent studies on growth of five hybrid poplar genotypes exposed to interacting elevated carbon dioxide (CO_2) and ozone showed that the fastest growing clones had the greatest response to ozone treatment. Added CO_2 did not ameliorate the detrimental effects of ozone (Dickinson *et al.*, 2002). Ozone effects on hybrid poplar clones include leaf necrosis, premature leaf senescence and abscission, reduced net photosynthesis, growth, and biomass accumulation (Harkov and Brennan, 1982; Reich *et al.*, 1987; Wang *et al.*, 1986).

The responsiveness of hybrid poplar to ozone exposure is strongly correlated to their genetic components. Davis *et al.* (1993) ranked 11 clones of hybrid poplar based on the degree of foliar injury following 4 hours exposure to 250 ppb ozone from most to least sensitive as being NE388, NE52, NE249, NE215, NE4, NE11, NE302, NE353, NE327, NE245, and NE48. Dickson *et al.* (1998) also reported that in an open-top chamber study with 100 ppb, 6hr/day, 5 days/week and 60 days of exposure the hybrid poplar clone (*Populus maximiwizii* x *trichocarpa*) having the fastest growth rate also had the greatest reduction in dry mass. Variation in sensitivity among clones of hybrid poplars may be related to their difference in growth rate and/or other physiological differences such as stomatal conductance (Dickson *et al.*, 1998, Wei *et al.*, 2004). Because hybrid poplar clones are being widely planted in reforestation and intensive culture system in the USA, as well as within many other countries around the world Wei *et al.* (2004) suggested that they might serve as good bio-indicators of ozone pollution.

1.8. Gas exchange

Because the primary pathway for ozone to contact plant tissue is through plant stomata (Fowler *et al.*, 1999), ozone effects on vegetation are directly related to the effective rate of ozone uptake (effective flux) and/or to the effective cumulative uptake amount (i.e., effective flux × time) (US EPA, 1996a; Musselman and Massman, 1999). Ozone uptake is the product of ozone exposure and stomatal conductance; hence many environmental factors such as soil water content, atmospheric humidity, temperature and sunlight play a major role in the appearance of foliar symptoms on forest species (Davis *et al.*, 1981; Reich, 1987; Tingey and Hogsett, 1985; Wieser and Havranek, 1995; Shaub *et al.*, 2003; Wei *et al.*, 2004). The interaction between soil moisture availability and predicted ozone effects changes during periods of drought is likely due to reduced stomatal conductance. High ozone concentrations often occur during hot, dry weather conditions, so periods of elevated ozone do not necessarily correspond to periods of maximal predicted ozone injury. Tingey and Hogsett (1985) found that bean plants growing under conditions of high soil moisture availability were more vulnerable to ozone exposures than were seedlings growing under low soil moisture availability. A 12-year study initiated to determine if the incidence of foliar injury on hybrid poplar induced by regional ambient ozone was influenced by local emissions from a complex of coal-burning power plants in southwestern Pennsylvania showed that water stress plays a major role in influencing symptom severity induced by ozone (Davis *et al.*, 1993). Differences in soil moisture regimes through the growing season have been demonstrated to be a controlling factor in affecting interactions with ambient ozone and subsequent physiological differences leading to alteration in ozone uptake (Schaub *et al.*, 2003). Numerous other studies indicate that water deficit alone reduced photosynthetic rate leading to loss in growth and plant productivity (Kramer and Kozlowski, 1979; Kozlowsiy *et al.*, 1991; Chappelka and Freer-Smith, 1995). Such consequences may not only reduce foliar injury but may also reduce photosynthetic activity and productivity. As a response to stress factors, plants exhibit not only changes in photosynthetic rates and growth rates but also changes in carbon allocation. Carbon allocation depends upon growth strategy and response to varying environments (Chapin, 1991; Lee and Jarvis, 1995; Loehle, 1996; Grulke *et al.*, 2002). Most genetically improved tree species have been selected based upon increased growth rate; but rapid growth rate likely also increases chances for increased sensitivity to ozone (Wei *et al.*, 2004).

Therefore taken together with genetic and environmental factors, visible foliar injury might serve as an indication not only of ozone stress but also as a tool to quantify ozone exposures. It might be possible that a multi-year field study of ozone effects on very sensitive genotypes of ozone bio-indicators, planted in a number of different environments along an O_3 gradient, would provide information that might be useful in quantitatively assessing air quality for ozone concentrations based upon foliar injury expression of these ozone sensitive genotypes.

1.9. Summary of the literature review

Forests and natural vegetation in northeastern USA have been impacted by the adverse effects of ozone exposures (Smith *et al.*, 2001). Field surveys have generally coupled forests condition with ambient ozone concentrations patterns. Native vegetation near the passive samplers and real-time ozone monitors has been evaluated for ozone-induced foliar injury (Skelly *et al.*, 2001; Yuska *et al.*, 2003). While there is general agreement that there is a direct relationship between previous exposure to ambient ozone and incidence of injury on leaves of sensitive vegetation, it is questionable whether a good statistical correlation can be made between the extent of foliar ozone injury on bio-indicator plants and average ambient ozone concentrations. It is now well known that established bio-indicators visibly respond to ozone exposures only when environmental conditions and genetic predisposition are conductive to ozone injury (Davis *et al.*, 1981; Reich, 1987; Tingey and Hogsett, 1985; Wieser and Havranek, 1995; Schaub *et al.*, 2003). Thus, ozone-risk assessments should measure ozone concentrations and account for the influence of atmospheric conditions, soil moisture and stomatal conductance. The response of the plants can be considered to be a relative index of ozone air quality, but it is not possible to use this response to quantitatively assess air quality for ozone (Smith *et al.*, 2001). It now follows to determine if the use of bio-indicator plants that have been previously screened for sensitivity to ozone (greenhouse screening), and then exposed to varying predisposing environments, would allow inferences about air quality for ambient ozone.

The high correlations between ozone monitoring with passive samplers and real-time ozone analyzers, make passive samplers perfect candidates for monitoring ambient ozone in remote and forested areas. The main limitation of the use of passive sampler approach is that the use of mean values over a weekly or a longer time period cannot explain the dynamics of the ozone exposures; therefore they cannot provide the descriptors for developing a model of the vegetation responses to long-term ozone exposures. Also because the passive samplers integrate ozone exposures over several days to weekly period, variations of ozone concentrations on a daily basis cannot be assessed. It is therefore necessary to develop models to predict hourly ozone concentrations from single passive sampler data (weekly averages) to simulate continuous measurements and subsequently to determine day vs. night exposures (Krupa *et al.*, 2001). An approach to describe the exposure-plant relationship is to determine temporal variability of ozone concentrations.

The primary path of the entry of the gaseous pollutant into the leaf is through stomata hence stomatal function must be coupled with the dynamics of ozone exposures on a diurnal basis in order to explain changes in pollutant uptake and the observed plant effects (Krupa and Kickert, 1997).

There is a significant temporal and spatial variability in the occurrence of ambient ozone. These variations are governed by the latitude, longitude, altitude, topography and also seasonal meteorology of a particular location. Although ambient O_3 concentrations at a given location vary from year to year, the overall pattern of the O_3 hourly concentrations follows the same distribution. In most cases such patterns exhibit a non-normal distribution, best described by the mathematical functions of the Weibull family (Legge and Krupa, 1990). Nosal *et al.* (2000) developed, and successfully applied a stochastic Weibull probability generator for replacing sequentially missing hourly ozone values. As an analogy, the single mean O_3 concentration obtained with a passive sampler can be considered as a product of all the unknown values that would be otherwise measured by a continuous monitor. The model can be further improved by including variables such as air temperature, relative humidity, solar radiation, and wind speed. Krupa *et al.* (2001) using data from passive ozone samplers and data sets from co-located continuous monitors, developed a multivariate, nonlinear statistical model to predict the hourly frequency distributions of ozone. Krupa *et al.* (2001) identified the main parameters affecting ozone concentrations as air temperature, relative humidity, solar radiation, and wind speed. Unfortunately passive samplers in most ecological studies are deployed at locations where data of the specified parameters are not available; therefore, most studies cannot make use of those parameters.

The north-central region of Pennsylvania, where high-elevation sites are located in the vicinity of low-elevation sites and exhibit differences in ozone concentrations throughout the summer season, is a desirable area for studies involving ozone exposures. The region also shows a verified pattern of spatial ozone distribution consisting in low ozone surrounded by high ozone concentration areas (Skelly *et al.*, 2001; Yuska *et al.*, 2002). Therefore this geographical area may serve several purposes; determining ozone fluctuations as a function of elevation, and determining the response of bio-indicators to these ozone exposures under natural conditions.

1.10. Statement of objectives and hypothesis

The main objective of this study was to evaluate the impact of site-specific environmental factors (elevation, soil moisture, meteorological conditions) in estimating ozone effects on bio-indicator tree species. We utilized (R-12) black cherry seedlings and NE388 hybrid poplar cuttings that are known to be sensitive to ozone. The final goal was to develop a reliable perennial bio-indicator of ozone air pollution. Investigations as to whether response of sensitive individuals to ozone exposures might be used to predict the quantity of ambient ozone exposures was one of our priorities. In addition, since cumulative exposures derived from prolonged sampling periods cannot account for the dynamics and flux of ozone occurrences and the corresponding plant response, a stochastic probability model to simulate hourly frequency distribution of ozone concentrations, using the weekly mean values obtained from passive samplers, was developed.

The specific objectives of the study were to;

1. Determine hourly frequency distribution of ozone concentrations during growing seasons within forested areas of north-central Pennsylvania, using weekly mean values obtained from passive samplers as to account for the hourly dynamics of ozone occurrences.
2. Determine spatial and temporal patterns of ozone concentrations across north-central Pennsylvania, and their relationship to foliar injury through the use of spatial analysis.
3. Determine the relationships between foliar symptoms incidence and severity expressed by sensitive genotypes of black cherry (R-12) and hybrid poplar clones (NE 388) and site-specific environmental factors (soil moisture, elevation meteorological conditions) as a response to ozone exposures found under natural forest conditions within north-central Pennsylvania, leading to the development of reliable perennial bio-indicators of ozone air pollution.

In order to meet the objectives as stated above, this study is designed to test the following hypothesis:

H_O I: There is no spatial and/or temporal pattern in ozone distribution throughout north-central Pennsylvania.

H_O II: There is no statistically significant difference between hourly ozone distributions of the generated synthetic data (using weekly averages) and the empirical continuous ozone monitoring distribution (as measured with continuous analyzers).

H_O III: There is no relationship between environmental factors and ozone exposures in determining leaf gas exchange in seedlings of black cherry (R-12) and hybrid poplar (NE 388).

H_O IV: There is no relationship between ambient ozone exposures, environmental factors and severity and incidence of visible foliar ozone-induced injury expressed by seedlings of black cherry (R-12) and hybrid poplar (NE 388).

H_O V: There is no relationship between ozone exposures, local environment and above ground biomass production for seedlings of black cherry and hybrid poplar.

References:

Alshuller, A.P., 1987. The role of nitrogen oxides in non-urban ozone formation in the planetary boundary layer over N. America, W. Europe and adjacent area of ocean. Atmospheric Environment 20, 245-268.

Aneja, V.P., Clairborn, C.S., Li, Z., and A. Murthy, 1994. Trends, seasonal variations and analysis of high elevation surface nitric acid, ozone and hydrogen peroxide. Atmospheric Environment 28, 1781-1790.

Aneja, V.P., Li, Z., and Das, M., 1994b. Ozone case studies at high elevation sites in the eastern United States. Chemosphere 29(8), 1711-1733.

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

Berrang, D., Karnosky, D.F., and Bennett, J.P., 1991. Natural selection for ozone tolerance in *Populus tremuloides*: an evaluation of nationwide trends. Canadian Journal of Forestry Research 21, 1091-1097.

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

Bytnerowicz, A., Tausz, M., Alonso, R., Jones, D., Johnson, R. Grulke, N., 2002. Summertime distribution of air pollutants in the Sequoia National Park, California. Environmental Pollution 118(2), 187-203.

Chapin, F.S., Moilanen, L., 1991. Nutritional controls over nitrogen and phosphorous resorption from Alaskan birch leaves. Ecology 72, 709-715.

Chappelka, A.H., Chevrone, B.I., 1986. White Ash seedlings growth response to ozone and simulated acid rain. Canadian Journal of Forest Research 16, 786-790.

Chappelka, A.H., Hildebrand, E.A., Skelly, J.M., Mangis, D., and Renfro, J.F., 1992. Effects of ambient ozone concentrations on mature eastern hardwood trees growing in Great Smoky Mountains National Park and Shenandoah National Park. Presented at 85th Annual Meeting of the Air and Waste Management Association, June 21-26, 1992.

Chappelka, A.H. and Freer-Smith, P.H., 1995. Predisposition of trees by air pollutants to low temperature and moisture stress. Environmental Pollution 87, 105-117.

Chappelka, A.H., Renfro J.R., and Somers G.L., 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.

Comrie, A.C., 1994. Tracking Ozone: Air Mass Trajectories and Pollutant Source regions Influencing Ozone in Pennsylvania Forests. Annals of the Association of American Geographic's 84,635-651.

Comrie, A.C.,1994a. A synoptic climatology of surface ozone in rural ozone pollution at three forest sites in Pennsylvania. Atmospheric Environment 28, 1601-1614.

Cox, R.M., 2003. The use of passive sampling to monitor forest exposure to O_3, NO_2 and SO_2 a review and some case studies. Environmental Pollution **126**, 301–311.

Davis, D. D. and Coppolino, J. B., 1974. Relative ozone susceptibility of selected woody ornamentals. Horticultural Science 9,537-539

Davis, D.D., Umbrach, D.M., and Coppolino, J.B., 1981. Susceptibility of tree and shrub species and response of black cherry foliage to ozone. Plant Disease 65, 904-907.

Davis D.D. and Skelly, J.M., 1992. Foliar sensitivity of eight eastern hardwood tree species to ozone. Water, Air, and Soil Pollution 62, 269-277.

Davis, D.D., Hutnik R.J., and McClenahen, J.R., 1993. Evaluation of Vegetation near Coal-burning Power Plants in Southwestern Pennsylvania: Ozone injury on foliage of Hybrid Poplar. Journal of Air Waste Management Association 43, 760-764.

Davis, D.D. and Orendovici, T., 2005. Impact of ozone on sensitive plants at selected USDI National Wildlife Refuges in Eastern United States. Environmental Pollution, in press.

Dickinson, R.E., Berry, J.A., Bonan, G.B., Collatz, G.J., Field, C.B., Fung, I.Y., Goulden, M., Hoffmann, W.A., Jackson, R.B., Myneni, R., Sellers, P.J., Shaikh, M., 2002. Nitrogen controls on climate model evapotranspiration. Journal of Climate 15, 278-295.

Dickson, R.E., Coleman, M.D., Riemenschneider, D.E., Isebrands, J.D., Hogan, G.D., Karnosky, D.F., 1998. Growth of five hybrid polar genotypes exposed to interacting elevated CO_2 and O_3. Canadian Journal of Forest Research 28, 1706-1716.

Ferdinand, J.A., 1997. Leaf morphology in relation to ozone sensitivity of black cherry (*Prunus serotina* Ehrh.) trees by tree size, genotype, and light environment. M.S. Thesis, Pennsylvania State University, PA.

Ferdinand, J.A., Fredericksen, T.S., Kouterick, K.B., and Skelly, J.M., 2000. Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. Environmental Pollution 108, 297-302.

Fiore, A., Jacob, D.J., Liu, H. Yantoska, R.M., Fairlie,T.D. and Li, Q., 2003. Variability in surface ozone background over the United States: Implications for air quality policy. Journal of Geophysical Research, 108 (D24), 4787.

Fishman, T.S., Brackett, V.G., and Fakhruzzaman, K., 1979. Distribution of tropospheric ozone from satellite and ozone sonde measurements. Journal of Waste Management and Terrestrial Physics 54, 589-597.

Fredericksen, T.S., Joyce, B.J., Skelly, J.M., Steiner, K.C., Kolb, T.E., Kouterick, K. Savage, J.E., and Snyder, K.R., 1995. Physiology, morphology and ozone uptake of leaves of black cherry, samplings and canopy trees. Environmental Pollution 89, 273-283.

Fowler, D., Cape, J.N., Coyle, M, Flechard, C., Kuylenstierna, J.,Hicks., K, Derwent, D., Johnson, C., Stevenson, D.,1999. The global exposure of forests to air pollutants. Water Air and Soil pollution 116, 5-32.

Gilliam, F.S. and N.L. Turrill, 1995. Temporal patterns of ozone pollution in West Virginia: implications for high elevation hardwood forests. Journal of Air and Waste Management Association 45, 621-626.

Grulke, N.E., Preisler, H.K., Rose, C., Kirsh, J., Balduman, L., 2002. Ozone uptake and drought stress affects on carbon acquisition of ponderosa pine in natural stands. New Phytologist 154, 621-631.

Harkvo, R., and Brennan, E., 1982. The effect of acute ozone exposures on the growth of hybrid poplar. Plant disease 66, 587-589.

Hayes, E.M. and Skelly, J.M., 1977. Transport of ozone from the northeastern Un into Virginia and its effects on eastern with pine. Atmospheric Environment. Plant disease Rep. 61, 778-782.

Hildebrand , E.S., Skelly, J.M., and Frederiksen, T.S., 1996. Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. Canadian Journal of Forest Research 26, 658-669.

Jagodinski, J.M., 2000. An analysis of tropospheric ozone transport in north-central Pennsylvania during the summer of 1999. M.S. Thesis, Pennsylvania Sate University, University Park, PA.

Junge, C.E., 1962. Global ozone budget and exacange between stratosphere and troposphere. Tellus 14, 363-377.

Karnosky, D.F. 1976. Threshold levels for foliar injury to Populus tremuloides by sulfur dioxide and ozone. Canadian Journal of Forest Resources 6,166-169.

Karnosky, D. F., Gagnon, Z.E., Dickson, R.E., Coleman, M.D., Lee, E.H., and Isebrands, J.G., 1996. Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. Canadian Journal of Forest Research 26, 23-37.

Kouterick, K.B., 1995. Comparisons of foliar ozone injury, leaf gas exchange and biomass response to ozone among black cherry (*Prunus serotina*) genotypes. M.S. Thesis, Pennsylvania State University, PA.

Koutrakis, P., Wolfson, J. M., Bunyaviroch, A., Froelich, S. E., Hirano, K., Mulik, J. D. 1993. Measurement of ambient ozone using a nitrite-saturated filter. Analytical Chemistry 65, 210-214.

Kozlowski, T.T., Kramer, P.J., and Pallardy, S.G., 1991. The physiological ecology of woody plants. Academic Press Inc., New York, NY, pp. 657.

Kramer P.J. and Kozlowsky, T.T., 1979. Physiology of woody plants. Academic Press Inc., New York, NY, pp. 811.

Krupa, S.V. and Kickert, R.N., 1997. Ambient ozone (O_3) and adverse crop response. Environmental Reviews 5, 55-77.

Krupa, S.V., Tonnejkck, A.E.G. and Manning, W.J., 1998. Ozone. Pp.2-1 to 2-23, in R.B.Flagler *et al.* (Eds.). Recognition of Air Pollution Injury to Vegetation: A pictorial atlas. Air and Waste Management Association, Pittsburgh, PA.

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

Krupa, S.V., Nosal, M., and Peterson, D.L., 2001. Use of passive ambient ozone (O_3) samplers in vegetation effects assessment. Environmental Pollution 112, 303-309.

Krupa, S.V., Nosal, M., Ferdinand, J. A., Stevenson R.E., and Skelly, J. M., 2003. A multi-variate statistical model integrating passive sampler and meteorology data to predict the frequency distributions of hourly ambient ozone (O_3) concentrations. Environmental Pollution 124, 173-178.

Lee, H.S.J., and Jarvis, P.G. 1995. Trees differ from crops and from each other in their responses to increases in CO_2 concentration. Journal of Biogeography 22, 323-330

Lee, J.C., Skelly, J.M., Steiner, K.C., Zhang, J.W., and Savage, J.E., 1999. Foliar response of black cherry (*Prunus serotina*) clones to ambient ozone exposure in central Pennsylvania. Environmental Pollution 105, 325-331.

Lee, J.C., Steiner, K.C., Zhang, J.W., Skelly, J.M., 2002. Heritability of ozone sensitivity in open-pollinated families of black cherry (*Prunus serotina* Ehrh.) In press.

Lefohn, A.S., Oltmans, S.J., Dann, T., and Singh, H.B. 2001. Present-day variability of background ozone in the lower troposphere. Journal of Geophysics Research 106(D9), 9945-9958.

Lege, A.H. and Krupa, S.V., (Eds.), 1990. Acidic deposition: Sulphur and nitrogen Oxides. Lewis Publishers, Chelsea, MI, USA.

Loehle, C., 1996. Forest responses to climate change. Journal of Forestry 94, 13-15.

Long, R.P., D.D. Davis, 1991. Black cherry growth response to ambiental ozone and EDU. Environmental Pollution 70, 241-257.

Manning, W.J., and Feder, W.A., 1980. Biomonitoring Air Pollutants with Plants. Applied Science Publishers, London, England, 142 pp.

Martinez, J.R. and Singh, H.B., 1979. Survey of the role of NO_x in nonurban ozone formation. Prepared by SRI International for the USA EPA, Research Triangle Park, NC. EPA- 450/4-79-035.

Mueller, P.K., and Hidy, G.M., 1983. The regional sulphur experiment: Report to findings, Rep. 1901 (3 vols.), Electric Power Resources Institute., Palo Alto, CA.

Musselman, R.C., Massman, W.J., 1999. Ozone flux to vegetation and its relationship to plant response and ambient air quality standards. Atmospheric Environment 33, 65-73.

Nosal, M., Legge, A. H., and Krupa, S.V., 2000. Applications of a stochastic, Weibull probability generator for replacing missing data on ambient concentrations of gaseous pollutants. Environmental Pollution 108, 439-446.

Neufeld, H.S., Renfro J.R., and Hacker, W.D., 1992. Ozone in Great Smokey Mountains national Park: dynamics and effects on plants. Air and Waste Management Association, Pittsburg, 594-617.

Neufeld, H.S., Lee, H.E., Renfro J.R., and Hacker, W.D., 1995. Sensitivity of seedlings of black cherry (Prunus serotina Ehrh.). New Phytologist 130, 447-459.

Pratt, G.C., Hendrickson, R.C., Chevone, B.I., Christopherson, D.A., and Krupa, S.W., 1983. Ozone and oxides of nitrogen in the rural upper-Midwestern United States. Atmospheric Environment 17, 2013-2023.

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

Richards, B.L., Taylor, O.C. and Edmunds, Jr. G.F. 1968. Ozone needle mottle of pine in southern California. Journal of the Air Pollution Control Association 18,73-77.

Schaub, M., Skelly, J.M., Steiner, K.C., Davis, D.D., Pennypacker, S.P., Zang, J., Ferdinand, J.A., Stevenson, R. E., Savage, J. E., 2003. Physiological and foliar injury of *Prunus serotina, Fraxinus Americana* and *Acer rubrum* seedlings to varying soil moisture and ozone. Environmental Pollution 124, 307-320.

Shaw, R.W., and Paur, R.G., 1983. Measurements of sulphur in gases and particles during sixteen months in the Ohio River Valley. Atmospheric Environment 17, 143-160.

Simini, M., Skelly, J.M., and Savage, J.E., 1991. Sensitivity of wild type and half-sibling families of black cherry to ambient doses of ozone in north-central Pennsylvania. Phytopathology 81, 124-131.

Simini, M., Skelly, J.M., Davis, D.D., Savage J.E., and Comrie, A. C., 1992. Sensitivity of four hardwoods species to ambient ozone in north central Pennsylvania. Canadian Journal Research 22, 1789-1799.

Singh, H.B., Ludwig, F.L., and Johnson, W.B., 1978. Tropospheric ozone concentrations and variabilities in clean remote atmospheres. Atmospheric Environment 12, 2185-2196.

Skelly, J.M., Davis, D.D., Millen, A.A., Cameron, A.E. Brown,, J.D, Drummond, D.B. and Dochinger, L.S., 1987. Diagnosing Injury to Eastern Forest Trees. USDA- Forest service, Vegetation survey Research Cooperative and The Pennsylvania State University, University Park, PA 122 pp.

Skelly, J.M., Savage, J.E., Snyder, K.R., Fredericksen, T.S., and Kouterick, K.B., 1994. Response of open-pollinated black cherry families to ambient ozone under open-top chamber conditions. Phytopathology 84, 547-554.

Skelly, J.M., Savage, J.E., Snyder, K.R., and Fredericksen, T.S., 1994a. Tropospheric ozone occurrences at remote sites in Pennsylvania: A database for evaluation of changing pollution scenarios. Environmental Research Institute, Pennsylvania State University, University Park, PA, USA, 25 pp.

Skelly, J.M., Chappelka, A.H., Laurence J.A., Fredericksen T.S., 1997. Ozone and its known potential effects on forests in eastern United States. In forest decline and Ozone, a comparision of controlled Chamber and field experiments. Berlin, Springer-verlag, 69-93.

Skelly, J.M., 2000. Tropospheric ozone and its importance to forests and natural plant communities of the northeastern United States. Northern Naturalist 7(3), 221-236.

Skelly, J.M., Ferdinand, J.A., Jagodzinski, J.A., Savage, J.E., and Mulik, J.D., 2001. A thirteen week comparison of passive and continuous ozone monitors at forested sites in north-central Pennsylvania. Journal of Air and Waste Management Association 51, 1280-1287.

Smith, G.C. and Manning, W.J., 1990. An ozone effects Monitoring program for the Class I Wilderness Areas in the White Mountain national forests of New Hampshire. University of Massachusetts, Amherst, Ma, 60 pp.

Smith, W.B., Vissage, J., Sheffield, R., Darr, D., 2001. Forest Statistics of the United States, 1997 (Gen. Tech. Rep. NC-219). US Department of Agriculture, Forest Service, St. Paul, MN.

Stull, R.B., 1988. An introduction to boundary layer meteorology (Kluwer Academic Publishers, Dordrecht).

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

U.S. Environmental Protection Agency (US EPA), 1996. Air quality criteria for ozone and related photochemical oxidants. Vol.1 of 3 reports no EPA/600/P-93/004aF. Research Triangle Park, North Carolina.

U.S. Environmental Protection Agency (US EPA), 1996a. Air quality criteria for ozone and related photochemical oxidants. Office of Research and development, Washinghton, D.C. EPA/600/P-93/004a-cF.

Wang, D., Karnovsky, D.F., Bormann, F.H., 1986. Effects of ambient ozone on the productivity of *Populus tremuloides* Michx. growth under field conditions. Canadian Journal of Forest Research 16, 47-55.

Wei, C., Skelly, J.M., Pennypacker, S.P., Ferdinand, J.A., Savage, J. E.,Stevenson, R. E., Davis, D.D., 2004. Influence of light fleck and low light on foliar injury and physiological responses of two hybrid poplar clones to ozone. Environmental Pollution 130, 215-227.

Wieser, G. and Havranek, W. M., 1995. Environmental control of ozone uptake in *Larix decidua* Mill.: a comparison between different altitudes. Tree Physiology 15, 253-258.

Winner, P.B., Lefohn, A.S., Cotter, I.S., Greitner, C.S., Nellesen, J., McEvoy, L.R., Olson, R.L., Atkinson, C.J., and Moore, L.D., 1989. Plant responses to elevational gradients of O_3 exposures in Virginia. Proc. Nat. acad. Sci. 86, 8828-8832.

Woodbury P.B., Laurence, J.A., and Hudler G.W., 1994. Chronic ozone exposure alters the growth of leaves, stems and roots of hybrid *Populus*. Environmental Pollution 85(1), 103-108.

Yuska D.E., J.M. Skelly, J.A. Ferdinand, R.E. Stevenson, J.E. Savage, J.D. Mulik and A. Hines, 2003. Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania. Environmental Pollution 125, 71-80.

CHAPTER II

Spatial and temporal ozone distribution across north-central Pennsylvania

"Capsule": Passive ozone sampling systems are efficient monitors for describing ozone patterns.

2.1. Abstract

Ozone concentrations were monitored from the end of May to the beginning of September for 3 consecutive years (2002-2004) in remote, forested areas of north-central Pennsylvania using Ogawa Passive ozone monitors. Ambient ozone concentrations were measured on a weekly basis at 20 monitoring sites to explore spatial and temporal patterns of ozone distribution. Passive samplers were sited at low-to-high elevations with three high elevation sites being co-located with real-time ozone analyzers; resulting data were used for cross-correlation and for synthetically generating hourly frequency distributions of ambient ozone. The efficiency of passive samplers was highly dependent upon climatic factors; i.e. temperature, wind speed and relative humidity. Ambient ozone concentrations were greater in years with greater temperatures and lower relative humidity. During all three monitoring seasons, ambient ozone levels were positively correlated to elevation. Ozone concentrations were greater in 2002 and moderate to low in 2003 and 2004. In addition spatial models of ozone distribution revealed that the central part of north-central Pennsylvania exhibits lower ozone concentrations than the surrounding areas.

Furthermore, the use of mean values over a week was not effective in describing the dynamics of the ozone exposures, and consequently cannot provide the descriptors to realistically describe the vegetation responses to long-term ozone exposures. A normal (Gaussian) distribution was used as the theoretical distribution to describe the ozone

dynamics. Small differences between the real data and synthetically generated data showed that our method of determining ozone distribution was fairly efficient.

Keywords: passive sampling, ozone, monitoring, north-central Pennsylvania, kriging, spatial and temporal patterns, climatic factors

2.2. Introduction

Tropospheric ozone above background concentrations is an anthropogenic air pollutant that affects the health and productivity of forests throughout the northeastern United States (US EPA, 1996). Tropospheric concentrations of ambient ozone air pollution impose an increasing stress on forest health and potentially on biodiversity (Krupa and Manning, 1988; Krupa *et al.*, 1998). Therefore, information about the spatial and temporal distribution of ozone is essential for estimating the risks associated with ozone air pollution and subsequently for understanding the potential stress affecting forests.

In the forested and mountainous areas of north-central Pennsylvania different spatial and temporal patterns of ozone distribution have been observed (Simini *et al.*, 1992; Skelly *et al.*, 1994a; Jagodzinski, (2000); Yuska *et al.*, 2003). Simini *et al.* (1992), Comrie (1994) and Skelly (1994a) suggested that a west-to-east gradient of decreasing ozone exists across Pennsylvania, most likely related to the distance and the direction of long range transport of ozone. Jagodzinski, (2000) noted a different ozone gradient across central Pennsylvania; lower ozone concentrations in the central region of the state surrounded by the more ozone-laden air, with usually the Southeast and Southwest regions of the state expressing the higher ozone concentrations. Skelly *et al.* (2001) also noted a trend of lower ozone concentrations in the north-central region of Pennsylvania surrounded by regions having higher ozone concentrations. They explained that this pattern is due to the fact that the air mass dominating the central area of Pennsylvania originates in northwest PA (Great Lakes) and is usually characterized by low ozone concentrations.

In the central region of PA, a vertical ozone gradient was observed in mountain/valley situations. Yuska *et al.* (2003) found a high correlation between ozone concentrations and elevation with just a few outliers not supporting this conclusion. They suggested that the lack of correlation between elevation and ozone concentrations observed in a few cases in the central area of PA might be due to the air mass trajectory pattern which originates in western NY and northwest PA.

Some authors have pointed out that the relationship between ozone concentrations and elevation depend upon time of day (Lefohn, 1992). During the evening, night, and early morning hours, sites located below the boundary layer usually experience a decrease in ozone concentration caused by chemical consumption and depositions, while sites at elevations above the boundary layer do not experience such a depletion of ozone due to the absence of mixing. A higher ozone concentration on a mountain top site as compared to an adjacent valley site was reported for the first time by Berry (1964). He observed that during daytime the low elevation site experienced higher concentrations than the high elevation site but during night time higher ozone was measured on the mountaintop and the overall highest average was measured on the mountain top.

To determine forest exposure to gaseous pollutants, to develop mesoscale spatial models, and to verify models of atmospheric transport, many recent studies have made use of passive sampler monitoring systems (Bytnerowicz *et al.*, 2002; Cox, 2003). Extensive monitoring to identify pollution exposure gradients or areas of potential impact can be conducted by using passive samplers because these systems are easy to use in remote and wilderness areas. The low cost and flexibility of placement for passive sampling also make them attractive alternatives for assessing exposures at locations that are difficult to access. Passive samplers for monitoring gaseous pollutant concentrations allow for large-scale coverage of the areas of interest (Krupa and Legge, 2000). Although these devices do not report real-time concentrations of pollutants, the time-integrated average concentrations allow determining trends in the spatial and temporal distribution of ozone.

The use of mean values representing ozone exposures over a week or a longer time period cannot explain the diurnal dynamics of the ozone exposures; that is passive samplers alone cannot provide the descriptors to model the vegetation responses to varying ozone exposures. Passive samplers when co-located and calibrated with active monitors can be used to make estimations of the real-time ozone concentrations (Krupa *et al.*, 2001; Tuovinen, 2002) which are needed for ground verification of ozone dispersion models and calculation of ozone uptake to plants. Legge *et al.* (1991) found that the frequency distributions of the hourly ambient ozone concentrations are best described by the mathematical functions of the Weibull family. Nosal *et al.* (2000) developed and successfully applied a stochastic Weibull probability generator for replacing as many as 100 sequentially missing hourly ozone values in field measurements over two consecutive years. Therefore, by applying Weibull probability to the passive sampler data and by using numerical parameters of the frequency distribution obtained from a co-located continuous monitor, hourly estimates of ozone concentrations can be reconstructed. This approach significantly extended the utility of the passive sampler measurements. The cumulative distribution function of the three-parameter Weibull distribution (shape, scale and location) can be used as the theoretical distribution to fit the data (Nosal *et al.*, 2000).

The specific objectives of the present study were, (1) to determine hourly frequency distributions of ozone concentrations within forested areas of north-central Pennsylvania, using weekly mean values obtained from passive samplers so as to account for the dynamics of ozone occurrences along with data from real-time ozone monitors, and (2) to determine the spatial and temporal patterns of ozone concentrations across north-central Pennsylvania.

2.3. Materials and methods

2.3.1. Research area and ozone monitoring

During 3 consecutive years we quantified the distribution of tropospheric ozone during the summer months in north-central Pennsylvania at 20 sites of varying elevation. To better explore and understand the spatial and temporal distribution of ozone across north-central Pennsylvania we chose 20 sites and during early summer of 2002 we started monitoring ozone concentrations. The 20 sites were located within well-exposed clearings representing rural and mostly forested areas in the sparsely populated areas of north-central Pennsylvania. The investigation area was part of a monitoring study conducted in previous years (Figure 1).

To study the relationship between elevation and ozone concentrations, we selected two relative elevation categories (i.e., high and low); 10 sites were established at high (> 550 m), and 10 sites at low altitudes (< 350 m) (Table 1a and 1b). We chose the locations such that any low-elevation site was located in the proximity (5 to 15 miles) of a high-elevation site for a valley/mountaintop study case, overall study employing 10 pairs of sites (Table 1a).

Ambient ozone concentrations were measured with Ogawa passive ozone samplers (Ogawa and CO., Inc.). Passive samplers were positioned at each site on a steel pipe at 1.5-2m above the soil surface and shielded from wind and rain under a 7.6 cm PVC cap. Each sampler contained two nitrite-saturated filters. The filter assemblies of the passive sampler for the 20 sites and two for control were prepared within a clean environment, on the evening preceding the weekly exchange and kept frozen until transferred to an insulated ice chest the next morning into the field travel. Respective sites were visited within the same hour of day as established by the first week's initial deployment. At each of the respective sites, the hour of each filter replacement was recorded for the purpose of accurately determining O_3 exposures. In the laboratory, passive-sampler filters were extracted within a clean environment, placed into labeled vials, and stored within a freezer until analyzed for nitrate concentration at the Environmental Resources Research Institute at The Pennsylvania State University.

Mean ozone concentration (ppb) was calculated for each passive-sampler site. The rate of conversion of nitrite (NO_2^-) to nitrate (NO_3^-) by ozone was determined by ion chromatography. For each week, the mean nitrate concentration obtained for the blank filters was subtracted from the values obtained from the filters exposed to ambient air at the monitored sites as follows:

$$E_{sample} = [(NO_{3(sample)})*Vol*6.572\times10^3]*R^{-1}$$

$$E_{blank} = [(NO_{3(blank)})*Vol*6.572\times10^3]*R^{-1}$$

$$E_{net} = [E_{(sample)}-E_{(blank)}]\{O_3\} = [E_{net}*t^{-1}]$$

E = ppb $\{O_3\}$ = ozone mixing ratio (ppb)

t = exposure time (h)

R = collection factor (21.6)

Vol = extraction volume (ml)

(NO_3) = nitrate determined by analysis (μg ml^{-1})

At three high-elevation sites, the passive samplers were co-located with real-time ozone analyzers (Table 1). Continuous API - Advanced Pollution Instrumentation - Model 400A in 2004, and TECO Model 49 in 2002 and 2003, ozone analyzers were operated as the real-time monitors. Real-time ozone analyzers provided data about seasonal and diurnal ozone patterns. Ozone values were monitored at 5-min time intervals and finally recorded as 1-hr averages using Odessa Engineering data-logger model DSM3260. Sensors were initially calibrated in early April at the beginning of the ozone-monitoring season for north-central Pennsylvania. Each analyzer performed a two-point auto-calibration check nightly. Calibration quality control measures followed the standards required by the Pennsylvania Department of Environmental Protection. Ozone concentration data were downloaded to a computer at The Pennsylvania State University twice a month throughout the study period. By comparing passive-sampler values to continuous hourly ozone values we determined the accuracy of the passive monitors. Data obtained from real-time ozone analyzers were used for cross-correlation and for determining and synthetically generating hourly frequency distribution of ambient ozone using weekly mean values obtained from passive samplers Nosal *et al.* (2000). To determine the precision of the passive sampler duplicate samplers were deployed at 3 of the sites (Table 1a).

In addition to passive samplers, meteorological data including temperature (Temp., ^{0}C), relative humidity (RH, %), precipitation (mm), and wind speed (WS, m s^{-1}) were recorded during the 2002-2004 seasons at the three air quality monitoring sites (Table 1a). A Campbell meteorological data system (Campbell Scientific Inc., Logan, UT) was operated at each air quality monitoring site, and resulting data were used to test the influence of environmental factors on the precision and accuracy of passive samplers.

The 2002 ozone monitoring study was conducted during a 12-week period, with an initial deployment of passive samplers on June 13th and last deployment on August 31st. The 2003 ozone monitoring study was conducted during a 13-week period, with the initial deployment of passive samplers on June 9th and the last deployment on September 2nd. The 2004 ozone monitoring study was conducted during a 14-week period, with the initial deployment of passive samplers on May 23rd and the last deployment on August 24th.

2.3.2. Data analysis

One-week average ozone concentrations were calculated for each passive sampler location. Sampler precision was obtained by calculating the percent difference (defined as the ratio of the absolute value of difference of the replicates to their average) between replicate filters for each week of the study period. Correlation analysis was used to compare seasonal and weekly ozone concentrations as measured by the passive samplers with the continuously monitored ozone. Plotting the 1-week average real-time monitors against the data from passive samplers, we tested the accuracy of the passive sampler. When necessary, linear regression was used to correct the ozone values measured with passive samplers. One-way ANOVA was used to test for significant differences among sites for each individual year. Data from sampling weeks were used to compute summer averages for each year/site. The seasonal average calculated for each site/year was also used to compare differences in ozone concentration at varying elevations and from an east to west direction. Regression analysis was used to describe the relationship between ozone concentrations and elevation.

This study was also concerned with the development of predictive models to generate maps of ozone exposures. The technique used to estimate spatial ozone patterns for the north-central Pennsylvania region made use of spatial data analysis. Spatial data consist of measurements taken at specific locations, also termed geostatistical data. A geostatistical analysis provides tools for data exploration and for creating surfaces that can be used to visualize, analyze, and understand the geographic phenomena of interest. Geostatistical data typically exhibit small-scale variations that might be modeled as spatial autocorrelation and incorporated into estimating procedures. The measure of the spatial correlation (how data are related with distance and direction) is illustrated by a variogram. When the data appear to be spatially correlated, kriging is a useful method to make predictions. Kriging is a linear interpolation method that allows predictions of unknown values of a random function from observations at known locations. The final step employs interpolation in order to generate intensity maps. By using this approach, a spatial model of prediction or estimation of the intensity of ozone concentrations can be derived (Kaluzny *et al.*, 1998). Preliminary evaluations were made to decide which geostatistical method (kriging, co-kriging) provided the most accurate estimate of ozone concentrations.

Our interest was to understand the spatial distribution of concentrations over the whole study region, given the concentrations at fixed sampling points. Ultimately these models were used to obtain good predictions of ozone values at points where the attribute has not been sampled. Our objective was to model the pattern of variability of ozone concentrations and to establish any factors to which they might relate. Intensity maps showing spatial distributions of ozone concentrations (annual and weekly) were generated to analyze and understand the ozone pattern in the area of study.

Hourly ambient ozone concentration data were used to determine the underlying distribution of the pollutant. We found that the frequency distribution of pollutant concentrations followed a normal, 'bell-shaped' distribution rather than a Weibull distribution. Therefore, we used normal (Gaussian) distribution as the theoretical distribution to fit the data. Hourly ozone data were synthetically generated by using distribution parameters specific to a normal distribution (mean and standard deviation). Mean of the distribution was assumed to be equal to the weekly ozone value of a passive sampler and standard deviation was assumed to be equal to that of the ozone distribution obtained from a continuous monitor. Results were comparable to the corresponding data from the continuous measurements.

Weekly means (co-located passive sampler) were used to synthetically generate normally distributed data which were then compared with the frequency distributions of continuous ozone data (using correlation, and t-test for paired data). Synthetically generated data were used to calculate SUM40, SUM60 and SUM80 for every location during the three seasons of monitoring. SUM40 is defined as the cumulative ozone concentrations greater than 40 ppb for the season. Similarly SUM60 is defined as the cumulative ozone concentrations of 60 ppb or greater for the season, and was of particular interest since this statistic has been correlated with foliar ozone injury in eastern USA (Hildebrand *et al.*, 1996). Likewise, SUM80 is defined as the cumulative ozone concentrations of 80 ppb or greater for the season. Generated data were used to identify the relationship between ozone-sensitive vegetation and ozone exposures (Chapter IV). Data analysis was made using the Statistical Analysis System (SAS Inc. 2000), MINITAB Release14 (Minitab Inc. 2003) and S-plus (Insightful Corporation, 1998).

2.4. Results

2.4.1. Sampler precision

Sampler precision was not determined for the 2002 monitoring season. For the 2003 monitoring season the sampler precision was determined by calculating the relative error or the percentage difference between replicates (relative error * 100). The Ogawa passive sampler had a good precision between 0 and 20%, except for two duplicate values (Figure 2a). The range of the differences (between the two replicate samplers) was (-5.25; 5.727 ppb), with one outlying value of 14.636 ppb.

The performance of the Ogawa passive sampler during the 2004 season was similar to that observed for 2003; that is a precision between 0 and 20% with the exception of two duplicates (Figure 2b). The range of the differences was close to that observed for 2003, (-5.4; 4.77 ppb), with two outlying values of 15.4 and 10.3 ppb.

2.4.2. Accuracy of measurements

Correlation analysis was used to compare weekly ozone concentrations measured via passive samplers to continuously monitored ozone via the three real-time ozone analyzers. For 2002 the correlation coefficient was 0.853 ($p < 0.001$) suggesting that Ogawa passives sampler proved to be efficient devices for characterizing ozone exposures. The determined linear regression equation was $Y = 5.895 + 0.868 * X$. The plot of passive data versus real-time data showed that there was a very slight departure from the 1:1 line (Figure 3a).

The correlation coefficient for the 2003 data was 0.911 ($p < 0.001$) for weekly data, but the plot of passive data versus real-time data (Figure 3b) showed that there was a significant departure from the 1:1 line, and therefore need for correction. It is known that low wind speeds may cause underestimates of pollutant gas concentrations, and one can use the relationship between wind speed and boundary layer resistance to correct air pollutant concentration estimates (Willems, 1993). The addition of weekly temperature average, relative humidity and wind speed averages to correct ozone concentrations increased the regression parameters (p-values of the parameters and R-sq values).

Considering that one uses passive samplers in remote areas, due to the usual lack of meteorological data it is not feasible to integrate meteorological data into a linear regression. Therefore simple (real-time and passive data) were used in linear regression to correct the ozone concentrations which were obtained from the passive data ($Y = 0.102 + 0\ 0.8013 * X$). The plot of corrected and passive data is shown in Figure 4a. The residuals appear to be were randomly distributed (Figure 5a).

The correlation coefficient of the 2004 weekly ozone values was 0.064 ($p = 0.561$), and the plot of passive versus real-time data (Figure 3c) showed no relationship between the two data sets. Turbulent transfer caused by wind incursion into the open-ended tube sampler likely caused overestimates of gas concentrations by as much as 30% (Campbell, 1994). Therefore care in placement and protection of the samplers, to minimize their differential exposure to the wind, might minimize the problem. When replacing the samplers, we suspected that the problem might have been caused by the persistent high wind speed at Gleason. Therefore we analyzed the data for each individual site where co-located continuous monitors were present. The plot of passive versus real-time data for each individual site showed that when the Moshannon and Tiadaghton sites where analyzed separately from the Gleason site the correlation was high ($\rho = 0.679$; $p < 0.001$). Even if there was departure from the 1:1 line, the regression and 1:1 lines were parallel (Figure 3d). When analyzing different parameters (meteorological site specific) to determine a good regression fit for the data we found that average wind speed was more influential than average temperature and relative humidity; wind speed was higher at Gleason (Table 3). Since wind speed data were not available for all 20 locations used in this study, we excluded the data from Gleason and used only data from Moshannon and Tiadaghton to correct the ozone concentration which were obtained from passives. Real-time and passive data from the two mentioned sites were used in a simple linear regression equation (Y = - 0.327 + 0 0.816* X). The resulting residuals were normally distributed (Figure 5b). The plot of corrected and passive data is shown in Figure 4b.

Once the corrections of ozone concentrations for every individual year of study were made, the ozone patterns were analyzed using the ozone concentrations that were the result of the corrections applied to the concentrations obtained from the Ogawa passive sampler.

2.4.3. Ozone and elevation

The significant positive high correlation between seasonal ozone concentrations and elevation at sampling locations ($\rho = 0.813$, $p < 0.001$) for 2002, and ($\rho = 0.877$, $p < 0.001$) for 2003 showed that ozone concentration increased with elevation in similar fashion to the results obtained by Winner *et al.* (1989), Skelly *et al.* (2001), and Yuska *et al.*, (2003). There was also a positive correlation in 2004 ($\rho = 0.518$, $p < 0.019$), but the plot of ozone seasonal averages against elevation (Figure 6b) showed a different trend in 2004 compared to 2002 and 2003. The slopes of the regression equations (1) and (2) were very close in value showing a similar pattern of increasing ozone concentrations with elevation for 2002 and 2003. From the values of the slope and intercept of the equation (3) can be implied a different pattern of ozone concentrations with elevation in 2004 than that observed in 2002 and 2003.

(1) OZONE'02 (ppb) = 20.5 + 0.0394*ELEVATION (m), R-Sq = 66.0%;

(2) OZONE'03 (ppb) = 12.5 + 0.0418*ELEVATION (m), R-Sq = 77.2%;

(3) OZONE'04 (ppb) = 29.0 + 0.00957*ELEVATION (m), R-Sq = 26.8%,

Elevation was significant in determining ozone concentrations over the study area but ozone concentrations might have been influenced by the meteorological conditions as well. The observed seasonal averages of temperature (^{0}F), relative humidity (%), and wind speed, at three air quality monitoring sites are shown (Figure 7 and Table 3). Lower temperatures and higher relative humidity values in 2004 might explain the change in the trend of ozone concentrations with elevation, observed in the previous 2 years. These factors are likely the reason of the lessened efficiency of passive samplers in 2004. During all three years of the study the highest ozone concentrations were monitored at the Mt. Pisgah County Park and Pete's Run which were located at two of the higher elevation sites in this study. The lowest ozone concentrations were monitored at the Hyner Park which was located at one of the lowest elevation sites in our study. The comparison of mountain vs. valley differences in ozone concentrations shows that, in 2002 and 2003 there were higher differences in seasonal ozone concentrations between a valley site and a mountain site from the same pair when the high-elevation site was located at more than 600 m altitude (Figure 8). In 2004 the differences in seasonal averages of ozone concentrations between a valley site and a mountain site forming a pair, were less than in the previous two years and the differences were not significant (Figure 8).

2.4.4. Hourly patterns of ozone

Data on the mean or total ozone concentrations over the sampling period cannot explain the underlying exposure dynamics and consequently the ozone exposure-plant response relationships (Kickert and Krupa, 1991). One goal of this study was to determine the hourly frequency distribution of ozone concentrations using weekly mean values obtained from passive samplers as to account for the dynamics of ozone occurrences and resulting plant response.

The three real-time ozone monitoring sites co-located with passive samplers monitors (Table 1a) provided not only a study of the accuracy of the passive sampler devices but also a study of daily ozone patterns. Daily ozone patterns were similar at all three sites and hourly ozone concentrations were highly positively correlated ($0.651 < \rho < 0.753$, $p < 0.001$), during all three years of the study. Diurnal distribution of ozone varied between individual days with the known daily ozone pattern: daily maximum during early afternoon hours and daily minimum during night-time hours. Examples of diurnal distribution for one randomly selected week of 2003 and 2004 are presented within Figure 9.

At any individual period of time ozone concentrations followed similar patterns. The Tiadaghton and Moshannon ozone values followed a 24-hr ozone pattern with high values during mid-day and early afternoon, and low values at night. However a different pattern was observed at Gleason. At Tiadaghton and Moshannon the ozone concentrations dropped during the nighttimes, but at Gleason ozone exhibited higher values during the night. This observation together with the spatial location (all three sites were located within forested area on the top of a mountain) of the three sites led us to conclude that the differences in total ozone concentrations for the region were primarily due to the differences in ozone levels between day and night and related to elevation. The Tiadaghton site was located at 550 m elevation and of the three sites Tiadaghton exhibited the lowest ozone values when average concentrations were high for the region. The Moshannon site was located at 660 m and showed higher values during day-time which dropped significantly at night. The Gleason site was located at 700 m and showed little day-to-night variation in ozone concentrations. Daily minimum and maximum concentrations were clearly dependent upon site elevation and meteorological conditions, and hourly values followed a similar pattern.

Since the continuous monitored air quality sites were fairly evenly distributed across the study area (one in each edge and one in the middle) (Figure 1), we assumed that the main differences in ozone levels among the 20 monitoring locations were in terms of magnitude (mean values), not based upon the underlying distribution of hourly values. Our preliminary study on the theoretical distribution of the hourly ozone showed that we can assume a normal distribution of the data.

Continuous data were collected during the monitoring seasons of 2002-2004 and were analyzed according to the following steps:

1. Based on previous studies (Nosal *et al.*, 2000) the cumulative distribution function of the three-parameter Weibull was first tested to identify the theoretical distribution to fit the data. The results showed that the data did not fit the assumed distribution and therefore we tested several frequently used distributions (Table 6), nevertheless none proved to perfectly fit the data. We used the Anderson–Darling (AD) statistic for the maximum likelihood and least squares estimation methods. The Anderson–Darling statistic is a measure of how far the plot points fall from the fitted line in a probability plot. The statistic is a weighted squared distance from the plot points to the fitted line with larger weights in the tails of the distribution. A smaller Anderson–Darling statistic indicates that the distribution fits data better. The three parameter Weibull and Normal distribution proved to have low Anderson–Darling values but neither the Weibull nor a normal distribution was statistically significant.

2. Cumulative frequency Distribution Functions [CDF (x)] of continuous ozone monitoring data at all three locations and for the 3 years of study were computed. Each year of study and each monitoring location were analyzed individually. Different distribution functions were considered; we found that the data best fitted to normal Gaussian and to three-parameter Weibull distributions (Figure 10).

3. Based on previous results, it was concluded that the three-parameter Weibull distribution was not the only way to determine hourly ozone distribution, and would not necessarily represent the true frequency distribution of the data. Therefore, in this paper we report the results of the application of a stochastic, normal probability distribution for generating data. The Cumulative Normal Distribution Function is defined as:

$$F(x) = P(X \leqslant x) = \int_{-\infty}^{x} \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{1}{2}[(y-\mu)/\sigma]^2} dy,$$

μ is the mean, and σ is the standard deviation of the sample.

Since passive samplers provided fairy accurate weekly ozone concentrations, the only assumption that had to be made was on the sample standard deviation. For 2002 and 2003 the ANOVA Tukey test revealed that the mean ozone concentrations could be grouped into three classes, which were also correlated with elevations in most cases, therefore we assumed one value of standard deviation for each class and week of the study (i.e., assuming that similar means corresponded to similar standard deviations). One monitoring site belonged to each class; therefore we assumed that standard deviation of ozone values for a particular site would be equal (on an weekly basis) to the known standard deviation as obtained from continuous data of that site found to be in the same class. For 2004 we assumed that the standard deviation for a location was equal to the standard deviation measured at one real-time monitor showing a similar mean (terms of not being statistically different) (Table 7, 2004a). But in 2004 there were no obvious groups for the weekly means, so in a second analysis we assumed that the standard deviation of hourly ozone values of one particular week was the mean standard deviation calculated for the values of the three air quality monitoring sites (Table 7, 2004 b).

In this study we claim as null hypothesis that the empirical weekly cumulative distribution F(x) obtained for the air quality monitoring is equal to the hypothetical distribution function $F_0(x)$ of a particular site. The mean of the distribution was known (passive ozone data) and the standard deviation was assumed known and equal to standard deviation of the real-time data.

Steps in obtaining hourly values of ozone:

1. Assumptions;
    1. Hourly values were normally distributed,
    2. Different sites on the same class would exhibit equal standard deviation on a particular week,

3. The true mean of the sample was the mean calculated from passive samplers;
2. From weekly means (passives) and standard deviations as assumed, we generated normally distributed data for every site and week of the monitoring period. The procedure was repeated 10 times.
3. Synthetically generated weekly means (averaged over the 10 identical trials) were compared with the real values as obtained at the air quality monitoring sites (Table 7). Correlation and t-test were performed to test for significant differences between real and generated data.
4. Synthetically generated hourly data were used to compute SUM40, SUM60, SUM80, N40, N60, and N80 for each week involved in this study and for each monitoring site. N40 is defined as the cumulative number of hours when ozone values were higher or equal to 40 ppb. Similarly N60 is defined as the cumulative number of hours when ozone values were higher or equal to 60 ppb. Likewise N80 is defined as the cumulative number of hours when ozone values were higher or equal to 80 ppb.
5. Synthetically generated computed SUM40, SUM60, SUM80, N40, N60, and N80 seasonal values were compared with the real values (Table 7).
6. Synthetically generated weekly means for all sites were compared with weekly mean values as provided by the passive samplers.

The accuracy of passive samplers in 2004 was clearly lower than in preceding years, therefore the differences in ozone concentrations between the real data and the synthetically generated data were higher in 2004 than in 2003 and 2002 (Table 7). The differences observed between the synthetically generated and monitored data are more likely due to the accuracy of the passive sampler than to the assumptions of normality of the distribution.

The correlation between the corrected passive weekly means and synthetically generated weekly means ($\rho = 0.998$, $p < 0.001$), and the T-Test of mean difference = 0 (vs. not = 0): T-test = -0.05, $p = 0.957$, DF = 1699 both showed that the assumptions regarding distribution and standard deviations were accurate. The performed tests showed that the differences in the mean ozone concentrations between the actual passive and the synthetic data are within 1.0 ppb and the significance values in predominant number of cases were > 0.05 (Figure 11). These results showed that the frequency distributions of the measured and the synthetic data were statistically indistinguishable at the 95% level depending on the sampling period or week, thus substantiating the utility of the method. Therefore, all null hypotheses can be accepted. The acceptance of the null hypotheses implies that the difference between the empirical and theoretical normal distribution curves were very close. Therefore, we can conclude that the empirical distribution came from a population of a sample exhibiting a normal distribution. The results of the performed tests demonstrated that the method used can achieve accurate estimates. These results appear to be the product of a non-systematic bias (based on the data from different sampling periods or weeks) in the mean values obtained from the passive sampler. Nevertheless, in the present case, independent of the bias, the data on the synthetic hourly ozone frequency distribution parameters (mean, and standard deviation) underlying the passive sampler mean values were quite comparable in most cases to the corresponding data from the continuous measurements (Table 7).

2.4.5. Spatial and annual variation

Weekly means of ozone concentrations were compared to determine significant differences among sites for every year (Figure 6a). Two-way ANOVA was used to test for significant differences and for patterns of ozone concentrations. Both year of study and location were significant sources of variance for mean ozone, but when Tukey's test was used, ozone concentrations were found to be different in 2002 (μ = 38.479; σ = 10.704) from 2003 (μ = 32.858; σ = 10.535) and 2004 (μ = 33.142; σ = 5.192), but there was not a significant difference between annual means of ozone in 2003 and 2004. In fact, the 2004 ozone concentrations decreased at high-elevation sites and increased at low-elevation sites compared to 2002 and 2003 (Figure 6a,b).

Ozone concentrations monitored during the study period also showed significant differences among locations. These differences were more obvious in 2002 and 2003, when similar ozone patterns were observed, and less pronounced in 2004 (Figure 6b). Also one-way ANOVA and Tukey's test used to test for significant differences among sites, showed that in 2002 and 2003 ozone concentrations could be grouped in similar classes closely related to elevation, but in 2004 ozone concentrations were grouped differently (Table 2).

Preliminary evaluation of geostatistical methods showed that an exponential variogram model and ordinary kriging method would provide an accurate estimation of the spatial ozone concentration. The main goal of a variogram analysis is to provide the best estimates of the autocorrelation structure of the underlying stochastic process. In order to ensure that the variance of predicted values was positive we fitted exponential, spherical and Gaussian variogram models. The theoretical variogram that best fit the data was the exponential model. Based on the lowest prediction errors criteria, the best model was also the exponential model. Therefore, the interpolation algorithm was selected based on statistical characteristics, such as the lowest errors criteria. Since the relationship between ozone and elevation was strong only in 2002 and 2003, we have not utilized co-kriging with elevation as a secondary variable for this study.

The model's ability to map ozone distribution for the entire north-central Pennsylvania area was limited because of the small number of monitoring sites. Nevertheless maps presenting individual annual averages for the 3 years of study showed several characteristics of ozone concentrations (Figures 12, 14, 16).

Maps characterizing weekly ozone averages for individual years were constructed to evaluate the seasonal pattern of ozone distribution. Weekly ozone distribution maps (Figures 13, 15, 17) showed similar trends in ozone concentrations from one week to another in 2002 and 2003; weekly trends were also similar to the seasonal trend for both years. Apparently there was a low variation in the spatial trend of ozone concentrations from one week to the next, therefore low temporal variation during 2002 and 2003. The ranges of ozone averages were (17.3 to 78.7) in 2002, (15.8 to 72.4) in 2003, and (23.7 to 58.4) in 2004.

The highest variability for ozone average concentrations was detected for locations at high-elevation sites that usually record higher ozone values. Other locations showed less variation in average ozone concentration (Table 2). In 2002 and 2003 there was a consistent trend of low ozone in the central part of the region, surrounded by high ozone concentrations. Even for the weeks during which very high ozone concentrations were recorded the central region exhibited less ozone (Figures 13, 15). In 2003 the ozone averages were significantly lower than in 2002 but the general spatial trend was consistent with that observed for 2002; that is low ozone in the central part of the region surrounded by areas with higher ozone concentrations. The main difference between the two years was in the calculated seasonal ozone mean. In 2004 the seasonal ozone averages were not significantly different from 2003, but the spatial pattern was different from that observed for 2003. There was low ozone in the central part of the region but that area extended less than in the previous 2 years and ozone concentrations were more uniformly distributed throughout the entire region. The seasonal maps for individual years showed a similar trend with the trend observed on a weekly basis (Figures 13, 15, 17).

Since the algorithm used to create prediction maps also provided calculations of standard errors of prediction, maps of prediction errors of ozone concentrations were generated (Figures 12, 14, 16). Table 8 shows the distance from monitoring sites up to which the estimated values were spatially autocorrelated. That distance can be used to confidently delineate areas of estimated ozone concentrations. The observed threshold was 10-12 km and typically a value of ozone concentration can be confidently estimated only within such a distance from the monitoring site. Even in the areas where the confidence of prediction was satisfactory, there was an error of estimation around 1 ppb. The error increased as the distance from the sampling site increased.

The geographic distribution of the higher and lower ozone concentration was not random, and in a typical year, sites at high elevations would exhibit higher ozone concentrations. Also the central region of the area exhibited lower ozone concentration regardless of the seasonal ozone means. Concentration of ozone in a region is known to be dominated not only by the sources of air pollution, but also by the meteorological conditions (Comrie, 1994). Ozone values at three air quality monitoring sites (Table 1a) were positively correlated with temperatures and wind speed in 2002 and 2003, but negatively correlated in 2004 (Table 4). Also there were strong negative correlations between ozone and relative humidity for all 3 years of study. High concentrations of ozone are likely to occur during periods of high temperature and low relative humidity. Therefore lower temperatures associated with high relative humidity and strong winds can result in changes in ozone distributions, as was observed for 2004. When meteorological conditions are not conductive to high ozone, as those observed for 2004, the spatial pattern of ozone distribution changes, and the prediction ability of any model decreases. To explain the variability of ozone concentrations with meteorological conditions, we performed multivariate polynomial regression of the continuous ozone values and meteorological variables (Table 5). This statistical approach was described by Krupa *et al.* (2003), when coupling passive ozone sampler data with climatological variables to mimic the frequency distributions of hourly ambient ozone concentrations obtained by continuous monitoring. Our results showed that all significant variables identified by the models were important factors regulating ambient ozone concentrations. And more importantly if the variability of ozone concentrations can be fairly explained by the meteorological variables (usually high R-sq values) then ozone distribution across the area would have a more predictable behavior.

2.5. Discussion

There is a significant temporal and spatial variability in the occurrence of ambient O_3 concentrations. These variations are usually governed by the latitude, altitude, location, season and synoptic meteorology Nosal *et al.* (2000) and Krupa *et al.* (2001).

The 3 years of ozone monitoring in north-central Pennsylvania region revealed that the efficiency of the passive sampler is highly dependent on wind velocity, ambient temperatures and relative humidity. The occurrences of these varying conditions are stochastic by nature, and care in placement and protection of the samplers might minimize the problem. Although a diffusion barrier is commonly used, such barriers may not always provide protection against changing wind turbulence. Our results proved that during years of low temperature and high winds the efficiency of passive samplers drops.

Ozone is an oxidant and there are other oxidants in the atmosphere, such as peroxy-acyl nitrates and oxides of nitrogen and sulfur, and interferences from these compounds can result in an overestimation or underestimation of the cumulative ozone concentration by the passive sampler (Campbell, 1994). Our results show that it is essential to have co-located continuous ozone analyzers along with passive samplers, in this case the passives data can be corrected and used for further analyses. Usually a simple linear regression would suffice to correct the passive data and the resulting ozone means would provide good estimates of ozone concentrations within the study area.

A strong positive correlation existed between ozone concentrations and temperature when the ozone levels were high to moderate, but negative when ozone levels were low (Table 4). Ozone pollution episodes are generally associated with slow-moving high-pressure systems, and meteorological conditions that are conducive to the photochemical production of ozone. If the variability of ozone values can be fairly explained by the meteorological variables (high R-sq values) then ozone distribution would be more predictable (Table 5).

The significant positive high correlation between seasonal ozone concentrations and elevation at our sampling locations suggests that ozone concentration increased with elevation in those years when meteorological conditions were conductive to production of greater ozone concentrations. These conclusions are reinforcing the results of Yuska *et al.* (2003), who found a similar pattern in ozone concentrations. Also the ANOVA results showed that increased ozone is to be expected at higher elevations. The differences between seasonal ozone concentrations at valley sites vs. mountain sites are likely to increase as the difference in elevation between the valley and the mountain increases. These differences would be less obvious in years with low ozone exposures. Since temperature is such an important factor determining ozone concentrations, part of the change in the effect of elevation observed in 2004 may be due to the lower temperatures that were recorded at higher elevations. The ozone concentrations were significantly higher in 2002 as compared to 2003 and 2004. In 2002 the average temperature was also significantly higher. Yuska *et al.* (2003) found that higher ozone concentrations across central PA observed in 2001 where most likely due to higher temperatures in 2001 as compared to 2000.

Simini *et al.* (1992) suggested a west-to-east decreasing gradient of ozone concentrations across central PA. Our results do not support their hypothesis; one explanation for differing results is that their eastern most site was located at Tiadaghton, which was located in the central area of the present study. The Tiadaghton site exhibiting lower ozone concentrations was located west of Mount Pisgah County Park a site exhibiting higher ozone concentrations than the Tiadaghton site. Our data did not show a west-east decreasing ozone gradient. In fact spatial analysis of the ozone distributions showed that there was a persistent pattern of low ozone in the central part of the region surrounded by higher ozone concentrations, as previously observed (Jagodzinski, 2000; Skelly *et al.*, 2001). The monitoring sites in the central area were all located at elevations below 600 m while the sites exhibiting the highest ozone concentrations were all located above 600 m (Table 2). The ANOVA test showed that ozone concentrations exhibited at locations below 600 m were not significantly different among themselves but significantly different from those exhibited at sites located above 600 m. Therefore lower ozone concentrations in the central area of north-central PA are most likely related to the topography of the area. The three higher elevation sites Pete's Run, Mt. Pisgah County Park, and Gleason supported this observation for 2002 and 2003; during 2004 weather conditions were so much different that any conclusion regarding ozone exposures might be premature. Two sites at lower elevation exhibited ozone concentrations somehow higher than some of the sites at higher elevation; Canton and Mt. Pisgah State Park, they both were located less than 6 miles from two of the highest elevation sites Mt. Pisgah County Park, and Gleason. We suspect that a close proximity of a low-elevation site to a high-elevation site increase ozone concentrations at the low-elevation site. This exchange results in an increase in ozone concentration at a lower elevation site. Six sites at higher elevation were located above 600 m supporting the conclusion that ozone exposures are significantly higher on the mountaintop than in the valley. For those sites located below 600 m the difference of ozone concentrations between mountaintop and valley was not significantly different.

Comparing the ozone exposures recorded at Moshannon and Tiadaghton sites from 1989 to 2004 we found that in 2002 ozone concentrations reached the highest level since 1989, while in 2004 ozone concentrations reached one of the lower levels since 1989, the lowest ozone concentrations were in 1990. The two sites were part of ozone monitoring program in rural north-central Pennsylvania that began in 1989 (Douglas, 1998; Skelly *et al.*, 2001; Yuska *et al.*, 2003).

Passive sampler data were used to estimate the real-time ozone concentrations (Krupa and Legge, 2000; Tuovinen, 2002) which are needed for calculation of ozone uptake by vegetation. Hourly ozone distributions can be assumed to be normally distributed with means provided by the passive samplers and standard deviations assumed equal to those obtained from real-time monitors. The high correlation between the synthetically generated data and the real data showed that the method used to generate continuously ozone values can provide good estimates of ozone distributions. The performed tests showed that the differences in the mean ozone concentrations between the actual passive and the synthetic data were within 1.0 ppb and the significance p-values in predominant number of cases were < 0.05 (Figure 11).

2.6. Conclusion

Passive ozone samplers have been useful in extending the ozone monitoring network within our study area. Seasonal averages or summed ozone concentrations from passive monitors were comparable to those obtained from continuous monitors. Weather-related factors greatly affected the accuracy of passive samplers, but a combination of passive and real-time monitors increased information about the distribution and regional spatial patterns of ambient ozone.

Spatial interpolation of data from a network of locations was used to identify the potential risk areas. However the spatial interpolation does not take into account topographic or climatologic factors, therefore not providing enough information on specific locations where exceedances of the standards are occurring. Nevertheless, such interpolations can provide reliable information about potential problem areas.

References

Berry, C.R., 1964. Differences in concentration of surface oxidant between Valley and Mountaintop conditions in the Southern Appalachians; Journal of Air Pollution Control Association, 14, 238-239.

Bytnerowicz, A., Tausz, M., Alonso, R., Jones, D., Johnson, R. Grulke, N., 2002. Summertime distribution of air pollutants in the Sequoia National Park, California. Environmental Pollution 118(2), 187-203.

Campbell, G.W., Stedman, J.R., Stevenson, A, 1994. A survey of nitrogen dioxide concentrations in the United Kingdom using diffusion tubes, July–December 1991. Atmospheric Environment 28, 477–486.

Comrie, A.C., 1994. Tracking Ozone: Air mass trajectories and pollutant source regions influencing ozone in Pennsylvania forests. Annals of the Association of American Geographic's 84,635-651.

Comrie, A.C.,1994a. A synoptic climatology of surface ozone in rural ozone pollution at three forest sites in Pennsylvania. Atmospheric Environment 28, 1601-1614.

Cox, R.M., 2003. The use of passive sampling to monitor forest exposure to O_3, NO_2 and SO_2 a review and some case studies. Environmental Pollution 126, 301–311.

Douglas B.C., 1998. An analysis of tropospheric ozone concentrations at four forested sites in north-central Pennsylvania from 1989-1997. M.S. Thesis, Pennsylvania Sate University, University Park, PA.

Hildebrand , E.S., Skelly, J.M., and Frederiksen, T.S., 1996. Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. Canadian Journal of Forest Research 26, 658-669.

Jagodinski, J.M., 2000. An analysis of tropospheric ozone transport in north-central Pennsylvania during the summer of 1999. M.S. Thesis, Pennsylvania Sate University, University Park, PA.

Kaluzny, S.P., Vega, Cardoso, S.C., T.P., Shelly, A.A., 1998. S+Spatial Stats: user's manual for Windows and UNIX. Springer-Verlag, New York, Inc., 175 Fifth Avenue, N.Y., 10010, USA.

Kickert, R.N., and Krupa, S.V., 1991. Modeling plant response to tropospheric ozone: a critical review. Environmental Pollution 70, 271–383.

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

Krupa, S.V., Tonnejkck, A.E.G. and Manning, W.J., 1998. Ozone. Pp.2-1 to 2-23, in R.B.Flagler *et al.* (Eds.). Recognition of Air Pollution Injury to Vegetation: A pictorial atlas. Air and Waste Management Association, Pittsburgh, PA.

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

Krupa, S.V., M. Nosal and D.L. Peterson, 2001. Use of passive ambient ozone (O_3) samplers in vegetation effects assessment. Environmental Pollution 112, 303-309.

Krupa, S.V., Nosal, M., Ferdinand, J. A., Stevenson R.E., and Skelly, J. M., 2003. A multi-variate statistical model integrating passive sampler and meteorology data to predict the frequency distributions of hourly ambient ozone (O_3) concentrations. Environmental Pollution 124, 173-178.

Lefohn, A.S., Knudsen, H.P., Shadwick, D.S., Herman, K.A., 1992. Surface ozone exposures in the eastern United States (1985-1989). In: Flager, R.B., ed. Transactions of the response of southern commercial forests to air pollution, specialty conference. Pittsburg, PA, Air and Waste Management Association, 81-93.

Legge, H., M. Nosal, G. E., McVehil and Krupa, S. V., 1991. Ozone and the clean troposphere: Ecological implications. Environmental Pollution 70, 157-175.

Minitab Inc., Quality Plaza, 1829 Pine Hall Road, State College, Pennsylvania, 16801, USA.

Nosal, M., Legge, A. H., and Krupa, S.V., 2000. Applications of a stochastic, Weibull probability generator for replacing missing data on ambient concentrations of gaseous pollutants. Environmental Pollution 108, 439-446.

Ogawa & Company, USA, Inc., 1998. NO–NO_2 simultaneous sampling protocol using Ogawa sampler. Pompano Beach, FL.

SAS Institute Inc., 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, SAS Institute Inc., Cary, NC, 1686 pp.

Simini, M., Skelly, J.M., Davis, D.D., Savage J.E., and Comrie, A. C., 1992. Sensitivity of four hardwoods species to ambient ozone in north central Pennsylvania. Canadian Journal Research 22, 1789-1799.

Skelly, J.M., J.E. Savage, K.R. Snyder and T.S. Fredericksen, 1994a. Tropospheric ozone occurrences at remote sites in Pennsylvania: A database for evaluation of changing pollution scenarios. Environmental Resources Institute, Pennsylvania State University, University Park, PA, USA, 25 pp.

Skelly, J.M., Ferdinand, J.A. Jagodzinski, J.M., Savage J.E. and Mulik, J.D., 2001. A thirteen week comparison of passive and continuous ozone monitors at forested sites in North-central Pennsylvania. Journal Air and Waste Management Association 51, 1280-1287.

S-PUS 6 for Windows, Insightful Corporation, Seattle, WA, USA.

Tuovinen, J.A., 2002. Assessing vegetation exposure to ozone: is it possible to estimate AOT40 by passive sampling? Environmental Pollution 119, 203-214

U.S. Environmental Protection Agency (US EPA), 1996. Air quality criteria for ozone and related photochemical oxidants. Vol.1 of 3 reports no EPA/600/P-93/004aF. Research Triangle Park, North Carolina.

U.S. Environmental Protection Agency (US EPA), 1996a. Air quality criteria for ozone and related photochemical oxidants. Office of Research and development, Washinghton, D.C. EPA/600/P-93/004a-cF.

Willems, J.J.H., 1993. Low-cost Methods for Measuring Air Pollutants (a Training Program). Report R-635, Wageningen (NI) Agricultural University, Department of Air Quality, Wageningen, The Netherlands.

Winner, P.B., Lefohn, A.S., Cotter, I.S., Greitner, C.S., Nellesen, L.R. McEvoy, C.J. Olson, R.L., Atkinson C.J., and Moore, L.D., 1989. Plant responses to elevational gradients of O_3 exposures in Virginia. Procedures at the National Academy of Science 86, 8828-8832.

Yuska D. E., Skelly, J.M., Ferdinand, J.A., Stevenson, R.E., Savage, J.E., Mulik J.D., and Hines, A, 2003. Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania. Environmental Pollution 125, 71-80.

CHAPTER III

Modeling stomatal conductance and ozone uptake (flux) in seedlings of *Prunus serotina* and cuttings of *Populus maximiwizii x trichocarpa*

"Capsule": Environmental factors explain less than 30% of the variation in stomatal conductance; any model of stomatal conductance should include plant specific physiological parameters.

3.1. Abstract

Studies were conducted at 20 sites located at low-to-high elevation in north-central Pennsylvania from early June to the end of August during 2003 and 2004. A linear model was developed to estimate the influence of local environmental conditions on stomatal conductance of two ozone sensitive tree species. One year-old seedlings of black cherry (R-12) (*Prunus serotina*) and ramets of hybrid polar clone NE388 (*Populus maximiwizii* x *trichocarpa)* were established in the early spring of the 2003 within open plots at the 20 locations.

Gas exchange measurements were performed at 12 sites (6 sites at high elevation and 6 sites at low elevation) under field conditions, on seedlings and cuttings of the two tree species. The measurements were performed three times a day under favorable weather conditions (clear sky) during both study seasons. Gas exchange data from the 12 sites were used to empirically model stomatal conductance and to model and map ozone flux for the two tree species throughout the study area. Ambient ozone concentrations at the canopy level were continuously monitored using passive ozone samplers.

Throughout both seasons, a significant variation was observed in measured stomatal conductance (g_s) and photosynthetic rates (P_n). The most significant factors explaining stomatal variance were: tree species, air temperature, elevation, and time of day. The most significant factors in limiting g_s were vapor pressure deficit (Vpdl) and air

temperature. Stomatal conductance was higher for hybrid poplar than for black cherry during both years. Stomatal conductance was greater at noon and in the early afternoon at high-elevated sites during 2003. During 2004 g_s rates were higher at low-elevation sites. Photosynthetic rates were greater at high-elevation sites in the morning and at noon during both study seasons. Apparently greater air temperature at the low-elevation sites inhibited g_s in 2003, reducing ozone uptake. Also, lower than normal air temperature values at the high elevation sites might have inhibited g_s rates in 2004. Since environmental factors explained less than 30% of the variation in stomatal conductance, Vpdl and P_n were used to increase the accuracy of the g_s model.

Although visible ozone induced injury better correlated with the ozone uptake/flux than with cumulative ozone exposures, the flux approach did not entirely explain the visible effects of ozone on the two plant species.

Keywords: stomatal conductance, ozone uptake, ozone flux

3.2. Introduction

Tropospheric concentrations of ambient ozone and its adverse effects on sensitive vegetation have been long recognized (Davis *et al.*, 1981; Tingey and Hogsett, 1985; Reich, 1987; Wieser and Havranek, 1995; Skelly *et al.*, 1997; Schaub *et al.*, 2003; Wei et al, 2004). A major area of interest has been to determine the impact of cumulative ozone exposures on individual plants, native species, and regional ecosystems. Foliar symptoms exhibited by any sensitive plant species are an indication of previous exposure of the plants to ozone when the environmental conditions are conductive to ozone uptake and consequently to cellular injury (Krupa *et al.*, 1998).

The primary pathway for ozone to contact plant tissue is through plant stomata (Fowler and Cape, 1982), and therefore ozone effects on vegetation are directly related to the effective rate of ozone uptake and/or to the effective cumulative uptake amount (i.e., effective uptake rate× time) (US EPA, 1996a; Musselman and Massman, 1999). The response of a plant to pollutant exposure is a consequence of the diffusion of the pollutant from the ambient air through stomata. This pollutant influx over time is defined as the effective dose (Grunhage *et al.*, 1999). There is general agreement that ozone uptake/flux or the instantaneous rate at which plant surfaces absorb ozone, can be used to determine biologically relevant estimates of ozone risks (Furher, 2000). However, implementation of this approach requires the development of appropriate ozone flux models that need to be rigorously tested against actual data collected under field conditions (Emberson *et al.*, 2000).

This approach emphasizes the fact that ozone effects on plants are related to the amount of ozone taken up by the foliage, which depends on the physical conditions in the boundary layer, species specific characteristics, and edaphic factors (Nussbaum, 2003). Thus, the prediction of the level of ozone injury should be based on measured canopy-level ozone concentrations and should account for modifying influence of environmental factors on stomatal conductance for a given species. Manning (2003 suggested that any threshold cumulative value air quality standard for predicting ozone injury on native plants that does not take into account the biological and environmental variables that affect ozone uptake via stomata has no biological basis or relevance.

Since ozone uptake is controlled by stomatal conductance, a range of environmental variables that exert a strong control on stomatal opening help determine the sensitivity of vegetation to pollutant exposure. The stomatal responses to air humidity, wind speed, and soil water status are mediated through their effects on the balance of water loss from the leaves and water uptake by the roots, which in turn influence the water status of the plant (Mott and Parkhurst, 1991). Exploring the relative contribution of the environmental factors such as solar radiation, air temperature, Vpdl, and soil water potential to g_s, Zierl (2001) found that soil moisture was the most important factor controlling the response of stomata to its environment during the growing season. To determine if g_s of forest trees could be predicted from measures of leaf microclimate Patterson *et al.*, (2000) found that only 30% of diurnal variation of stomatal conductance was explained by photosynthetically active radiation (PAR) and (Vpdl). Hildebrand *et al.* (1996) found that more foliar injury was observed on yellow-poplar (*Liriodendron tulipifera*) and black cherry growing on wetter soils as compared with drier sites. In some cases, environmental factors might even control ozone uptake. For example, in dry years with high ozone concentrations sensitive vegetation might be protected from leaf injury by stomatal closure, limiting gas uptake. In wet years with relatively low ozone concentrations ozone damage might be high, as ozone uptake is not limited by the stomatal regulations under wet conditions.

The efficiency of plant water use depends both on g_s and on the difference in vapor pressure (Vpdl) in the intercellular air spaces and the surrounding air. Temperature also affects the Vpdl in the leaf, and therefore temperature has a pronounced effect on water-use efficiency, which in turn has a direct effect on stomatal opening/closure. Temperature also has a major effect on enzymatically catalyzed reactions and therefore affects photosynthesis. Differences among plant capacity to perform at high temperatures correlate with the plant capacity to photosynthesize at these temperatures.

Internal conductance varies widely among species and roughly correlates with the photosynthetic capacity of the leaf (Lambers *et al.*, 1998). After entering leaves, ozone affects the internal tissue, resulting in reduction of carbon assimilation and leaf life-span and consequently inducing early senescence (Skelly *et al.*, 1999). Also when ozone enters plant stomata, defensive mechanisms in the tissue will start to detoxify and neutralize the ozone to prevent the oxidizing effect of the ozone (Massman *et al.*, 2000). Defense systems vary with time according to plant health, stage of the plant development, status of available resources, and within plant species variation due to inherent genetic differences. Defensive capabilities are reduced when photosynthetic rates are low; that is, without photosynthesis basic metabolic processes are slow. In a study of the influence of the light flecks on foliar injury of poplar species, Wei *et al.* (2004) found that lower photosynthetic rates were associated with increased injury due to the limited defense capacity as a result of constant ozone uptake but decreased photosynthesis. Biological relevance of ozone effects in terms of plant response should include factors that influence flux (concentration and conductance) and effective absorbed dose (Musselman and Massman, 1999).

In the present study empirical parameterization of the stomatal conductance response was used for estimating ozone flux for seedlings of black cherry and hybrid poplar. This modeling approach has been developed allowing for between-species variability. The model incorporated both physiological and site-specific environmental factors, such as P_h, Vpdl, elevation, and temperature through generalized relationships.

The responses of plants to ozone vary with tree size. Previous work established greater rates of stomatal conductance and leaf ozone uptake for open-grown seedlings than for mature black cherry trees (Fredericksen *et al.*, 1995), so extrapolations from seedlings to mature trees must be carefully considered.

In this study ozone flux was estimated from multiplication of stomatal conductance as generated by the model by cumulative ozone (sum of hourly averages of ozone values over the growing season) (Jarvis, 1976; Emberson *et al.*, 2000). The goals of this study were; (1) to model stomatal conductance for seedlings of two tree species growing in different environmental conditions, and (2) to determine the spatial distribution of ozone flux throughout the study area.

3.3. Materials and methods

3.3.1. General considerations

The main objective of this study was to evaluate the impact of site-specific environmental factors in estimating ozone flux for two ozone sensitive tree species. The environmental regulation of stomatal conductance usually results in large spatial variation of ozone flux even in conditions of equal exposures. If one model of ozone flux is to be used in large-scale estimation, the model should require a minimum input of plant characteristics and be driven by the environmental data that are readily available (Uddling *et al.*, 2004).

This study utilized black cherry seedlings and hybrid poplar cuttings. Black cherry is one of the most ozone sensitive tree species within mixed-hardwood forests of the northeastern USA. Poplar species (*Populus* sp.) and hybrid poplars have also been demonstrated to be among the most ozone sensitive tree species of eastern USA (Berrang *et al.*, 1991; Woodbury *et al.*, 1994; Karnosky *et al.*, 1996). Both plant species response to ozone exposures have been extensively studied in terms of foliar injury, leaf morphology and whole-plant physiology (Davis *et al.*, 1977; Long and Davis, 1999;

Bennett *et al.*, 1992; Davis and Skelly 1992; Fredericksen *et al.*, 1996; Ferdinand *et al.*, 2000; Schaub *et al.*, 2003; Wei *et al.*, 2004).

3.3.2. Research sites

In order to explore spatial variation of stomatal conductance and consequently of ozone flux, we established 20 research sites in wooded clearings within rural and forested areas in north-central Pennsylvania. To determine the influence of elevation on stomatal conductance and on ozone flux, 10 sites were established at higher (> 550 m) and 10 sites at lower elevations (< 350 m) (Table 1a). At each site 5 seedlings of black cherry and 5 seedlings of hybrid poplar were planted in early spring of 2003. Ambient ozone concentrations were measured at the seedling canopy height with Ogawa passive ozone samplers (Ogawa and CO., Inc.). The accuracy of ozone measurements was determined by comparing passive-sampler values to continuous hourly ozone values from the co-located Advanced Pollution Instrumentation (API) Model 400-A ozone monitors. The continuous API ozone analyzers were operated as real-time monitors at three air quality monitoring sites (Table 1a). The 2003 study was conducted during a 13-week period from June 9 to September 9. The 2004 study was conducted during a 14-week period from May 23 to September 2. Weekly ozone mean concentrations were used to synthetically generate hourly values and determine SUM40, SUM60 and SUM80 (Chapter II) for each location, and consequently calculate ozone flux throughout the study area. The summary of ambient ozone exposures are presented in Table 9.

Soil samples were taken from each location and analyzed for N, P, K, Ca, Mg, and pH. Soil acidification and the disruption of nutrient cycles appear to be important factors that weaken the resistance of sensitive tree species to abiotic and biotic stressors (St.Clair and Linch, 2004).

3.3.3. Plant material

Black cherry seedlings used in this study were derived from open-pollinated seeds collected in the fall of 2001 from a single mother tree (R-12) within a black cherry seed orchard at Penn State. One year-old seedlings of black cherry half-sib selection “R-12”, and ramets of hybrid polar clone NE388 were planted within the 20 open plots during May 2003. Black cherry seedlings used in this study had been previously tested for ozone sensitivity and/or tolerance during the 2002 summer season under controlled conditions within CSTR chambers. The predominant symptom induced by the previous ozone treatments on the cherry seedlings was adaxial light brown to black stipple. On very sensitive individuals stipple was quickly followed by early leaf senescence and drop. The percentage of leaves showing symptoms in the controlled experiments was recorded as the amount of injury per plant (% AMT). The Horsfall-Barratt rating system (Horsfall and Barratt, 1945) used by the Forest Health Expert System (Nash *et al.*, 1992) was utilized to assess the severity of injury of the affected leaves (% SEV) for symptomatic leaves on each plant, with observations recorded as 0, 3, 6, 12, 25, 50, 75, 88, 94, 97, and 100% area affected. The injury index was calculated (% INJ = % AMT * % SEV) to evaluate whole plant injury.

Based on the response of the plants to ozone exposures, to gain uniformity among plots, and to establish an equal number of seedlings at each plot, the seedlings were classified as follows: Class 0: no visible symptom response to ozone exposures (tolerant), Class 1: 1-6% INJ, Class 2: 7-15% INJ, Class 3: 16-30% INJ, and Class 4: more than 30% INJ, (very sensitive). Following exposure and evaluations seedlings were tagged according to their sensitivity class, and maintained under greenhouse conditions during the next winter. In May 2003 one seedling from each sensitivity class was planted at each plot.

In March of 2003 ramets of hybrid poplar clone NE388 were started by cutting dormant scionwood with viable buds. One-year old branches with diameter of 3-10 mm of clone NE388 were collected from clonal outplantings in central Pennsylvania. Upper crown branches were cut into 15 cm section and inserted 10 cm deep into Metro-Mix 250 within plastic pots. Potted ramets were maintained in the greenhouse facility and were planted in the field in May 2003. The seedlings were planted in natural soil, in two concentric circles within 8 feet diameter plot which was fenced for protection against rodents and deer. Following initial watering after planting, seedlings were subjected to natural conditions for the duration of the study and were not fertilized.

3.3.4. Physiological measurements

Gas exchange was measured at the leaf level under field conditions. Leaf gas exchange measurements including photosynthesis, stomatal conductance, and Vpdl, were measured at constant CO_2 (400 µmol/mol) using a Li-Cor 6400 (Li-Cor Inc., Lincoln, NE) open-gas exchange photosynthesis system with an internal light source. The measurements were taken during June-August of 2003 and 2004 at 12 different sites when weather conditions were favorable (Table 10). Leaf gas exchange measurements were made only during optimal light and environmental conditions to avoid outliers that occur under extreme ambient conditions.

At each site all 10 seedlings were sampled three times a day. One measurement was taken between 0900h and 1100 h, a second between 1200 h and 1400 h and a third measurement between 1500 h and 1700 h. Only leaves that appeared healthy and were fully exposed to the sun were measured. After the first measurement the shoots and leaves were tagged. After the initiation of ozone injury in 2004, one uninjured and one ozone-injured leaf per plant were measured at the onset of visible ozone injury. Measurement time per leaf was approximately 1.5 min, which minimized the time for conductance to acclimatize to the conditions in the leaf chamber. The differences in microclimate between the chamber and the ambient air were minimized by controlling CO_2 concentrations (400 µmol/mol), light (800 PAR), and temperature (20^0C). Chamber pressure was matched with that of ambient air by passing a portion of the air flow through the dessicant. Environmental variables including temperature, relative humidity, and wind speed were measured with a portable pocket weather meter (Kestrel 3000). Soil moisture was measured with gypsum blocks, the average soil moisture was constantly high (> 98 % soil moisture availability) for both years and there were no differences among sites. Due to the lack of changes in this variable, it was not introduced into the model and will not be discussed further.

3.3.5. Data analysis

All data collected during the 2003 and 2004 study periods were analyzed using the Statistical Analysis System (SAS Inc. 2000), MINITAB Release14 (Minitab Inc.) and S-plus (Insightful Corporation, 1998). Statistical significance was reported at α-level = 0.05. Stomatal conductance and photosynthetic rate were analyzed using the general mixed effects models. Means of gas exchange parameter averaged over the entire monitoring season were compared by Tukey's multiple range tests. Diurnal gas exchange data measured in 2003 and 2004 were used to construct site-specific empirical models of stomatal conductance for each year. The resulting models were compared in order to determine those patterns in gas exchange that were due to the local environmental conditions, wind speed, precipitation, temperature, plant-specific physiological data and elevation which were used as input parameters. The years 2003 and 2004 had greater than average precipitation. Therefore the models do not account for stomatal closure due to drought stress, but do help explain variations induced by other environmental factors.

For ozone uptake calculations, g_s was derived from those values measured in the field under conditions assumed optimal for g_s and pooled to give an average g_s corresponding to different spatial locations ignoring obvious outliers. The model was developed to calculate site-specific ozone fluxes to the canopy using ozone concentration at the 1-2 m height.

3.4. Results

3.4.1. General considerations

Due to the small differences in the measured soil moisture at the study sites, stomatal conductance response to soil moisture deficit could not be parameterized into the model. Results of the soil tests at the 20 study sites revealed that parameters measured at higher elevations were slightly different from those measured at sites situated at lower elevation, but the difference was not significantly different at a 0.05 level for any parameter (Table 12). However the elements Ca and N were statistically significant sources of variation for g_s and were therefore introduced into the general model of stomatal conductance. Wind speed and relative humidity were recorded at every site during gas exchange measurements these two environmental factors were not significant sources of variation for g_s and therefore were not used in the g_s model.

During both years of investigation (2003 and 2004) plant species, time of the measurement, elevation, and air temperature were significant sources of variation in stomatal conductance. However these four parameters together explained less than 35 % of the variation (Table 12). Regardless of elevation, time of measurement, and year, overall the cuttings of the hybrid poplar showed higher stomatal conductance rate than the seedlings of black cherry (Figure 18 a, b). The difference between average stomatal conductance for black cherry and hybrid poplar was not significant in 2003 ($t = -1.31$, $p = 0.189$, $DF = 424$), and significant in 2004 ($t = -6.81$, $p < 0.001$, $DF = 551$). During both seasons of the investigation, stomatal conductance was positively correlated ($\rho = 0.225$, $p < 0.001$ in 2003) ($\rho = 0.542$, $p < 0.001$ in 2004) to the photosynthetic rate (Figure 21 a, b). Net photosynthetic rates were significantly different in both years between the two tree species. Hybrid poplar exhibited higher P_n rates than black cherry regardless time of the measurement (Figure 21). Both plant species showed a significant decrease in photosynthetic rates on a daily basis, P_n rates were higher in the morning than at noon and in the afternoon (Figure 21). During both years of study g_s was negatively correlated with Vpdl ($\rho = -0.302$, $p < 0.001$ in 2003) ($\rho = -0.201$, $p < 0.001$ in 2004) (Figure 20). There were no decreasing trends in P_n and g_s rates as the seasons progressed (Figures 23 and 24) ; during both seasons of the study gs and P_n for non-symptomatic leaves were higher in August than in July (Figure 26).

The significant positive correlation between air temperature and Vpdl for both years of the study ($\rho= 0.370$, $p < 0.001$ in 2003 and $\rho= 0.611$, $p < 0.001$ in 2004) showed that in the absence of soil water stress, plants are still stressed by the high Vpld. Because of the high Vpdl plants indirectly respond to temperature stress by stomatal closure (Figure 20). The P_n/g_s ratio showed a significant decline from the morning measurements to the noon and afternoon measurements for both plant species equally. There were no significant differences in the P_n/g_s ratio between the two plant species in 2003 ($t = 0.27$, $p = 0.784$, $DF = 424$), but differences were significant in 2004, black cherry seedlings had higher P_n/g_s ratio ($t = 4.06$, $p < 0.001$, $DF = 524$) (Figure 22).

3.4.2. *Seasonal variation in stomatal conductance*

During both years of investigation the date in the season was a significant source of variation in stomatal conductance of both species grown in different environments ($p < 0.001$). During both seasons of the investigation there were no obvious trends of decreasing stomatal conductance rates toward the end of the season (Figure 23). When analyzed separately, only injured leaves showed a slight but not significant decrease of stomatal conductance rates as the 2004 season progressed.

During the 2003 study season there were no repeated measurements at any site. Both site and date in the season when the measurements were taken were statistically significant sources of variance for the g_s, but they were confounded factors. Their effects were hardly distinguished from each other. During the 2004 study season there were two measurements per each site, the first measurement was taken at the beginning of the season (June, mid July) and a second measurement was taken at the end of the season (mid July, August). Site and measurement date were significant sources of variance for the g_s, but there was no decreasing trend toward the end of the season (Figure 23).

Due to low ozone exposures that were characteristic for the two years of the study, the deleterious effect of cumulative ozone was not observed. On the contrary, monthly means of g_s were higher in August than in July or June. During both years of the study, time of the day was a significant source of variation in stomatal conductance; g_s values were lower in the morning and higher later in the day (Figure 25).

During the 2003 study season there was a general trend of greater stomatal conductance at high elevation sites than at low elevation sites. During the 2004 season the observed trend was opposite of that observed in 2003, with greater stomatal conductance being observed at low elevation sites for both plant species (Figure 18).

3.4.3. *Inter-annual differences in daily conductance*

Seasonal means of g_s were higher in 2004 than in 2003 except for maximum daily g_s values, which were higher in 2003. At the end of both growing seasons (August), mean daily g_s values were also higher in 2004 than in 2003 (Figure 25).

3.4.4. Estimation of the stomatal conductance model

Diurnal gas exchange data measured in 2003 and 2004 were used to construct the empirical model of g_s. A General Linear Model (GLM) was used to determine those environmental and physiological parameters which were statistically significant. The models were parameterized only for fully exposed leaves of the seedlings with a healthy appearance. Due to the significant yearly variances in measured g_s, a different model was considered for each year of the study.

Although the weather related conditions were different between the two years of the study the parameterized models exhibited only small differences from one year to the other. The general g_s model contained the same parameters but the coefficients were different for each year due to the division of the data in two separate sets. When all the data were combined the model lost half of its predicting capabilities.

The models for both years explained 57% percent of the variation observed in measured g_s (Table 13).

3.4.5. Estimation of stomatal uptake of ozone

Time specific cumulative ozone uptake was calculated for each tree species as the product of the g_s mean values for different intervals of the day; that is morning, noon and afternoon, with seasonal cumulative ozone recorded for the same time intervals. Night-time ozone uptake was considered to be negligible. The following formula was used to calculate ozone flux at each one of the 20 locations selected for this study:

$F_{O3} = c_1 * g_{s1} + c_2 * g_{s2} + c_3 * g_{s3}$

F_{O3} is the ozone flux,

c_1 is the ambient cumulative ozone for interval 1 (from 8 AM to 12 AM),

c_2 is the ambient cumulative ozone for interval 2 (from 12 AM to 4 PM),

c_3 is the ambient cumulative ozone for interval 3 (from 4 PM to 8 PM),

g_s is the average stomatal conductance calculated for the same three intervals of the day.

Intensity maps of spatial distribution of the ozone flux were generated to analyze and understand the spatial pattern of the ozone flux and then compared with the spatial pattern of cumulative ambient ozone within the study area (Figures 27-32).

3.4.6. Visible foliar ozone induced injury

Ambient ozone concentrations were significantly lower during 2003 and 2004 than those that usually occur during summer months in north-central Pennsylvania. Nevertheless they were sufficient to induce typical adaxial foliar symptoms on cherry and poplar during both years of investigation. Initial injury was recorded on July 22 in 2003, and on August 4 in 2004 on both tree species. For the 2003 season, black cherry exhibited a higher percentage injury than hybrid poplar but in 2004 hybrid poplar exhibited a higher percentage injury than black cherry (Figure 37). During both seasons more foliar injury was observed at higher elevation sites than at lower elevation sites (seasonal average). Overall during 2003 average percent injury across all sites for cherry was 2.5% at low elevation and 6% at high elevation, for hybrid poplar 0.25% at low elevation and 2.2% at high elevation. During the 2004 season, average percent injury across all sites at low elevation was 3.2% for cherry and 4.2% for poplar. At high elevation sites average percentage injury was 1.8% for cherry and 10.2% for poplar. The end of the season visible foliar injury better correlated with cumulative seasonal exposures expressed as SUM40, than with SUM0 or SUM60 (Table 14). Therefore SUM40 was used to calculate maps of ozone exposures rather than SUM0 or SUM60.

3.5. Discussions

There is no one model to fully explain stomatal behavior. The best way to determine canopy uptake is by direct measurements of stomatal conductance, but this is not possible for the majority of the stands. Therefore estimates of ozone uptake are needed (Grulke *et al.*, 2002). In most studies to estimate ozone uptake into plants, different models were used and cumulative O_3 uptake (CUO_3) was calculated as the uptake rate integrated over time (by multiplying the average ozone concentrations with stomatal conductance and the duration of the experiment). The most used formula was $F_{O3}=(c_a-c_i)\times g_c\times 0.613$, where

F_{O3} is the ozone flux or uptake rate, c_a the ambient ozone concentration, c_i the internal ozone concentration (approx. zero), g_s the stomatal or whole-tree canopy conductance, and 0.613 is a conversation factor accounting for the lower diffusivity of ozone relative to water vapor in the air. In some studies when the ozone was measured at a different level than the stomatal conductance, some adjustments to the measured ozone concentrations were necessary. For this study, tree foliage was sampled between 1 and 1.5 m from the ground, ozone concentrations were measured at 1.5 m, therefore no adjustments were made to the measured ozone concentrations.

Our study showed that there was a significant diurnal variation in g_s, a significant variation in g_s between the two plant species, and also a significant variation in g_s between the two elevation categories. Therefore for ozone uptake estimations these three factors were introduced into the general equation of g_s.

Seasonal means of g_s were higher in 2004 than in 2003. Plant species tend to adapt to environmental conditions by physiological adjustments at different time scales. Therefore higher g_s during 2004 were possibly a consequence of the adaptation to the new environment. The tree seedlings were established in the early spring of 2003 with a prior development under green house optimal conditions. The sudden exposure of the seedlings to the new environmental conditions might have caused a decrease in the metabolic rates.

An alternative explanation of the inter-annual differences in g_s relates to the difference in average temperatures between the two summers of the study. During the summer of 2004 the temperatures were significantly lower than during the summer of 2003. During day-light hours, higher temperatures induce higher Vpdl pressure deficit and as a consequence plants lower they stomatal conductance even in conditions of higher soil moisture. Soil moisture is one the most important parameter in regulating stomatal opening/closure and therefore ozone flux into the plants (Hildebrand *et al.*, 1996; Schaub *et al.*, 2003) but the results of the present study showed that in conditions of high soil moisture availability there are other factors regulating stomatal opening/closure.

The significant variation in g_s between the two plant species indicate that if the actual ozone impact on different receptors is to be assessed, a very detailed database concerning the physiological behavior of individual species is needed, not only including the variations in g_s induced by the environment but also its intraspecific variability. Therefore our modeling approach to estimate ozone flux across north-central Pennsylvania for juvenile black cherry and hybrid poplar integrates the intraspecific variability observed during this study.

The model incorporates the effects of sites-specific environmental factors, Vpdl pressure deficit and photosynthesis. The environmental regulation of the g_s resulted in significant diurnal, annual and spatial variation. Stomatal conductance showed similar daily patterns during both seasons; higher during mid-day and lower during morning and afternoon. Significant variation in stomatal behavior was found between plants at low versus high elevation, but the differences were inconsistent from one year to the next. The difference in elevation between sites located at high versus low altitude was usually less than 400 m. Therefore we assumed that the observed differences in stomatal behavior were most likely due to the heterogeneous environmental conditions among locations; air temperature, wind speed, relative humidity and possibly their interactions.

Nevertheless, in this study the environmental parameters used to model g_s resulted in low R^2 values ($R^2 < 0.35\%$) with the performance of the model significantly increasing when environmental and physiological parameters were combined ($R^2 = 0.57\%$). However the g_s model including all environmental and physiological significant parameters explained only 57% of the stomatal variation. This indicates that if the impact of ozone on the vegetation is based on estimations of ozone uptake, then uncertainties are to be expected, that further reduce the predictive capability of the approach.

Vpdl and P_n were highly correlated with stomatal conductance and proved to be significant sources of variance for the g_s. Regardless of the observed periodicity of the stomatal aperture, our study showed that models of g_s that do not include Vpdl and P_n are incomplete. In a study investigating the relevance of critical ozone levels for acute injury on plants, Karlsson *et al.* (2003) showed that using a stomatal conductance model driven by solar radiation, air temperature and Vpdl, the correlation between modeled and observed effects were considerably improved.

Stomatal conductance and net photosynthesis have been shown to be strongly and positively correlated to one another (Kozlowsky *et al.*, 1991). Empirical models that assume leaf conductance and photosynthesis linked and not functioning independently have been developed (Ball *et al.*, 1987). In our study there was a strong increasing linear relationship between P_n and g_s during both study seasons. These two physiological functions are so strongly related that is difficult to distinguish their individual effects. Although P_n and g_s are related, stomatal function can be impaired by ozone, which uncouples CO_2 uptake and consequently photosynthetic response from stomatal conductance (Tjoelker *et al.*, 1995). However the effects of the ozone-induced reductions in photosynthesis cannot always be attributed strictly to changes in stomatal conductance (Pell *et al.*, 1992, Clark *et al.*, 1996). Furthermore caution must be exercised in generalizing conductance models.

Ozone flux into the plants can cause biochemical changes that may result in decreased photosynthesis or decreased stomatal conductance. Due to low ozone exposures, which were characteristic for the two years of the study, the deleterious effect of cumulative ozone on g_s was not observed. To the contrary, monthly means of g_s were higher in August than in July or June. Physiological activity and conductance decline with leaf age for *Populus tremuloides* and ozone exposure usually accelerates the decline (Clark *et al.*, 1996). During both seasons of the investigation there were no trends of decreasing stomatal conductance rates toward the end of the season as leaves aged. Our results showed that even for ozone injured leaves, P_n and g_s rates only slightly declined toward the end of the season.

The role that Vpdl plays in limiting g_s has been substantiated for other species (Emberson *et al.*, 2000). They concluded that Vpdl was the main variable limiting g_s and hence ozone flux. High air temperatures are usually associated with high ozone exposures, providing an additional explanation for stomatal closure during high-ozone episodes. Under high temperatures, even well-watered plants are mildly stressed as a consequence of the high Vpdl (Filella *et al.*, 1998). The g_s seasonal values were lower in 2003 with higher temperatures recorded on the days of the measurements than in 2004, when the temperatures were significantly lower.

Even low ambient ozone concentrations can injure seedlings of sensitive tree species such as black cherry and poplars and induce physiological deleterious effects (Lee *et al.*, 1999). Overall, average ambient ozone concentrations in north-central Pennsylvania were slightly greater in 2003 than in 2004 but significantly lower than in previous years. Nevertheless ozone concentrations were sufficiently high during both seasons to induce typical ozone injury on both plant species.

The two tree species used in this study showed significant differences in P_h and g_s rates during both seasons. Plant species was a significant source of variation for g_s, but the differences in sensitivity of the two species could not be totally explained through their differences in stomatal behavior. Stomatal conductance, and therefore ozone flux, was always greater for hybrid poplar than for cherry, but the amount of injury induced by ozone was not consistent with this pattern. The percent of visible ozone injury was greater for cherry in 2003 and greater for poplar in 2004. The lack of correspondence between species differences in g_s and visible foliar injury was also reported by Kouterick *et al.* (2000) further supporting the concept that visible foliar symptoms not always relates to levels of ambient ozone.

The final goal of the study was to relate ozone foliar injury with ozone uptake/exposures. Therefore several parameters that may relate to plant defense mechanisms P_n/g_s ratio were also evaluated. Lower P_n/g_s may be consistently associated with greater visible ozone injury due to decreased defense capacity injury (Wei *et al.*, 2003). The difference in P_n/g_s ratio was significant between the 2 years of the study and corresponded to the amount of injury observed for the two plant species. P_n/g_s ratio was greater in 2003 when the amount of injury was lower and the P_n/g_s ratio was lower in 2004 when the amount of injury was greater. Between plant species the P_n/g_s ratio was always greater for the species expressing less injury. If the P_n/g_s ratio accounts for the defense mechanisms at the cellular level, our study shows that differences observed in ozone-induced foliar injury between the two species might be explained by the differences in P_n/g_s ratio, and that P_n/g_s ratio might be an important factor of plant defense against the deleterious effects of ozone.

During both years of the study, time of the day was a significant source of variation in stomatal conductance, g_s values were always lower in the morning when the plants defense mechanisms (Massman *et al.*, 2000) were higher, and higher later in the day when the plant defense mechanisms were lower. This fact, coupled with the daily behavior of the ozone that exhibits lower values in the morning and higher values late in the afternoon, might result in more plant injury at those sites where ozone exposures are very high in late afternoon. The lack of temporal coherence between maximum g_s and maximum ambient ozone concentration was taken into account when calculating ozone uptake.

If ozone uptake is calculated as the product of the seasonal average of g_s with cumulative ambient ozone, then ozone uptake into the vegetation is overestimated. Therefore in this study a weighted flux formula was used to adjust for the temporal differences in the diurnal stomatal conductance values and diurnal ozone concentrations. Modeled flux was used to map mean seasonal ozone uptake for individual species as compared with maps describing the cumulative ozone exposures. The intensity maps of estimated cumulative ozone concentrations, expressed as SUM40 and estimated ozone flux for both tree species, showed that there is a high degree of association between ozone flux and ambient ozone concentrations (Figures 27-32). This suggests that if soil moisture is not a limiting factor for stomatal conductance, then it is relatively simple to associate ozone exposures with ozone flux. Because descriptions of ozone flux-response relationship require site-specific conditions combined with plant species physiological properties, a generalized large scale flux model might be more difficult to construct. The degree of uncertainty of the flux model depends on the predictions of many environmental variables, as well as on the plant defensive mechanisms which must be better understood. This study also suggests that the AOT40 index as currently used in Europe as a descriptor of ozone exposures (Karlsson *et al.*, 2004) can be a reliable predictor of ozone stress during conditions when there are no soil water limitations.

3.6. Conclusions

Plant responses to ozone are highly dependent upon flux but there are many other factors that influence plant response. The model used in this study for estimating g_s included time of the measurement, elevation, air temperature, and soil characteristics as environmental variables. It also used Vpld and P_n as plant specific physiological factors. This study revealed that environmental factors play an important role in variability of stomatal conductance. Furthermore, our data showed that the diurnal variation in stomatal conductance plays an important role in determining ozone flux into the plants. The direct cause-effect relationship between ozone uptake and ozone injury cannot be completely understood without a consideration of plant defense mechanisms. The P_n/g_s ratio might be a measure of plant defense against ozone, but further studies are required before this parameter can be introduced into the ozone flux models.

References:

Ball, J.T., Woodrow, I.E., Berry, J.A., 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In: Biggins, I. (Ed.), Progress in Photosynthesis Research, Vol. IV. Proceedings of the International Congress on Photosynthesis. Martinus Nihjoff, Dordrecht, 221-224.

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

Berrang, D., Karnosky, D.F., and Bennett, J.P., 1991. Natural selection for ozone tolerance in *Populus tremuloides*: an evaluation of nationwide trends. Canadian Journal of Forestry Research 21, 1091-1097.

Campbell, G.W., Stedman, J.R., Stevenson, A, 1994. A survey of nitrogen dioxide concentrations in the United Kingdom using diffusion tubes, July–December 1991. Atmospheric Environment 28, 477–486.

Clark, C.S., Weber, J.A., Lee, E.H., Hogsett, W.E., 1996. Reducttions in gas exchange of *Populus tremuloides* caused by leaf aging and ozone exposures. Canadian Journal of Forest Research 26, 1484-1391.

Davis, D.D., Miller, C.A., Coppolino, J.B., 1977. Foliar response of eleven woody species to ozone with emphasis on black cherry. Phytopathology, 67, 185-189.

Davis, D.D., Umbrach, D.M., and Coppolino, J.B., 1981. Susceptibility of tree and shrub species and response of black cherry foliage to ozone. Plant Disease 65, 904-907.

Davis, D.D. and Skelly, J.M., 1992. Foliar sensitivity of eight eastern hardwood tree species to ozone. Water, Air, and Soil Pollution 62, 269-277.

Emberson, L.D., Ashmore, M.R., Cambridge, H.M., Simpson, D., Tuovinen, J.P., 2000. Modelling stomatal ozone flux across Europe. Environmental Pollution 109, 403–412.

Ferdinand, J.A., Fredericksen, T.S., Kouterick, K.B., and Skelly, J.M., 2000. Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. Environmental Pollution 108, 297-302.

Filella, I., Llusià, J., Piñol, J., and Peñuelas, J., 1998. Leaf gas exchange and fluorescence of Phillyrea latifolia, Pistacia lentiscus and Quercus ilex saplings in severe drought and high temperature conditions. Environmental and Experimental Botany 39, 213-220.

Fredericksen, T.S., Joyce, B.J., Skelly, J.M., Steiner, K.C., Kolb, T.E., Kouterick, K.B. Savage, J.E., and Snyder, K.R., 1995. Physiology, morphology and ozone uptake of leaves of black cherry, samplings and canopy trees. Environmental Pollution 89, 273-283.

Fredericksen, T.S., Skelly, J.M., Steiner, K.C., Kolb, T.E., Kouterick, K.B., 1996. Size-mediated foliar response to ozone in black cherry trees. Environmental Pollution 91, 53-63.

Fowler, D., Cape, J.N., Coyle, M, Flechard, C., Kuylenstierna, J.,Hicks., K, Derwent, D., Johnson, C., Stevenson, D.,1999. The global exposure of forests to air pollutants. Water Air and Soil pollution 116, 5-32.

Fuhrer, J., 2000. Introduction to the special issue on ozone risk analysis for vegetation in Europe. Environmental Pollution 109, 359-360.

Grulke, N.E., Preisler, H.K., Rose, C., Kirsh, J., Balduman, L., 2002. Ozone uptake and drought stress affects on carbon acquisition of ponderosa pine in natural stands. New Phytologist 154, 621-631.

Grünhage, L., Jäger, H.J., Haenel, H.D., Löpmeier, F.J. and Hanewald, K., 1999. The European critical levels for ozone: improving their usage. Environmental Pollution 105, 163–173.

Hildebrand , E.S., Skelly, J.M., and Frederiksen, T.S., 1996. Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. Canadian Journal of Forest Research 26, 658-669.

Horsfall J.G. and Barratt, R.W., 1945. An improved grading system for measuring plant disease. Phytopatology **35**, pp. 655–660.

Jarvis, P.J., 1976. The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. Phil. Trans. Regal Society London 273, 593–610.

Karlsson, Pihl G., Karlsson, P.E., Danielsson, H., Pleijel, H., 2003. Clover as a tool for bioindication of phytotoxic ozone—5years of experience form southern Sweden—consequences for the short-term critical level. Science of the Total Environment 301, 205–213.

Karlsson, G.P., Karlsson, P.E., Soja, G., Vandermeiren, K., Pleijel, H., 2004. Test of short-term critical levels for acute ozone injury on plants-improvements by ozone uptake modeling and the use of an effect threshold. Atmospheric Environment 38, 2237-2245.

Karnosky, D. F., Gagnon, Z.E., Dickson, R.E., Coleman, M.D., Lee, E.H., and Isebrands, J.G., 1996. Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. Canadian Journal of Forest Research 26, 23-37.

Kauterick, K.B., Skelly, J.M., Fredericksen, T.S., Steiner, K.C., Kolb, T.E., Ferdinand J.A., 2000. Foliar ozone injury, leaf gas exchange and biomass response to ozone among black cherry (*Prunus serotina*) genotypes. Environmental Pollution 107, 117-126.

Kozlowski, T.T., Kramer, P.J., and Pallardy, S.G., 1991. The physiological ecology of woody plants. Academic Press Inc., New York, NY, pp. 657.

Krupa, S.V., Tonnejkck, A.E.G. and Manning, W.J., 1998. Ozone. Pp.2-1 to 2-23, in R.B.Flagler *et al.* (Eds.). Recognition of Air Pollution Injury to Vegetation: A pictorial atlas. Air and Waste Management Association, Pittsburgh, PA.

Lambers, H., Chapin, F.S., Pons, T.J., 1998. Plant Physiological Ecology. Springer-Verlag, New York Inc., USA.

Lee, J.C., Skelly, J.M., Steiner, K.C., Zhang, J.W., and Savage, J.E., 1999. Foliar response of black cherry (*Prunus serotina*) clones to ambient ozone exposure in central Pennsylvania. Environmental Pollution 105, 325-331.

LiCor Bioscience, 4441 Superior St. Lincoln, NE, USA.

Long, R.P. and Davis, D.D., 1999. Growth variation of white oak subjected to historic levels of fluctuating air pollution. Environmental Pollution, 106, 193-202.

Manning, W.J., 2003. Detecting plant effects is necessary to give biological significance to ambient ozone monitoring data and predictive ozone standards. Environmental Pollution 126, 375-379.

Massman, W.J., Musselman, R.C., and Lefohn, A.S., 2000. A conceptual ozone dose-response model to develop a standard to protect vegetation. Atmospheric Environment 34, 745–759.

Minitab Inc., Quality Plaza, 1829 Pine Hall Road, State College, Pennsylvania, 16801, USA.

Mott, A., and D.F. Parkhurst. D.F., 1991. Stomatal responses to humidity in air and helox. Plant, Cell and Environment 14, 509-515.

Musselman, R.C., Massman, W.J., 1999. Ozone flux to vegetation and its relationship to plant response and ambient air quality standards. Atmospheric Environment 33, 65-73.

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

Nussbaum, S., and Fuhrer, J., 2000. Difference in ozone uptake in grassland species between open-top chambers and ambient air. Environmental Pollution 109, 463-471

Ogawa & Company, USA, Inc., 1998. NO–NO_2 simultaneous sampling protocol using Ogawa sampler. Pompano Beach, FL.

Patterson, M.C., Samuelson, L., Somers, G., and Mays, A., 2000. Environmental control of stomatal conductance in forest trees of the Great Smoky Mountains National Park. Environmental Pollution 100, 225–233.

Pell, E.J., Eckardt N. and Enyedi, A.J., 1992. Timing and ozone stress and resulting status of ribulose bisphosphate carboxylase/oxygenase and associated net photosynthesis. New Phytologist 120**,** 397–405.

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

SAS Institute Inc., 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, SAS Institute Inc., Cary, NC, 1686 pp.

Schaub, M., Skelly, J.M., Steiner, K.C., Davis, D.D., Pennypacker, S.P., Zang, J., Ferdinand, J.A., Stevenson, R. E., Savage, J. E., 2003. Physiological and foliar injury of *Prunus serotina, Fraxinus Americana* and *Acer rubrum* seedlings to varying soil moisture and ozone. Environmental Pollution 124, 307-320.

Skelly, J.M., Chappelka, A.H., Laurence J.A., Fredericksen T.S., 1997. Ozone and its known potential effects on forests in eastern United States. In forest decline and Ozone, a comparision of controlled Chamber and field experiments. Berlin, Springer-verlag, 69-93.

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

S-PUS 6 for Windows, Insightful Corporation, Seattle, WA, USA.

St.Clair S.B., and Linch L.P., 2004. Element accumulation patterns of deciduous and evergreen tree seedlings on acid soils: implications for sensitivity to manganese toxicity. Tree physiology 25, 85-92.

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

Tjoelker M.G., Volin J.C., Oleksyn J. and Reich P.B., 1995. Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. Plant Cell and Environment 18, 895-905

Uddling, J., Günthardt-Goerg, M.S., Matyssek, R., Oksanen, E., Pleijel, H., Selldén, G., and Karlsson, P. E., 2004. Biomass reduction of juvenile birch is more strongly related to stomatal uptake of ozone than to indices based on external exposure. Atmospheric Environment 38, 4709-4719.

U.S. Environmental Protection Agency (US EPA), 1996. Air quality criteria for ozone and related photochemical oxidants. Vol.1 of 3 reports no EPA/600/P-93/004aF. Research Triangle Park, North Carolina.

U.S. Environmental Protection Agency (US EPA), 1996a. Air quality criteria for ozone and related photochemical oxidants. Office of Research and development, Washinghton, D.C. EPA/600/P-93/004a-cF.

Wei, C., Skelly, J.M., Pennypacker, S.P., Ferdinand, J.A., Savage, J. E.,Stevenson, R. E., Davis, D.D., 2004. Influence of light fleck and low light on foliar injury and physiological responses of two hybrid poplar clones to ozone. Environmental Pollution 130, 215-227.

Wieser, G. and Havranek, W. M., 1995. Environmental control of ozone uptake in *Larix decidua* Mill.: a comparison between different altitudes. Tree Physiology 15, 253-258.

Woodbury, P. B., Laurence, J. A., and Hudler G. W., 1994. Chronic ozone exposure alters the growth of leaves, stems and roots of hybrid *Populus*. Environmental Pollution 85(1), 103-108.

Yuska D. E., J. M. Skelly, J. A. Ferdinand, R. E. Stevenson, J. E. Savage, J. D. Mulik and A. Hines, 2003. Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania. Environmental Pollution 125, 71-80.

Zierl, B., 2001. A water balance model to simulate drought in forested ecosystems and its application to the entire forested area in Switzerland. Journal of Hydrology 242, 115–136

CHAPTER IV

The influence of ozone on the foliar symptoms and growth of *Prunus serotina* seedlings and *Populus maximiwizii* x *trichocarpa* cuttings

"Capsule": Ozone flux is more related to plant response than is ambient ozone concentration, but within heterogeneous environments ozone flux does not completely explain the high variation observed in visible injury.

4.1. Abstract

The influences of ozone on foliar symptoms and relative growth parameters of two ozone-sensitive species growing in different environmental regimes was investigated during the 2003 and 2004 growing seasons. Our main objective was to determine whether foliar injury expressed by genetically stable ozone-sensitive plants can provide quantitative information about ozone exposures. One-year-old seedlings of black cherry (R-12) (*Prunus serotina*) and ramets of hybrid polar (*Populus maximiwizii* x *trichocarpa)* clone USDA Forest Service NE388 were established in the spring of 2003 within 20 open plots ranging from low-to-high elevation, within central-Pennsylvania. Ambient ozone concentrations were measured weekly at the 20 sites using Ogawa passive ozone monitors and visible ozone-induced foliar injury was assessed weekly. Above-ground relative growth was measured at the end of the experiment as relative height (H) and basal diameter (D). During both years of investigation ambient ozone and environmental conditions were conducive to development of typical ozone-induced visible symptoms on both tree species. Percentage of total leaf area affected (expressed as percent injury %INJ) was positively correlated with cumulative ozone exposures. Foliar injury was better correlated with a flux-based approach than for an exposure-based approach. The best correlations in terms of exposure-based approach were obtained when the SUM40 ozone exposure index was used. More severe foliar injuries were observed on plants growing on plots located at higher elevation sites. The onset of foliar injury for both tree species was first observed at sites located at higher elevation during both years. At the end of the 2003 study season, black cherry seedlings expressed more foliar injury (7.28 % INJ) than did the hybrid poplar clone (1.73 % INJ). In 2004 the poplar clone seedlings expressed more foliar injury (15.42 % INJ) than did black cherry (7.32 % INJ). The inconsistent plant response to ozone exposures was likely due to the influence of environmental factors and possibly to plant defense mechanisms. We found that more study is required before making quantitative estimations of ozone exposures based on plant foliar injury.

Keywords: Ozone uptake, foliar injury, growth, bio-indicators

4.2. Introduction

Tropospheric ozone is an anthropogenical air pollutant that occurs in concentrations great enough to affect the health and productivity of forests throughout the northeastern Unites States (US EPA, 1996). Concentrations of ambient ozone have become, in the past decades, an increasing stress for forest health generating a potential for reducing biodiversity (Krupa and Manning, 1988; Krupa *et al.*, 1998). Ambient ozone has been demonstrated to cause a range of effects including visible leaf injury, growth and yield reduction, and altered sensitivity to biotic and abiotic stressors (Skelly *et al.*, 1987; Chappelka *et al.*, 1992; Fredericksen *et al.*, 1995). Increasing ozone exposure has been positively correlated to increasing foliar injury on sensitive species, with ozone-induced injury progressing as upper leaf surface pigmentation, (i.e., stippling) to premature leaf senescence. Adaxial leaf surface stipple has been described as the classic symptom of ozone injury on broadleaf species (Skelly, 2000). Premature leaf drop of the symptomatic leaves for individual species may occur as early as mid to late July depending upon ozone concentrations and local environmental conditions.

A strong correlation between increasing ozone exposure and symptom expression of sensitive plant species such as black cherry (*Prunus serotina* Ehrh.) and poplar species (*Populus* spp.) has been well documented under controlled and natural forest conditions within the eastern USA (Davis *et al.*, 1981; Davis and Skelly, 1992; Simini *et al.*, 1992; Davis *et al.* 1993; Fredericksen *et al.*, 1995; Hildebrand *et al.*, 1996; Karnosky *et al.*, 1996; Chappelka *et al.*, 1997). Hildebrand *et al.* (1996) found an exponential increase in foliar ozone symptoms with cumulative ambient ozone concentrations for black cherry in the Shenandoah National Park. Many results showed that foliar injury was positively correlated to cumulative ozone exposures, and that local site factors (e.g., edaphic factors, weather, and light regime) played a decisive role in determining plant response to ozone exposures (Hildebrand *et al.*, 1996; Schaub *et al.*, 2003; Wei *et al.*, 2004).

In order to examine the impact of pollutants on forest ecosystems, the USDA-Forest Service (USDA-FS) Forest Health Monitoring programs in the northeast USA have developed a series of observational surveys on native vegetation (Smith and Manning, 1990). A large part of the activity of these surveys has involved the use of tree and other native plant species that have been identified as sensitive to ozone and consequently used as bio-indicators of ozone exposures (Chappelka *et al.*, 1986; Davis and Skelly, 1992; Neufeld *et al.*, 1992, 1995). Advantages of native plants as bio-indicators are that they can be utilized even in the most remote forests, have biological relevance, and provide solid evidence of plant stress. There are, however, problems with assessing the impact of ozone on naturally growing bio-indicator populations. To be a good indicator a plant species should exhibit a distinct, verified response, have a few or no confounding disease or pest problems, and exhibit genetic stability. The last requirement is seldom accomplished when working with natural populations. Therefore, it is more useful to utilize sensitive perennial bio-indicators of ozone air pollution in northeastern forests such as black cherry and hybrid poplar (Davis and Skelly, 1992; Simini *et al.*, 1992).

Leaf symptoms induced by ambient ozone in natural forests have been increasingly reported but few effects on tree growth have been documented. Since forests are continuously exposed to ozone, foliar injury and growth reduction for ozone sensitive species are serious issues. Predictions about the effects of the ozone on tree growth suggests that growth reduction for some ozone sensitive species in the eastern USA might be as high as 12% (Chappelka and Samuelson, 1998; Ollinger *et al.*, 1997), however the causal role of ozone in reducing radial growth of the trees has not yet been demonstrated (Manning, 2004). Verifying the actual loss in forest conditions is difficult and most of the research has focused on controlled ozone exposures. Studies are needed to establish if exposure to ambient ozone actually affects tree growth (Sandermann *et al.*, 1997; Chappelka and Samuelson, 1998, Karlssson *et al.*, 2003). Given the many interacting variables involved, this is a difficult task. Some researchers have suggested that by a careful selection of the sites, tree, and ozone exposure the inherent variability might be reduced (Karlsson *et al.*, 2003, Manning, 2004).

The relationship between ozone exposures and induced effects on very sensitive plants, often have been studied employing exposure chambers and laboratories. Field studies were limited to open-top chambers (OTC), therefore controlled environments, or reduced to one site location. Extrapolations from exposure chambers to differing field condition are difficult. Therefore field studies allowing exposure of plants to natural environmental conditions and to the effect of fluctuating ozone concentrations constitute the ideal alternative.

In the present study we investigated the effects of differing environmental factors on the response of sensitive tree species exposed to fluctuating ambient ozone exposures over two growing seasons. Our objectives were: (1) to determine whether genetically stable populations of ozone-sensitive plant species are more reliable as bio-indicators than natural populations (in terms of similar response to similar exposures), (2) to examine the effects of environmental conditions on the response of two bio-indicators to ozone, (3) to determine the effects of combined ozone exposures and environmental factors on growth parameters of sensitive tree species, and (4) to investigate if foliar injury can provide quantitative information about ozone exposures.

4.3. Materials and methods

4.3.1. General considerations

The goal of this study was to determine if visible foliar injury in conjunction with genetic, environmental, and edaphic factors, might serve as an indication not only of ozone stress but also as a tool to quantify ozone exposures. We studied the possibility that a 2-year field study of ozone effects on two very sensitive genotypes of ozone bio-indicators, planted in a number of different environments along an ozone gradient, may provide enough information to quantitatively assess air quality for ozone exposures based upon foliar injury. Furthermore, to determine or to predict the actual effects of ambient ozone on plants and to develop air quality indices to protect vegetation, we studied the relationship between ozone exposures and tree growth. Using ozone-sensitive trees of the same age with genetic uniformities, we tried to quantify the adverse effects of ambient ozone on relative growth. The effect of ozone on seedlings survival and competitive ability is important for regeneration of forest trees. Our study provides information on how ambient ozone and environmental factors interact to affect seedling development in uncontrolled field conditions.

4.3.2. Study sites

The study was conducted at 20 research sites located in clearings within forested areas in rural north-central Pennsylvania. Past studies (Skelly *et al.*, 2001; Yuska *et al.*, 2003) showed that ozone exposures in north-central Pennsylvania were highly correlated with elevation. Consequently an ozone gradient was indirectly induced by the different elevations in the study area. Therefore we selected two relative elevation categories with ten sites established at higher elevations (> 550 m), and 10 sites at lower elevation (< 350 m) (Table 1). At each site five seedlings of black cherry and five cuttings of hybrid poplar were planted in early spring of 2003; 10 plants were established in natural, unfertilized soil in two concentric circles within an 8 foot diameter plot which was fenced for protection against rodents and deer. Seedlings and cuttings were watered at the time of planting; thereafter they were exposed only to natural conditions for the next 2 years. The 2003 study was conducted during a 13-week period, from June 9 to September 9. The 2004 study was conducted during a 14-week period, from May 23 to September 2.

4.3.3. Plant material

In March of 2003 ramets of hybrid poplar were started by cutting dormant, upper crown scionwood with viable buds. One-year old, 3-10 mm diameter, branches were collected from clonal outplantings in the field. Branches were cut into 15-cm sections and inserted 10 cm deep into 15 cm diameter plastic pots containing Metro-Mix 250. Potted ramets were maintained in the greenhouse until planting in May 2003.

All cherry seedlings were derived from open-seeds collected in the fall of 2001 from a single mother tree within a local black cherry seed orchard. The black cherry seedlings had been previously tested for ozone sensitivity under controlled conditions during 2002. The predominant symptom induced by the ozone under controlled conditions on black cherry seedlings was an adaxial light brown to black stipple. The percentage of leaves showing symptoms was recorded as amount of injury per plant (% AMT). The Horsfall-Barratt rating system (Horsfall and Barratt, 1945) developed for the Forest Health Expert System (Nash *et al.*, 1992) was used to assess the severity of injury of the affected leaves (% SEV) for symptomatic leaves on each plant. Injury was recorded as 0, 3, 6, 12, 25, 50, 75, 88, 94, 97, and 100% area affected. An injury index was calculated as (% INJ = % AMT * % SEV) to evaluate whole plant injury. Based on the response of the plants to ozone exposures, to increase uniformity among plots, and to establish an equal number of seedlings at each plot, the seedlings were classified as follows: Class 0, no visible symptom response to ozone exposures, (i.e., tolerant); Class 1, 1-6% INJ; Class 2, 7-15% INJ; Class 3, 16-30% INJ; and Class 4, more than 30% INJ, (i.e., very sensitive).

The classification had two goals, one goal being a possible comparison between responses observed during greenhouse exposures and the responses observed in the field under ambient ozone exposures and natural environmental conditions. The second goal was to gain uniformity among plots. The seedlings were tagged according to their sensitivity class and maintained under greenhouse conditions during the 2002/2003 winter. In May 2003 one seedling from each sensitivity class was planted within the enclosure at every plot.

4.3.4. Measurements

4.3.4.1. Ozone and environmental monitoring

During the 2003 and 2004 studies ambient ozone concentrations were measured at the canopy level with Ogawa passive ozone samplers (Ogawa and Co., Inc.). Passive samplers were positioned at each site on a steel pipe at 1.5-2 m above the soil surface and shielded from wind and rain under a 7.6 cm PVC cap. The accuracy of measurements was determined by comparing passive-sampler values to continuous hourly ozone values from the co-located API - Advanced Pollution Instrumentation - Model 400-A ozone monitor. The API continuous ozone analyzers were operated as real-time monitors at three air quality monitoring sites (Table 1). Ozone values were monitored at 5-min time intervals and finally recorded as 1-hr averages using Odessa Engineering DSM3260 data-logger model.

Weekly ozone mean concentrations were used to synthetically generate hourly values and determine SUM40, SUM60 and SUM80 (Chapter II) for each location. The SUM40 index is defined as sum of the positive differences between the hourly mean ozone concentrations at a threshold of 40 ppb. In general, AOT40 (sum of the positive differences between hourly means and 40 ppb, during daylight hours) ozone exposure index is shown to better correlate with foliar injury because it imposes a differential weighting on the peak values. However in this study we used passive samplers to assess ozone exposures and synthetically generated hourly values were used to determine SUM40.

The cumulative ozone exposures were used to calculate site-specific ozone fluxes throughout the study area (Chapter II).

Soil water availability was calculated from electrical conductance measurements with Gypsum Soil Moisture Probes (Soil Moisture Tester Model KS-D1, Delmohorst Instr. Co., Towaco, NJ, USA) at each plot. Rain was recorded at each site with rain gauges. Meteorological data inclusive of temperature (Temp., ^{0}C), relative humidity (RH, %), precipitation (mm), and wind speed (WS, $msec^{-1}$) were recorded during the 2003-2004 seasons at three air quality monitoring sites (Table 1). A Campbell meteorological data system (Campbell Scientific Inc., Logan, UT) was operated at each air quality monitoring site. To further characterize the sites soil samples were taken from each plot and analyzed for N, P, K, Mg, Ca and pH.

4.3.4.2. Visible foliar injury

Visible ozone-induced injury (%INJ) was rated on a weekly basis, at the same approximate hour of the day, coincident with the replacement of the Ogawa passive filter. Injury assessments were made from the time of full leaf expansion at the end of May until early in September. The percentage of leaves showing symptoms was recorded as amount of injury per plant (% AMT), with 5% increments. The Horsfall-Barratt rating system (Horsfall and Barratt, 1945) as modified by Nash *et al.*, (1992) was used to assess the severity of injury of the affected leaves (% SEV) for symptomatic leaves on each plant with observations recorded as 0, 3, 6, 12, 25, 50, 75, 88, 94, 97, and 100% area affected. An injury index was calculated as (% INJ = % AMT * % SEV) to evaluate whole plant injury.

4.3.4.3. Growth measurements

During the first week of September 2004, height (H) and basal diameter (D) of the main stem was measured on all seedlings. Stem diameter was measured to the nearest 0.01 mm using a digital caliper. Stem height was measured to the nearest 1 cm using a meter stick.

4.3.5. Data analysis

Data were analyzed using the Statistical Analysis System (SAS Inc. 2000), MINITAB Release14 (Minitab Inc.) and S-plus (Insightful Corporation, 1998). The relationship between visible ozone-induced injury and different exposure indices and/or ozone uptake was determined using Pearson's correlation index, and the level of significance was determined using the Student's t-test. All data were tested for normality and homogeneous variance. Leaf gas exchange data were analyzed using the General Linear Model (GLM) and ANOVA was used to test for significant differences in visible injury among sites for each year.

This study was also concerned with the development of predictive models to generate maps of visible foliar injury through the study region. Intensity maps (Figures 38-41) were generated to analyze and understand the correlation between ozone exposure/dose and foliar injury.

4.4. Results

4.4.1. Ozone exposures

A summary of ozone concentrations and cumulative rain recorded at each site during the two years of the study is presented in Table 15. Ambient ozone concentrations during both years of the study were lower than those usually expected for the north central Pennsylvania.

Weekly means of ozone concentrations were compared to determine significant differences in ozone levels among sites for each study year. There was not a significant difference between annual means of ozone in 2003 and 2004 (μ = 32.858 ppb, σ = 10.535 ppb) in 2003 and (μ = 33.142 ppb, σ = 5.192 ppb) in 2004. However there was a significant difference in ozone concentrations among sites for each year (Figure 33). ANOVA results showed that increased ozone concentrations were positively correlated with increased elevations. In 2003 there was a significant positive high correlation between seasonal ozone and elevation ($\rho = 0.877$, $p < 0.001$). In 2004 the correlation was also significant but less pronounced ($\rho = 0.518$, $p = 0.019$). Also in 2004 ozone concentrations decreased at higher elevation sites and increased at lower elevation sites compared to 2003 (Table 15).

The spatial trend of ozone exposures was different in 2003 compared with 2004, even if seasonal averages were not significantly different. In 2003 there were low ozone concentrations in the central part of the region surrounded by areas with higher ozone. In 2004 there was also lower ozone in the central part of the region but on a less extended area than in the previous year, resulting in more uniform ozone concentrations distributed throughout the entire region. The maps of seasonal ozone averages for individual years are presented in (Figures 34-35).

4.4.2. Environmental conditions and edaphic factors

Seasonal temperature averages monitored at the three air quality monitoring sites were slightly higher in 2003 than in 2004 (Table3). Continuous monitored air quality sites were fairly evenly distributed across the study area (one at each edge and one in the middle). We assumed that the pattern observed in seasonal means of the temperature, relative humidity and wind speed for the respective sites would be approximately similar for the study region.

Cumulative precipitations were also slightly higher in 2003 than in 2004, but were not significantly different ($t = 1.35$, $p = 0.192$, $DF = 33$). Soil water potential was at maximum values for both years of the study and not significantly different between years or among sites. Therefore we consider that for this particular study, soil water potential was not a factor determining the variation in visible ozone-induced foliar injury among plots.

The lowest soil pH value at the 20 study sites was 3.8 and the highest 7 (Table 11) which are within the typical range for north-central Pennsylvania soils. Sites situated at higher elevation showed a slightly lower pH than did sites situated at lower elevation. However the difference was not significantly different at a 0.05 level ($t = -1.86$, $p = 0.080$, $DF = 17$).

4.4.3. Visible foliar injury

During both years of the study ambient ozone concentrations were sufficiently high to induce typical adaxial brown stipple on black cherry and chlorotic spots and irregular brown-black necrotic spots on the hybrid poplar. Seasonal ambient ozone concentrations were positively correlated with visible ozone injury in both 2003 and 2004 (Table 14). The positive correlations between visible ozone injury and different ozone indices, for both tree species, were stronger in 2003 than in 2004. The SUM40 ozone index was slightly better correlated with visible injury than any other ozone indices.

Both tree species expressed more injury in 2004 although the ozone averages were only slightly higher compared to 2003. Cherry seedlings showed a similar seasonal average injury for both years (7.28% in 2003 vs. 7.32% in 2004), but the maximum observed injury was higher in 2004 (33.1% in 2003 vs. 45% in 2004), as calculated at the end of the season. In 2004 the incidence and severity of the necrotic spots on the hybrid poplar were significantly greater than in 2003, with premature senescence and leaf abscission observed later in the season (mid August). Poplar cutting showed a significantly higher difference in foliar injury for both, average and maximum observed injury, 1.73% in 2003 vs. 15.42 in 2004 in terms of average and 9% in 2003 vs. 69.33% in 2004 in terms of maximum injury, calculated at the end of the season.

The progress of site average injury (% INJ) for black cherry and hybrid poplar is presented within Figure 36. During both years of investigation both tree species growing at higher elevation sites showed more severe injury than those growing in plots located at lower elevation (Figure 37a). During both years of investigation there was significant difference in ozone exposures between sites located at higher elevation and sites located at lowerer elevation ($t = 4.62$, $p < 0.001$, $DF = 135$ in 2003 and $t = 2.30$, $p = 0.023$, $DF = 161$ in 2004.

In 2003, the initial injury was observed for hybrid poplar on July 22 and for black cherry on July 28 at higher elevation sites. At low elevation sites the first ozone induced foliar symptoms were recorded on August 11. In 2004 the initial onset of injury was observed on black cherry on June 30 and on poplar clones on July 20 at higher elevation sites. At low elevation sites first injury was observed on black cherry on July 21. During the 2003 season, average foliar injury for black cherry was greater than for poplar clone. In contrast, during 2004 the average foliar injury was greater for the poplar clone (Figure 37a). Average foliar injury for poplar was significantly greater in 2004 than in 2003 ($t = -3.07$, $p = 0.006$, $DF = 108$) and developed around the same date (July 20). For black cherry the average foliar injury was similar during the two study seasons ($t = -0.03$, $p = 0.974$, $DF = 141$), but developed earlier in the 2004 season (end of June) compared to

2003 (mid July). Results of site comparisons showed that in 2003 the highest seasonal injury combined for both species was recorded at Gleason site (17.3%). In 2004 the highest seasonal injury combined for both species was recorded at Moshannon site (54.37 %) (Figure 37b). When comparing sites from the same pair (high vs. low elevation) the results showed that during both seasons the highest seasonal injury was recorded at the high-elevation site of the pair regardless of the spatial location of the sites (Figure 37-c). When comparing the 10 pairs of sites the results showed that during both seasons at pair 2, 4, 8, and 9 (Table 1) was recorded the highest seasonal injury.

During both years of this study there were no significant differences in foliar injury in terms of class sensitivity of black cherry. The response to ambient ozone was uniform among seedlings within the same plot. Therefore our results do not allow comparisons between foliar injury responses observed during greenhouse CSTR exposures and foliar responses observed in the field under ambient ozone exposures and natural environmental conditions.

For both years of investigation visible foliar injury observed on black cherry was significantly and positively correlated with the P in the soil ($\rho = 0.584$, $p = 0.007$ in 2003; $\rho = 0.619$, $p = 0.004$ in 2004). None of the other soil parameters measured was correlated with visible ozone injury on either black cherry or hybrid poplar.

4.4.4. Ozone uptake and foliar injury

Gas exchange was measured at the leaf level under field conditions at constant CO_2 using a Li-Cor 6400 (Li-Cor Inc., Lincoln, NE) open-gas exchange photosynthesis system with an internal light source. The gas exchange measurements were taken in June-August of 2003 and 2004 at 12 different sites when the weather conditions were favorable (Table 10). All measurements were made only during optimal light and environmental conditions to avoid outliers and other values usually observed when measured under extreme ambient conditions. At each site all 10 seedlings were sampled three times a day. One measurement was taken between 0900h and 1100 h, a second between 1200 h and 1400 h and a third measurement taken between 1500 h and 1700 h. Diurnal gas exchange data measured on 2003 and 2004 were used to construct empirical models of stomatal conductance (g_s) for each year. The results showed that maximum g_s occurs at mid to late morning. Maximum ozone concentration occurs at mid to late afternoon. The lack of temporal coherence between maximum g_s and maximum ambient ozone concentration was taken into account when calculating ozone uptake. A weighted formula was used to adjust for the temporal differences in the diurnal stomatal conductance values and diurnal ozone concentrations (Chapter III).

Time specific cumulative ozone uptake was calculated for each species as the product of the g_s mean values for morning, noon and afternoon with seasonal cumulative ozone recorded for the same time periods. We assumed that nocturnal uptake was very low (therefore negligible) due to the general low ozone exposures during both years of the study with even lower values during night time.

The correlations between visible ozone injury and ozone uptake calculated with diverse ozone indices were stronger for both tree species when SUM40 was used as ozone index (Table 16). Correlations between visible ozone injury and ozone uptake, for both tree species, were stronger in 2003 than in 2004.

The spatial trend of ozone uptake was similar for both plant species but different between the two years of the study. The maps of seasonal ozone uptake for individual years and plant species are presented in (Figures 38-41).

4.4.5. Relative growth parameters

4.4.5.1. Black cherry

Relative growth (D*H) decreased slightly with increasing ozone exposures but the decrease was not statistically significant ($\rho = -0.303$, $p = 0.195$). The average stem diameter (D) and height (H) values were slightly greater for seedlings growing at the lower elevation sites, but the difference was also not significantly different. The results of the Two-sample T-test are: $t = -1.32$, $p = 0.203$, DF = 15 for diameter, and $t = -0.60$, $p = 0.556$, DF = 15 for height.

There were no significant effects of the measured soil parameters on the relative growth of the black cherry seedlings.

4.4.5.2. Hybrid poplar

Relative growth (D*H) decreased slightly with increasing ozone exposures, but the decrease was not significant (ρ = - 0.361, p = 0.117). The average stem diameter (D) and height (H) values were slightly higher for seedlings growing to lower elevation sites, but again the difference was not significant. The results of the Two-sample T-test are: t = -0.91 p = 0.384, DF = 10 for diameter, and t = -1.27 p = 0.232, DF = 10 for height. Stem diameter (D) and height (H) significantly increased with increasing calcium and pH; there were no significant effects of the other mineral nutrients present in the soil.

4.5. Discussion

Black cherry and poplar clone NE388 are among the most ozone-sensitive trees used as bio-indicators. The main goal of this study was to investigate the use of genetically stable bio-indicators for determining air pollution exposures based on the severity and the amount of injury expressed by biological indicators. Once achieved, these very sensitive plant species could therefore be planted and used as ozone monitors, rather than as indicators of ozone stress.

The range of seasonal ozone concentrations was greater in 2003 (23.3 to 47.7 ppb) than in 2004 (26.4 to 38.7 ppb). The seasonal average over the study area was slightly greater in 2004 (33.1 ppb) than in 2003 (32.8 ppb) but the differences were not significant. There were only a few episodic peaks above 80 ppb, usually occurring early in the growing season (June), which typically occur with a higher frequency during summer months in Pennsylvania (Simini *et al.*, 1992; Comrie, 1994, 1994a). The formation of ozone is generated photochemically from precursors such as NO_x and hydrocarbons, on hot sunny days. High concentrations of ozone are likely to occur during periods of high temperature and low relative humidity. Meteorological data showed that in 2003 and 2004 average temperatures were lower than those typical for central Pennsylvania and hence were less conducive to ozone build-up. Therefore, the relatively cool and humid summers of 2003 and 2004 resulted in much lower ozone exposures.

Previous studies (Pell *et al.*, 1991) have shown acceleration of senescence associated with low ozone exposures for numerous plant species. Although low during both years of investigation, ambient ozone concentrations, were sufficient to induce typical foliar injury on both cherry and poplar clone as originally described on grapes by Richards *et al.*, 1958) and later by Karnosky, 1976; Davis and Coppolino,1977; Davis and Skelly, 1992; Lee *et al.*,1999; Schaub *et al.*, 2003; Wei *et al.*, 2004. Foliar injury observed on black cherry advanced as adaxial purple to brown stipple followed by chlorosis, premature leaf senescence and leaf abscission. Visible foliar injury on the poplar clone developed as general yellowing followed by bifacial necrotic spots and leaf abscission as soon as mid-August.

Results from several studies (Winner *et al.*, 1989; Hildebrand *et al.*, 1996; Lefohn *et al.*, 1997; Schaub *et al.*, 2003) indicate that local site and associated environmental factors play an important role in determining the incidence and severity of ozone injury on forest trees. During another study within the same study area Yuska *et al.*, (2003) showed that although ambient ozone concentrations were higher in 2001 than in 2000, visible foliar injury was higher in 2000. He assumed that this result was due to lower soil moisture availability in 2001 than in 2000. Obviously ozone-induced foliar injury depends on many variables not solely on ozone fluctuations (Davis and Orendovici in press).

As one of the controlling factors of ozone uptake, stomatal conductance plays an important role in determining plant sensitivity to ozone exposures (Reich, 1987; Kolb *et al.*, 1997; Schaub *et al.*, 2003). Any environmental factor that influences stomatal conductance might influence ozone uptake. However Schaub *et al.* (2003) found that the amount of variation in stomatal conductance cannot be explained only by the soil moisture availability.

Within the current study, during the 2003 study season, there was a general trend of greater stomatal conductance at higher elevation sites than at lower elevation sites. In 2004 the general trend was the opposite with both plant species showing greater stomatal conductance at low elevation sites. During 2003 and 2004 both tree species growing at higher elevation sites showed more severe injury than those growing in plots located at lower elevation.

Apparently there is a discrepancy between foliar injury, stomatal conductance and ozone exposures. However, our results showed that regardless of this discrepancy the severity of foliar injury was positively correlated with ozone concentrations and with ozone uptake during both seasons (Table 14 and 16). A possible explanation is because gas exchange measurements were made only during optimal light and environmental conditions, and there were few clear days across the study area during 2003 and 2004, therefore g_s likely was overestimated. Thus the discrepancy between stomatal conductance and foliar injury might be due to the lack of data for cloudy days.

Another possible explanation is night-time ozone uptake which we considered to be negligible. There have been numerous reports of small but significant nocturnal leaf conductance (Emberson *et al.*, 2000; Musselman and Minnick, 2000). It was also shown that ozone up-taken during the night might be more harmful to plants than the ozone up-taken during the day (Matyssek *et al.*, 1993). Nevertheless, due to the uncertainties regarding nocturnal ozone uptake and to the effects of the nocturnal uptake the estimation of the effect of the ozone uptake on the vegetation is further complicated and correlations between visible injury and ozone uptake harder to prove.

Regardless of the variation in environmental factors during the 2 years of the study, overall cuttings of hybrid polar clone showed a greater stomatal conductance than those of black cherry. The expression of foliar injury was not consistent with this trend, since in 2003 poplar clone expressed less injury than cherry. Our results indicate that the actual ozone impact on different receptors, although induced by the environment may also be induced by species-specific characteristics that control plant response to a particular air pollutant. The interpretation of ozone sensitivity based only on stomatal conductance clearly oversimplifies plant response to ozone, complete stomatal closure, however is very important. Ozone injury is evidently related not only to stomatal conductance, but also to chemical processes within the leaf that might trigger plant defensive mechanisms.

In this study soil moisture availability was not a source of variance in ozone injury among study sites. Therefore in this study the observed variance in stomatal conductance was not explained by the variance in soil moisture availability, but rather by other environmental factors. The genetic variance within injury among plots was reduced by the use of poplar clones and a known sensitive open-pollinated seed source of black cherry. Therefore the observed variance in foliar injury among plots cannot be explained by the genetic variances among, trees but rather by the ozone levels and perhaps the age of the trees. During both years of the study the variance of foliar injury within the same plot was not statistically significant regardless plant species used. Therefore, by using genetically similar families of bio-indicators, plant response to ozone exposures can be more easily assessed than by using natural populations.

During both years of study, the black cherry seedlings responded to similar ozone exposures with similar amounts of foliar injury. In contrast, the poplar clone showed a significantly higher sensitivity to ozone in the second year of the study when ozone exposure regimes were similar. The difference in foliar ozone expression might be related to the age of the trees. In the first year of investigation (2003) black cherry seedlings were already 1-year-old whereas poplar seedlings were started from cuttings in March 2003 and perhaps more susceptible to adaptation stress. *Populus* species in general have rapid growth rates (rapid leaf production, rapid height growth) making them more sensitive to environmental stress. Such species store relatively little carbon during the growing season, and respond to stress with changes in carbon allocation within the plant (Pell *et al.*, 1994; Dickson *et al.*, 1998). Therefore the higher sensitivity of the poplar might have been a result of carbon allocation for growth, instead of compensating for ozone stress. The onset of premature leaf senescence and abscission also decrease total carbon availability and the ability to compensate for further ozone stress as observed for poplar species.

Several ozone fumigation experiments on black cherry have consistently showed biomass and stem growth reduction (Neufeld *et al.*, 1995; Loats and Rebbeck, 1996). Although there were negative correlations between ozone exposures and growth parameters for both cherry and poplar no significant correlations were found among ozone concentrations, foliar injury and any of the relative growth parameters which were measured. There were differences in growth between plants growing at low elevation sites vs. those growing at high elevation sites, but the difference were not statistically significant. Possible explanation is that a two years study is not enough to detect the toxic effect of ozone on growth, but in time the difference would became significant considering that the ozone exposures are higher at high elevation sites. Therefore a multi-year study on the effects of ozone on the growth of the forest trees should lead to a better understanding of the interactions which mostly influence their development. Tree morphology changes with age this also affecting ozone uptake and complicating the extrapolation from seedlings to forest trees (Ferdinand et al, 1998: Bennet *et al.*, 1992). Comparison of ozone induced responses of seedlings to those of mature tree will most likely introduce bias in predicting ozone effects on mature trees.

The various ozone statistics that may be correlated with ozone injury include SUM0, SUM40, and SUM60. Hildebrand *et al.*, 1996 reported that SUM60 was the ozone statistic best correlated with total leaf injury within Shenandoah National Park. SUM 60 was also the index selected for models describing biomass components (Neufeld *et al.*, 1995). By contrast, in a study of the various measures of ozone exposures that best correlated with the ozone uptake by a pine forest in California, Panek *et al.* (2002) found that SUM0 best corresponded to ozone uptake. In this study we also compared the actual measurement of ozone flux with the most common ozone exposure metrics to determine which best correlated with foliar injury. Of the metrics we assessed, SUM40 best correlated with total visible injury for both plant species (Table 14).

The high correlation between visible ozone injury and ozone uptake suggests that a flux-based approach is likely to be more precise in assessing plant injury than an exposure-based approach. At present no dose-based standard is likely to be fully understood without an understanding of the chemical and physiological processes involved in plant defense to ozone toxicity.

The spatial trend of ozone uptake was similar for both plant species but different between the two years of the study. This result is related to the differences in ambient ozone levels between 2003 and 2004. There was not a perfect overlay between ozone uptake and foliar injury but there seems to be a higher degree of association between ozone uptake and foliar injury than between average ozone and foliar injury.

As a result of the low ozone exposures during 2003 and 2004 across north-central Pennsylvania the severity of foliar injury was much lower than that observed by Skelly *et al*. (2001), Yuska *et al*., (2003), and Wei *et al.* (2004) on similar vegetation at sites located in the same area. The present study shows that low ozone exposures results in low or no ozone–induced foliar injury.

A simple assumption that injury is proportional to the ozone uptake rate and therefore making possible estimations of ozone exposures based solely on foliar injury appears not to be applicable. More information is required about the feedback among ozone uptake, plant defense, and plant injury within each species. Also more studies of the nocturnal interactions between plant stomatal conductance, ozone exposures and plant damage are necessary.

4.6. Conclusions

Our results confirm that environmental factors play an important role in determining plant response to ozone stress. In addition to stomatal behavior which controls the ozone uptake into the leaves, the response to ozone seems to be controlled by different characteristics which are species specific and also site specific. During years with high soil moisture the relationship between soil moisture and foliar injury is not as direct as reported in previous studies. Therefore, although plant response to ozone is highly related to ozone uptake/flux, there are many other factors that may influence plant response, resulting in a lack of a direct cause/effect relationship between ozone uptake and injury or retardation of growth. Thus, estimations of ozone exposures based on foliar injury expressed by sensitive tree species cannot be done.

The direct and harmful influence of ambient ozone on growth of black cherry and hybrid poplar was not detected in our results. Since we observed a slight but not statistically significant adverse effect of ozone exposures on growth, the most logical explanation is that 2 years of observations combined with low ozone exposures were not enough time to detect growth changes. Of the metrics which were used to estimate ozone exposures for vegetation, SUM40 best related to foliar injury, yet flux-based estimations seemed most closely related to foliar injury. More important, our study shows that low ozone exposures result in low ozone induced injury even on very sensitive vegetation.

References

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

Chappelka, A.H., Chevrone, B.I., 1986. White Ash seedlings growth response to ozone and simulated acid rain. Canadian Journal of Forest Research 16, 786-790.

Chappelka, A.H., Hildebrand, E.A., Skelly, J.M., Mangis, D., and Renfro, J.F., 1992. Effects of ambient ozone concentrations on mature eastern hardwood trees growing in Great Smoky Mountains National Park and Shenandoah National Park. Presented at 85th Annual Meeting of the Air and Waste Management Association, June 21-26, 1992.

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

Chappelka, A.H., Renfro J.R., and Somers G.L., 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.

Comrie, A.C.,1994a. A synoptic climatology of surface ozone in rural ozone pollution at three forest sites in . Atmospheric Environment 28, 1601-1614.

Davis, D.D., Miller, C.A., Coppolino, J.B., 1977. Foliar response of eleven woody species to ozone with emphasis on black cherry. Phytopathology, 67, 185-189.

Davis, D.D., Umbrach, D.M., and Coppolino, J.B., 1981. Susceptibility of tree and shrub species and response of black cherry foliage to ozone. Plant Disease 65, 904-907.

Davis D.D. and Skelly, J.M., 1992. Foliar sensitivity of eight eastern hardwood tree species to ozone. Water, Air, and Soil Pollution 62, 269-277.

Davis, D.D., Hutnik R.J., and McClenahen, J.R., 1993. Evaluation of Vegetation near Coal-burning Power Plants in Southwestern : Ozone injury on foliage of Hybrid Poplar. Journal of Air Waste Management Association 43, 760-764.

Dickson, R.E., Coleman, M.D., Riemenschneider, D.E., Isebrands, J.D., Hogan, G.D., Karnosky, D.F., 1998. Growth of five hybrid polar genotypes exposed to interacting elevated CO_2 and O_3. Canadian Journal of Forest research 28, 1706-1716.

Emberson, L.D., Ashmore, M.R., Cambridge, H.M., Simpson, D., Tuovinen, J.P., 2000. Modelling stomatal ozone flux across Europe. Environmental Pollution 109, 403–412.

Ferdinand, J.A., 1997. Leaf morphology in relation to ozone sensitivity of black cherry (*Prunus serotina* Ehrh.) trees by tree size, genotype, and light environment. M.S. Thesis, Pennsylvania State University, PA.

Fredericksen, T.S., Joyce, B.J., Skelly, J.M., Steiner, K.C., Kolb, T.E., Kouterick, K. Savage, J.E., and Snyder, K.R., 1995. Physiology, morphology and ozone uptake of leaves of black cherry, samplings and canopy trees. Environmental Pollution 89, 273-283.

Hildebrand , E.S., Skelly, J.M., and Frederiksen, T.S., 1996. Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. Canadian Journal of Forest Research 26, 658-669.

Horsfall J.G. and Barratt, R.W., 1945. An improved grading system for measuring plant disease. Phytopatology **35**, pp. 655–660.

Karlsson, Pihl G., Karlsson, P.E., Danielsson, H., Pleijel, H., 2003. Clover as a tool for bioindication of phytotoxic ozone—5years of experience form southern Sweden—consequences for the short-term critical level. Science of the Total Environment 301, 205–213.

Karnosky, D.F., 1976. Threshold levels for foliar injury to *Populus Tremuloides* Michx. by sulfur dioxide and ozone. Canadian Journal of Forest research 6, 166-169.

Karnosky, D. F., Gagnon, Z.E., Dickson, R.E., Coleman, M.D., Lee, E.H., and Isebrands, J.G., 1996. Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. Canadian Journal of Forest Research 26, 23-37.

Kolb, T.E., Fredericksen, T.S., Steiner, K.C., and Skelly, J.M., 1997. Issues in scaling tree size and age response to ozone: A review. Environmental Pollution 98, 195-208.

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

Krupa, S.V., Tonnejkck, A.E.G. and Manning, W.J., 1998. Ozone. Pp.2-1 to 2-23, in R.B.Flagler *et al.* (Eds.). Recognition of Air Pollution Injury to Vegetation: A pictorial atlas. Air and Waste Management Association, Pittsburgh, PA.

Lee, J.C., Skelly, J.M., Steiner, K.C., Zhang, J.W., and Savage, J.E., 1999. Foliar response of black cherry (*Prunus serotina*) clones to ambient ozone exposure in central Pennsylvania. Environmental Pollution 105, 325-331.

Lefohn, A.S., Jackson, W., Shadwick, D.S., Knodsen, H.P., 1997. Effect of surface ozone exposures on vegetation grown in the Southern Appalachian Mountains; Identification of possible areas of concern. Atmospheric Environment 31, 695-708.

LiCor Bioscience, 4441 Superior St. Lincoln, NE, USA.

Loats, K. V. and Rebbeck, J., 1996. Interactive effects of ozone and elevated carbon dioxide on the growth and physiology of black cherry, green ash, and yellow-poplar seedlings. Environmental Pollution, Volume 106, 237-248.

Manning, W.J., Cooley, D.R., Tuttle, A.F., Frenkel, M. A., Bergweiler, C.J., 2004. Assessing plant response to ambient ozone: growth of young apple trees in open-top chambers and corresponding air plots. Environmental Pollution 132, 5003-508.

Matyssek, R., Keller T. and Koike T., 1993. Branch growth and leaf gas exchange of Populus tremula exposed to low ozone concentrations throughout two growing seasons. Environmental Pollution 79, 1-7.

Musselman R.C., and Minnick T.J., 2000. Nocturnal stomatal conductance and ambient air quality standards for ozone. Atmospheric Environment 34, 719-733.

Minitab Inc., Quality Plaza, 1829 Pine Hall Road, State College, , 16801, USA.

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

Neufeld, H.S., Renfro J.R., and Hacker, W.D., 1992. Ozone in Great Smokey Mountains national Park: dynamics and effects on plants. Air and Waste Management Association, Pittsburg, 594-617.

Neufeld, H.S., Lee, H.E., Renfro J.R., and Hacker, W.D., 1995. Sensitivity of seedlings of black cherry (Prunus serotina Ehrh.). New Phytologist 130, 447-459.

Ogawa & Company, USA, Inc., 1998. NO–NO_2 simultaneous sampling protocol using Ogawa sampler. Pompano Beach, FL.

Ollinger, S.V., J.D. Aber, and P.B. Reich. 1997. Simulating ozone effects on forest productivity: interactions among leaf-, canopy- and stand-level processes. Ecological Applications. 7, 1237-1251.

Panek, J.A., Kurpius, M.R., and Goldstein, A.H., 2002. An evaluation of ozone exposure metrics for a seasonally drought-stressed ponderosa pine ecosystem. Environmental Pollution, Volume 117, 93-100.

Pell, E.J., Dann, M.S., 1991. Multiple stress-induced foliar senescence and implications for whole-plant longevity. In: Mooney H.A., Winner, W. E., Pell, E.J. (Eds), Response of Plant to Multiple Stresses. Academic Press, San Diego, CA, pp. 189-204.

Pell, E.J., temple, P.J., Friend A.L., Mooney, H.A., and Winner, W.E., 1994. Compensation as a plant response to ozone and associated stresses-an analysis of ROPIS experiments. Journal of Environmental Quality 23, 429-436.

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

Richards, B.L., Middleton, J.T., And Hewitt W.B., 1958. Air pollution relation to agronomic crops: Oxidant stipple on grapes. Agronomic Journal 125, 373-379.

Sandermann, H., A.R. Wellburn, and R.H. Heath. (eds). 1997. Forest decline and ozone: Ecological Studies 127. Springer Verlag Berlin Heidelberg, 400 pp.

SAS Institute Inc., 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, SAS Institute Inc., Cary, NC, 1686 pp.

Wei, C., Skelly, J.M., Pennypacker, S.P., Ferdinand, J.A., Savage, J. E.,Stevenson, R. E., Davis, D.D., 2004. Influence of light fleck and low light on foliar injury and physiological responses of two hybrid poplar clones to ozone. Environmental Pollution 130, 215-227.

Simini, M., Skelly, J.M., Davis, D.D., Savage J.E., and Comrie, A. C., 1992. Sensitivity of four hardwoods species to ambient ozone in north central Pennsylvania. Canadian Journal Research 22, 1789-1799.

Skelly, J.M., Davis, D.D., Millen, A.A., Cameron, A.E. Brown,, J.D, Drummond, D.B. and Dochinger, L.S., 1987. Diagnosing Injury to Eastern Forest Trees. USDA- Forest service, Vegetation survey Research Cooperative and The State University, University Park, PA 122 pp.

Skelly, J.M., 2000. Tropospheric ozone and its importance to forests and natural plant communities of the northeastern United States. Northern Naturalist 7(3), 221-236.

Skelly, J.M., Ferdinand, J.A. Jagodzinski, J.M., Savage J.E. and Mulik, J.D., 2001. A thirteen week comparison of passive and continuous ozone monitors at forested sites in North-central Pennsylvania. Journal Air and Waste Management Association 51, 1280-1287.

Smith, G.C. and Manning, W.J., 1990. An ozone effects Monitoring program for the Class I Wilderness Areas in the White Mountain national forests of New Hampshire. University of Massachusetts, Amherst, Ma, 60 pp.

S-PUS 6 for Windows, Insightful Corporation, Seattle, WA, USA.

U.S. Environmental Protection Agency (US EPA), 1996. Air quality criteria for ozone and related photochemical oxidants. Vol.1 of 3 reports no EPA/600/P-93/004aF. Research Triangle Park, North Carolina.

U.S. Environmental Protection Agency (US EPA), 1996a. Air quality criteria for ozone and related photochemical oxidants. Office of Research and development, Washinghton, D.C. EPA/600/P-93/004a-cF.

Wei, C., Skelly, J.M., Pennypacker, S.P., Ferdinand, J.A., Savage, J. E.,Stevenson, R. E., Davis, D.D., 2004. Influence of light fleck and low light on foliar injury and physiological responses of two hybrid poplar clones to ozone. Environmental Pollution 130, 215-227.

Winner, P.B., Lefohn, A.S., Cotter, I.S., Greitner, C.S., Nellesen, J., McEvoy, L.R., Olson, R.L., Atkinson, C.J., and Moore, L.D., 1989. Plant responses to elevational gradients of O_3 exposures in Virginia. Proc. Nat. acad. Sci. 86, 8828-8832.

Yuska D. E., J. M. Skelly, J. A. Ferdinand, R. E. Stevenson, J. E. Savage, J. D. Mulik and A. Hines, 2003. Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania. Environmental Pollution 125, 71-80.

APPENDIX A

Tables

Table 1a. Location of the study sites across north central Pennsylvania.

Site name	County	Latitude	Longitude	Elevation (m)	Pair
CURWENSVILLE	CLEARFIELD	40 56 54	78 31 99	320	1
LUTHERSBURG	CLEARFIELD	41 04 15	78 41 84	540	1
PENFIELD	CLEARFIELD	41 12 65	78 34 71	390	2
MOSHANON S.F.*	CLEARFIELD	41 07 02	78 31 61	660	2
MEDIX RUN	ELK	41 17 35	78 23 67	300	3
PIPER	CLEARFIELD	41 13 01	78 09 58	660	3
HYNER PARK	CLINTON	41 21 49	77 37 74	285	4
PETE’S RUN	CLINTON	41 15 10	77 45 50	690	4
CEDAR RUN	LYCOMING	41 30 44	77 27 42	250	5
GAUGE ROAD.	LYCOMING	41 23 69	77 32 37	570	5
PINE LAKE	LYCOMING	41 21 80	77 21 47	250	6
TIADAGHTON*	LYCOMING	41 20 05	77 26 95	550	6
ROTE	CLINTON	41 04 84	77 27 90	250	7
PINE ROAD	CLINTON	41 04 21	77 18 65	550	7
CANTON	TIOGA	41 39 74	76 50 12	350	8
GLEASON*	TIOGA	41 38 74	76 56 37	700	8
MT. PISGAH ST.	BRADFORD	41 48 39	76 40 27	350	9
MT. PISGAH CO.	BRADFORD	41 48 63	76 42 58	680	9
OGDONIA	SULLIVAN	41 24 65	76 42 28	300	10
WORLD'S END	SULLIVAN	41 25 63	76 36 43	600	10

* Site contains duplicate passive monitors co-located with real-time continuous ozone analyzers, as well as a meteorological station. Other sites contain only passive ozone monitors

Table 1b. Description of the study sites across north central Pennsylvania.

Site	Site name	Description of the location
1	CURWENSVILLE	State Park location, in a valley close to Curwensville Lake.
2	LUTHERSBURG	Rural site, on the top of the mountain surrounded by forest.
3*	MOSHANON S.F.	Rural site, on the mountain surrounded by forest
4	PENFIELD	Urban site, in a valley, in the back-yard of the Penfield Elementary School
5	MEDIX RUN	Rural site, close to a river valley.
6	PIPER	Rural site, on the mountain surrounded by forest
7	PETE’S RUN	Rural site, on the mountain surrounded by forest
8	HYNER PARK	State Park location, close to a river valley.
9	GAUGE ROAD.	Rural site, on the mountain surrounded by forest
10*	TIADAGHTON	Rural site, on the mountain surrounded by forest
11	ROTE	Urban site, in a valley, in the back yard of a police station
12	PINE ROAD	Rural site, on the mountain surrounded by forest
13	PINE LAKE	State Park location, in a river valley close to Pine Lake
14	CEDAR RUN	Urban site, in the valley of the Pine Creak, in the back yard of a fire station
15*	GLEASON	Rural site, on the mountain surrounded by forest
16	CANTON	Urban site, located in a valley
17	MT. PISGAH CO.	County Park location, clearing in a forest on the mountain hill
18	MT. PISGAH ST.	State Park location, located in a valley
19	WORLD'S END	Rural site, clearing in a forest on the mountain hill
20	OGDONIA	Rural site, clearing in a forest located in a valley

* Site contains duplicate passive monitors co-located with real-time continuous ozone analyzers, as well as a meteorological station. Other sites contain only passive ozone monitors

Table 2. The results of the ANOVA Tukey's test for significant differences in ozone concentrations as measured in north-central Pennsylvania during 2002-2004, means with the same letter are not significantly different, ozone concentrations were measured with Ogawa passive samplers.

Year	Site	Ozone (ppb)	Standard deviation	Tukey grouping
2002	GLEASON	54.08	10.35	A
	MT. PISGAH CO.	53.38	10.87	A
	PETE'S RUN	53.04	8.19	A
	MOSHANNON	46.39	8.35	B A
	WORLD'S END	46.08	10.23	B A
	PIPER	44.11	4.04	B A C
	CANTON	40.25	8.19	B D C
	MT. PISGAH ST.	40.10	7.13	B D C
	TIADAGHTON	37.10	8.51	B E D C
	PINE ROAD.	36.46	5.04	B E D C
	LUTHERSBURG	35.77	5.83	B E D C
	CURWENSVILLE	34.89	4.85	E D C
	GAGE ROAD	34.01	9.10	E D C
	PINE LAKE	33.91	4.99	E D C
	ROTE	32.31	5.07	E D
	CEDAR RUN	32.14	5.30	E D
	PENFIELD	30.98	4.78	E D
	MEDIX RUN	30.38	3.90	E D
	OGDONIA	28.46	7.69	E
	HYNER PARK	26.77	5.54	E
2003	PETE'S RUN	47.73	11.50	A
	MT. PISGAH CO.	47.22	8.66	B A
	GLEASON	45.41	7.37	B A C
	MOSHANNON	40.12	7.29	B D A C
	WORLD'S END	37.61	8.46	B D E C
	PIPER	37.18	8.03	B D E C
	TIADAGHTON	32.76	6.00	F D E G
	MT. PISGAH ST.	30.96	8.45	H F D E G
	LUTHERSBURG	30.35	5.22	H F E G
	PINE ROAD	29.06	7.09	H F E G
	GAGE ROAD	28.96	5.76	H F E G
	CANTON	28.54	7.16	H F E G
	CURWENSVILLE	27.88	6.94	H F G
	PENFIELD	27.78	5.91	H F G
	ROTE	26.58	5.86	H G
	OGDONIA	25.14	7.76	H G
	PINE LAKE	24.82	4.90	H G
	CEDAR	23.76	6.02	H G
	MEDIX RUN	23.29	5.02	H G
	HYNER PARK	22.30	7.05	H
2004	MT. PISGAH CO.	38.76	4.11	A

WORLD’S END	37.95	5.40			A	
PETE’S RUN	37.79	3.19			A	
PIPER	36.98	6.85	B		A	
MOSHANNON	36.39	1.62	B		A	C
CURWENSVILLE	34.84	4.93	B	D	A	C
PINE ROAD	34.68	4.95	B	D	A	C
CANTON	34.57	4.52	B	D	A	C
GAGE ROAD	33.91	4.95	B	D	A	C
LUTHERSBURG	33.71	4.71	B	D	A	C
MT. PISGAH ST.	32.34	3.48	B	D		C
OGDONIA	32.10	4.27	B	D		C
PENFIELD	31.99	4.33	B	D		C
TIADAGHTON	31.59	2.17	B	D	E	C
ROTE	31.41	4.64		D	E	C
CEDAR RUN	31.01	4.23		D	E	
PINE LAKE	30.82	4.03		D	E	
MEDIX RUN	30.60	4.87		D	E	
HYNER PARK	29.90	4.54		D	E	
GLEASON	26.46	2.76			E	

Table 3. Seasonal averages of temperature, relative humidity and wind speed as measured at three air quality monitoring sites in north-central Pennsylvania during 2002-2004.

Site	Year	Temp.(C)	Std. dev.	Humidity (%)	Std. dev.	Wind (M/Hr.)	Std dev.
GLEASON	2002	19.85	2.60	73.33	17.82	7.72	3.59
	2003	18.53	2.09	81.05	17.34	7.36	3.64
	2004	17.06	2.04	84.32	15.85	7.76	3.79
MOSHANNON	2002	20.14	2.72	74.61	18.53	6.55	3.52
	2003	18.81	2.17	81.87	18.05	6.37	3.85
	2004	17.79	2.25	83.6	16.49	6.74	3.82
TIADAGHTON	2002	20.34	3.02	75.94	20.11	4.69	3.81
	2003	19.02	2.46	83.96	17.96	4.16	3.42
	2004	17.99	2.40	84.72	17.44	4.14	3.45

Table 4. Correlation coefficients between ambient ozone concentrations, temperature, relative humidity and wind sped recorded at three air quality monitoring sites in north-central Pennsylvania during 2002-2004.

Year	Variables	Correlation coefficient	P
2002	O_3, Temperature	0.738	< 0.001
	O_3, Relative humidity	-0.582	< 0.001
	O_3, Wind speed	0.342	< 0.001
2003	O_3, Temperature	0.614	< 0.001
	O_3, Relative humidity	-0.575	< 0.001
	O_3, Wind speed	0.478	< 0.001
2004	O_3, Temperature	-0.034	0.003
	O_3, Relative humidity	-0.101	< 0.001
	O_3, Wind speed	-0.070	< 0.001

Table 5. Regression equation of ozone with temperature, relative humidity and wind speed as predictors, data were collected at three air quality monitoring sites in north-central Pennsylvania during 2002-2004.

Regression equation for 2002
OZONE(ppb) = - 41.1 + 1.45*TAAVG - 0.150*RHAVG + 0.0410*WINDMPH
S = 14.2341   R-Sq = 55.4%   R-Sq(adj) = 55.4%

Regression equation for 2003
OZONE (ppb) = - 0.44 + 0.794*TAAVG - 0.228*RHAVG + 1.08* WINDMPH
S = 12.5337   R-Sq = 47.2%   R-Sq(adj) = 47.2%

Regression equation for 2004
OZONE (ppb) = 307 - 2.97*TAAVG - 1.62*RHAVG + 2.76*WINDMPH
S = 193.483   R-Sq = 2.2%   R-Sq(adj) = 2.2%

Year	Predictor	Coefficient	Std. error	T	P
	Constant	-41.09	2.74	-15.01	< 0.001
2002	TAAVG	1.45	0.03	52.32	< 0.001
	RHAVG	-0.15	0.01	-10.33	< 0.001
	WINDMPH	0.04	0.06	0.72	0.472
	Constant	-0.44	2.57	-0.17	0.864
2003	TAAVG	0.79	0.03	28.71	< 0.001
	RHAVG	-0.23	0.01	-18.66	< 0.001
	WINDMPH	1.08	0.05	23.63	< 0.001
	Constant	306.90	29.58	10.37	< 0.001
2004	TAAVG	-2.97	0.33	-9.09	< 0.001
	RHAVG	-1.62	0.16	-9.90	< 0.001
	WINDMPH	2.76	0.64	4.33	< 0.001

TAAVG - hourly temperature average (^{0}F)

RHAVG - hourly relative humidity average (%)

WINDMPH - hourly wind speed (miles/hour)

Table 6. Results of the Anderson-Darling (AD) statistic test of ozone data from three air quality monitoring sites in north-central Pennsylvania during 2002-2004 (end of May to mid-September).

Year		2002		2003		2004	
Site	Distribution	AD	P	AD	P	AD	P
Three sites	Normal	13.31	<0.005	5.20	<0.005	6.07	<0.005
	3-Parameter Lognormal	3.49	*	2.37	*	7.13	*
	2-Parameter Exponential	729.7	<0.010	883.1	<0.010	835.5	<0.010
	3-Parameter Weibull	2.89	<0.005	4.86	<0.005	7.86	<0.005
	Smallest Extreme Value	109.4	<0.010	136.2	<0.010	92.08	<0.010
	Largest Extreme Value	22.58	<0.010	42.39	<0.010	67.70	<0.010
	3-Parameter Gamma	3.22	*	2.45	*	9.99	*
	Logistic	16.40	<0.005	7.12	<0.005	11.92	<0.005
	3-Parameter Loglogistic	9.23	*	6.30	*	12.75	*
	Normal	5.99	<0.005	5.83	<0.005	13.65	<0.005
	Lognormal	10.31	<0.005	14.29	<0.005	105.5	<0.005
	3-Parameter Lognormal	2.03	*	2.72	*	15.09	*
	Exponential	373.0	<0.003	464.3	<0.003	189.5	<0.003
	2-Parameter Exponential	263.9	<0.010	289.9	<0.010	165.3	<0.010
Gleason	Weibull	2.88	<0.010	9.22	<0.010	27.62	<0.010
	3-Parameter Weibull	0.83	<0.03	4.98	<0.005	17.48	<0.005
	Smallest Extreme Value	40.70	<0.010	71.93	<0.010	41.07	<0.010
	Largest Extreme Value	6.41	<0.010	13.09	<0.010	26.27	<0.010
	Gamma	3.05	<0.005	5.41	<0.005	48.34	<0.005
	3-Parameter Gamma	1.80	*	2.90	*	17.51	*
	Logistic	7.46	<0.005	2.78	<0.005	17.15	<0.005
	Loglogistic	8.72	<0.005	8.83	<0.005	73.82	<0.005
	3-Parameter Loglogistic	4.96	*	2.27	*	18.64	*
	Normal	12.51	<0.005	2.94	<0.005	1.53	<0.005
	3-Parameter Lognormal	4.45	*	1.18	*	1.56	*
	2-Parameter Exponential	244.0	<0.010	274.4	<0.010	406.2	<0.010
	3-Parameter Weibull	7.14	<0.005	2.90	<0.005	3.10	<0.005
Moshannon	Smallest Extreme Value	46.51	<0.010	44.80	<0.010	33.65	<0.010
	Largest Extreme Value	5.31	<0.010	14.24	<0.010	31.00	<0.010
	3-Parameter Gamma	4.49	*	1.22	*	3.53	*
	Logistic	12.14	<0.005	3.35	<0.005	1.87	<0.005
	3-Parameter	5.14	*	2.09	*	1.88	*

	Loglogistic						
	Normal	11.05	<0.005	6.71	<0.005	5.15	<0.005
	Lognormal	39.74	<0.005	39.94	<0.005	30.31	<0.005
	3-Parameter Lognormal	6.30	*	1.65	*	1.31	*
	Exponential	150.2	<0.003	219.5	<0.003	371.0	<0.003
Tiadaghton	2-Parameter Exponential	124.6	<0.010	202.7	<0.010	351.0	<0.010
	Weibull	4.69	<0.010	1.45	<0.010	1.32	<0.010
	3-Parameter Weibull	5.42	<0.005	0.88	0.02	1.30	<0.005
	Smallest Extreme Value	44.15	<0.010	55.20	<0.010	52.68	<0.010
	Largest Extreme Value	8.44	<0.010	5.89	<0.010	10.13	<0.010
	Gamma	13.54	<0.005	11.71	<0.005	9.48	<0.005
	3-Parameter Gamma	7.41	*	1.67	*	1.29	*
	Logistic	11.46	<0.005	6.34	<0.005	5.50	<0.005
	Loglogistic	27.84	<0.005	20.58	<0.005	16.12	<0.005
	3-Parameter Loglogistic	9.08	*	4.04	*	3.34	*

Table 7. Results of the correlation and T-test between real time data and synthetically generated data based on assumption of normality of distributions with mean as measured by the passive samplers at three air quality monitoring sites in north-central Pennsylvania, during 2002-2004.

Year	Variable/Test	Correlation	P	T-test	P
2002	PASSIVE, REAL-TIME	0.858	< 0.001	0.370	0.714
	PASSIVE, GENERATED	0.999	< 0.001	-0.160	0.874
	REAL-TIME, GENERATED	0.861	< 0.001	-0.390	0.702
	SUM0, SUM0G	0.731	< 0.001	-0.144	0.159
	SUM40, SUM40G	0.851	< 0.001	-0.790	0.435
	SUM 60, SUM 60G	0.839	< 0.001	0.800	0.430
	SUM80, SUM80G	0.590	< 0.001	0.960	0.390
	N40,N40G	0.809	< 0.001	-3.080	0.004
	N60, N60G	0.822	< 0.001	-0.720	0.474
	N80, N80G	0.782	< 0.001	0.970	0.341
2003	PASSIVE, REAL-TIME	0.911	< 0.001	-17.460	< 0.001
	PREDICTED(1), REAL TIME	0.911	< 0.001	-0.360	0.726
	PREDICTED, GENERATED	0.999	< 0.001	-0.800	0.428
	REAL-TIME, GENERATED	0.913	< 0.001	0.300	0.767
	SUM0, SUM0G	0.913	< 0.001	0.300	0.767
	SUM40, SUM40G	0.946	< 0.001	1.460	0.148
	SUM60, SUM60G	0.964	< 0.001	3.130	0.002
	SUM80, SUM80G	0.977	< 0.001	2.190	0.031
	N40, N40G	0.893	< 0.001	0.350	0.729
	N60, N60G	0.926	< 0.001	3.090	0.003
	N80, N80G	0.956	< 0.001	1.950	0.060
2004a	PASSIVE, REAL-TIME	0.064	0.561	-1.410	0.164
	PASSIVE, PREDICTED	0.087	0.736	-1.570	0.119
	PREDICTED(2), REAL-TIME	0.736	< 0.001	< 0.001	1.000
	REAL-TIME, GENERATED	0.728	< 0.001	0.070	0.946
	PREDICTED, GENERATED	0.997	< 0.001	0.830	0.407
	SUM0, SUM0G	0.719	< 0.001	-0.170	0.869
	SUM40, SUM40G	0.742	< 0.001	2.680	0.009
	SUM60, SUM60G	0.569	< 0.001	2.270	0.026
	SUM80, SUM80G	0.060	0.580	-3.360	< 0.001
	N40, N40G	0.708	< 0.001	-1.660	0.101
	N60, N60G	0.755	< 0.001	4.100	< 0.001
	N80, N80G	-0.039	0.720	-0.280	0.783
2004b	PASSIVE, REAL-TIME	0.064	0.561	-1.410	0.164
	PASSIVE, PREDICTED	0.087	0.736	-1.570	0.119
	PREDICTED,REAL-TIME	0.736	< 0.001	< 0.001	1.000
	REAL-TIME, GENERATED	0.735	< 0.001	-0.020	0.981
	PREDICTED, GENERATED	0.997	< 0.001	-0.300	0.763
	SUM0, SUM0G	0.726	< 0.001	-0.260	0.797
	SUM40, SUM40G	0.737	< 0.001	2.380	0.019
	SUM60, SUM60G	0.683	< 0.001	2.420	0.018
	SUM80, SUM80G	0.510	< 0.001	-4.290	< 0.001

N40, N40G	0.678	< 0.001	1.490	0.139
N60, N60G	0.767	< 0.001	4.010	< 0.001
N80, N80G	0.391	< 0.001	0.380	0.708

Where correlation represents the Pearson correlation coefficient, p r p of the correlation coefficient or the of the t-test, T-value represents the value of the T-Test of mean difference = 0

(1) PREDICTED (2003) = 6.65+1.03*PASSIVES

(2) PREDICTED (2004) = 18.5+0.564*PASSIVES

(G) represents generated data using normal distributions

(N) represents the number of hours above the specified value

2004 a – data were generated using standard deviation of weekly ozone as measured at each site

2004 b – data were generated using standard deviation of weekly ozone for all three continuous monitors

Table 8. Geostatistical parameters used to generate prediction maps of ambient ozone concentrations in north-central Pennsylvania during 2002-2004.

year	interpolation	model	Range (km)	partial sill	nugget	lag (km)	mean errors of estimation
2002	kriging	exponential	29.04	50.83	33.27	12	5.7
2003	kriging	exponential	33.01	53.75	32.36	10	8.4
2004	kriging	exponential	74.89	9.54	7.08	12	3.4

range - the neighborhood within which measurements are related to each other
partial sill - value of spatial variation at the distance where a maximum value is reaches
nugget - includes sum of errors (measurement errors, location error)
lag - the distance up to which the correlation between sample point exists

Table 9. Summary of ambient ozone concentrations at study sites in north-central Pennsylvania, ozone was monitored from June 9th to September 9th in 2003 and from May 23rd to September 2nd in 2004.

Year	Site	Ozone (ppb)	SUM0 (ppb)	SUM40 (ppb)	SUM60 (ppb)	SUM80 (ppb)
2003	PETE'S RUN	47.73	104292	23862.10	5543.70	788.71
	MT. PISGAH CO.	47.22	103198	22185.80	3957.27	446.63
	GLEASON	45.41	198422	38025.80	5882.85	673.09
	MOSHANNON	40.12	175491	29063.60	4749.32	589.66
	WORLD'S END	37.61	82213	12557.20	2180.39	264.98
	PIPER	37.18	81037	11722.90	2161.30	346.50
	TIADAGHTON	32.76	143138	14224.30	1445.39	73.97
	MT. PISGAH ST.	30.96	67307	6676.30	750.60	24.14
	LUTHERSBURG	30.35	66261	5399.70	464.25	23.35
	PINE ROAD	29.06	63594	5273.10	462.04	14.67
	GAGE ROAD	28.96	62871	4574.10	344.21	14.68
	CANTON	28.54	62154	4868.70	396.98	14.69
	CURWENSVILLE	27.88	60681	4548.70	440.30	19.46
	PENFIELD	27.78	61081	4311.20	311.53	12.54
	ROTE	26.58	57967	3638.90	245.80	4.67
	OGDONIA	25.14	53539	2996.20	201.55	2.93
	PINE LAKE	24.82	54173	2777.40	149.60	3.07
	CEDAR	23.76	51519	2691.80	168.11	2.52
	MEDIX RUN	23.29	50678	2315.70	122.06	2.49
	HYNER PARK	22.30	48490	2453.90	153.11	5.64
2004	MT. PISGAH CO.	38.76	90813	11560.90	995.67	24.05
	WORLD'S END	37.95	89599	11393.10	957.63	18.54
	PETE'S RUN	37.79	88723	10469.40	817.76	17.17
	PIPER	36.98	87114	10851.20	1241.64	63.53
	MOSHANNON	36.39	171210	18074.20	1196.84	20.47
	CURWENSVILLE	34.84	81692	8173.20	549.67	9.84
	PINE ROAD	34.68	81903	8250.20	542.60	6.31
	CANTON	34.57	81624	8058.80	510.40	7.79
	GAGE ROAD	33.91	79412	7349.30	496.55	18.72
	LUTHERSBURG	33.71	79225	7370.60	434.51	3.92
	MT. PISGAH ST.	32.34	75704	5921.50	309.44	2.06
	OGDONIA	32.10	75742	6073.30	335.65	5.34
	PENFIELD	31.99	75155	5799.60	239.56	2.48
	TIADAGHTON	31.59	148515	10551.20	493.99	5.48
	ROTE	31.41	74072	5851.40	272.59	2.08
	CEDAR RUN	31.01	72849	5273.10	310.63	2.92
	PINE LAKE	30.82	72209	4955.60	231.62	3.66
	MEDIX RUN	30.60	71870	5076.00	269.64	2.47
	HYNER PARK	29.90	70904	4781.10	250.98	2.76
	GLEASON	26.46	124728	5807.50	201.33	0.86

Table 10. Sites and dates when measurements of leaf gas exchange were taken during 2003 and 2004, study sites were located in north-central Pennsylvania.

Site	2003	2004
Canton	25-July	7-June, 22-July
Gleason	25-July	7-June, 22-July
Medix Run	31-July	4-June, 8-July
Moshannon St. Forest	17-July, 21-August	1-July, 9- August
Penfield	17-July, 21-August	1-July, 9- August
Pine Lake	3-July, 28- August	21-June, 2- August
Pine Road	14- August	3-June, 2-July, 23- August
Piper	31-July	4-June, 8-July
Mt. Pisgah Co.	7- August	24-June, 29-July
Mt. Pisgah St.	7- August	24-June, 29-July
Rote	14-August	3-June, 2-July, 23-August
Tiadaghton	3-July, 28-August	21-June, 2-August

Table 11. Chemical analysis of soil samples collected in the fall 2004 at the 20 study sites located in north-central Pennsylvania.

Site	pH	Phosphate (P2O5) kg/Ha	Potassium (K2O) kg/Ha	Magnesium (MgO) kg/Ha	Calcium (CaO) kg/Ha	Nitrogen (N) %	Elevation[1]
GAGE ROAD	3.8	119.44	226.36	125.13	503.91	0.19	H
GLEASON	5.6	56.88	161.53	668.85	2517.29	0.20	H
LUTHERSBURG	5.1	52.33	163.80	252.53	1390.03	0.21	H
MOSHANNON	4.9	646.10	183.14	131.95	600.60	0.11	H
MT. PISGAH ST.	5.5	276.41	546.00	381.06	3790.15	0.38	H
PETE’S RUN	4.2	192.24	229.78	275.28	764.40	0.37	H
PINE ROAD.	5.6	458.41	177.45	286.65	2653.79	0.24	H
PIPER	3.8	72.80	81.90	86.45	404.95	0.16	H
TIADAGHTON	5.1	427.70	138.78	180.86	2045.23	0.20	H
WORLD’S END	6.5	30.71	188.83	1556.10	3832.24	0.17	H
CANTON	5.9	98.96	737.10	592.64	2946.13	0.16	L
CEDAR RUN	5.0	167.21	144.46	257.08	1893.94	0.30	L
CURWENSVILLE	5.4	98.96	226.36	291.20	3587.68	0.35	L
HYNER PARK	5.0	36.40	163.80	131.95	539.18	0.11	L
MEDIX RUN	5.6	125.13	489.13	498.23	1726.73	0.17	L
MT. PISGAH CO.	7.0	114.89	253.66	702.98	8321.95	0.34	L
OGDONIA	5.2	155.84	134.23	245.70	2757.30	0.27	L
PENFIELD	5.8	78.49	316.23	680.23	4643.28	0.33	L
PINE LAKE	5.0	135.36	257.08	362.86	2525.25	0.35	L
ROTE	7.0	114.89	298.03	626.76	8925.96	0.34	L

[1] Elevation represents the elevation category of the site

Table 12. Degrees of freedom (DF), F-values, and p-values for the general linear model for stomatal conductance (R-Sq = 35.57% for 2003 and R-Sq = 34.35% for 2004). Measurements were taken three times a day at 12 sites on north-central Pennsylvania using black cherry and hybrid poplar seedlings during 2003 and 2004.

Year	2003			2004		
Source of variance	DF	F-value	P	DF	F-value	P
Time	2	12.85	< 0.001	2	33.34	< 0.001
Tree species	1	7.01	0.008	1	71.79	< 0.001
Elevation	1	10.35	0.001	1	6.3	0.012
Temperature (Tair)	1	5.09	0.025	1	79.36	< 0.001
Time*Elevation	2	17.77	< 0.001	2	10.02	< 0.001
Elevation*Tair	1	12.58	< 0.001	1	4.81	0.029
Time*Tair	2	16.56	< 0.001	2	30.43	< 0.001
Time*Elevation*Tair	2	21.93	< 0.001	2	8.38	< 0.001
Error	417			561		

Table 13. Degrees of freedom (DF), F-values, and p-values for the general linear model for stomatal conductance (R-Sq = 56.94% for 2003 and R-Sq = 57.28% for 2004). Measurements were taken three times a day at 12 sites on north-central Pennsylvania using black cherry and hybrid poplar seedlings during 2003 and 2004.

Year	2003			2004		
Source	DF	F-value	P	DF	F-value	P
Time	2	26.34	< 0.001	2	20.65	< 0.001
Tree	1	7.02	0.008	1	3.22	0.073
Elevation	1	13.55	< 0.001	1	0.59	0.445
Temperature (Tair)	1	50.67	< 0.001	1	10.24	<0.001
Time*Elevation	2	8.98	< 0.001	2	9.38	< 0.001
Elevation*Tair	1	16.57	< 0.001	1	0.34	0.560
Time*Tair	2	32.22	< 0.001	2	18.17	< 0.001
Time*Elevation*Tair	2	11.7	< 0.001	2	7.94	< 0.001
VpdL	1	26.42	< 0.001	1	20.18	< 0.001
Photosynthesis	1	3.27	0.071	1	133.7	< 0.001
Calcium (CaO)	1	25.97	< 0.001	1	8.34	0.004
Nitrogen	1	51.38	< 0.001	1	14.96	< 0.001
Tair*VpdL	1	31.91	< 0.001	1	12.81	< 0.001
Error	395			556		

Table 14. Correlation coefficients and p-values between cumulative ozone concentrations and percent injury (%INJ) for black cherry and hybrid poplar in 2003 and 2004, in north-central Pennsylvania.

Year	Variable	%INJ (poplar)	%INJ (cherry)	Ozone statistic SUM0	SUM40	SUM60
2003	%INJ (Black cherry)	0.266 0.258				
	SUM0	0.552 0.012	0.745 < 0.001			
	SUM40	0.706 0.001	0.751 < 0.001	0.936 < 0.001		
	SUM60	0.692 0.001	0.762 < 0.001	0.842 < 0.001	0.972 < 0.001	
	SUM80	0.642 0.002	0.761 < 0.001	0.767 < 0.001	0.927 < 0.001	0.986 < 0.001
2004	%INJ (Black cherry)	0.481 0.032				
	SUM0	0.572 0.008	0.598 0.005			
	SUM40	0.565 0.009	0.551 0.012	0.730 < 0.001		
	SUM60	0.495 0.027	0.336 0.147	0.432 0.057	0.895 < 0.001	
	SUM80	0.283 0.226	0.085 0.723	0.145 0.541	0.560 0.010	0.833 < 0.001

Table 15. Seasonal averages of ozone (ppb) concentrations and cumulative precipitation (mm) recorded during 2003-2004 in north-central Pennsylvania, at the 20 study sites, and the results of the ANOVA test for significant differences, means with the same letter are not significantly different.

Year	Site	Precipitation (mm)	Ozone (ppb)	St. Dev.	ANOVA
2003	Pete's Run	273	47.73	11.50	A
	Pisgah Park	397	47.22	8.66	B A
	Gleason	423	45.41	7.37	B A C
	Moshannon	397	40.12	7.29	B D A C
	World's End	365	37.61	8.46	B D E C
	Piper	403	37.18	8.03	B D E C
	Tiadaghton	336	32.76	6.00	F D E G
	Pisgah Lake	362	30.96	8.45	H F D E G
	Luthersburg	377	30.35	5.22	H F E G
	Pine Road	404	29.06	7.09	H F E G
	Gage Road	297	28.96	5.76	H F E G
	Canton	353	28.54	7.16	H F E G
	Curwensville	638	27.88	6.94	H F G
	Penfield	452	27.78	5.91	H F G
	Rote	319	26.58	5.86	H G
	Ogdonia	367	25.14	7.76	H G
	Pine Lake	362	24.82	4.90	H G
	Cedar Run	410	23.76	6.02	H G
	Medix run	341	23.29	5.02	H G
	Hyner Park	386	22.30	7.05	H
2004	Pisgah Park	416	38.76	4.11	A
	World's End	496	37.95	5.40	A
	Pete's Run	364	37.79	3.19	A
	Piper	452	36.98	6.85	B A
	Moshannon	394	36.39	1.62	B A C
	Curwensville	392	34.84	4.93	B D A C
	Pine Road	392	34.68	4.95	B D A C
	Ccanton	478	34.57	4.52	B D A C
	Gage Road	466	33.91	4.95	B D A C
	Luthersburg	448	33.71	4.71	B D A C
	Pisgah Lake	434	32.34	3.48	B D C
	Ogdonia	371	32.10	4.27	B D C
	Penfield	341	31.99	4.33	B D C
	Tiadaghton	386	31.59	2.17	B D E C
	Rote	342	31.41	4.64	D E C
	Cedar Run	465	31.01	4.23	D E
	Pine Lake	344	30.82	4.03	D E
	Medix Run	338	30.60	4.87	D E
	Hyner Park	476	29.90	4.54	D E
	Gleason	414	26.46	2.76	E

Table 16. Correlation coefficients and p-values between ozone uptake calculated with different cumulative ozone indices and percent injury (%INJ) for black cherry and hybrid poplar in 2003 and 2004, in north-central Pennsylvania.

Year	Tree species		%INJ	uptake (SUM0)	uptake (SUM40)
2004	black cherry	uptake (SUM0)	-0.216		
			0.361		
		uptake (SUM40)	0.552	-0.027	
			0.012	0.910	
		uptake (SUM60)	0.486	-0.177	0.890
			0.030	0.456	< 0.001
	hybrid poplar	uptake (SUM0)	-0.093		
			0.695		
		uptake (SUM40)	0.547	-0.107	
			0.012	0.652	
		uptake (SUM60)	0.319	-0.266	0.876
			0.171	0.258	< 0.001
2003	black cherry	uptake (SUM0)	0.443		
			0.050		
		uptake (SUM40)	0.781	0.394	
			< 0.001	0.085	
		uptake (SUM60)	0.769	0.437	0.970
			< 0.001	0.054	< 0.001
	hybrid poplar	uptake (SUM0)	0.115		
			0.629		
		uptake (SUM40)	0.614	0.505	
			0.004	0.023	
		uptake (SUM60)	0.593	0.534	0.970
			0.006	0.015	< 0.001

APPENDIX B

Figures

Figure 1. Location of ozone passive monitoring sites in north-central Pennsylvania, (*) site contains duplicate passive monitors co-located with real-time continuous ozone analyzers, as well as a meteorological station (air quality monitoring site) other sites contain only passive ozone monitors.

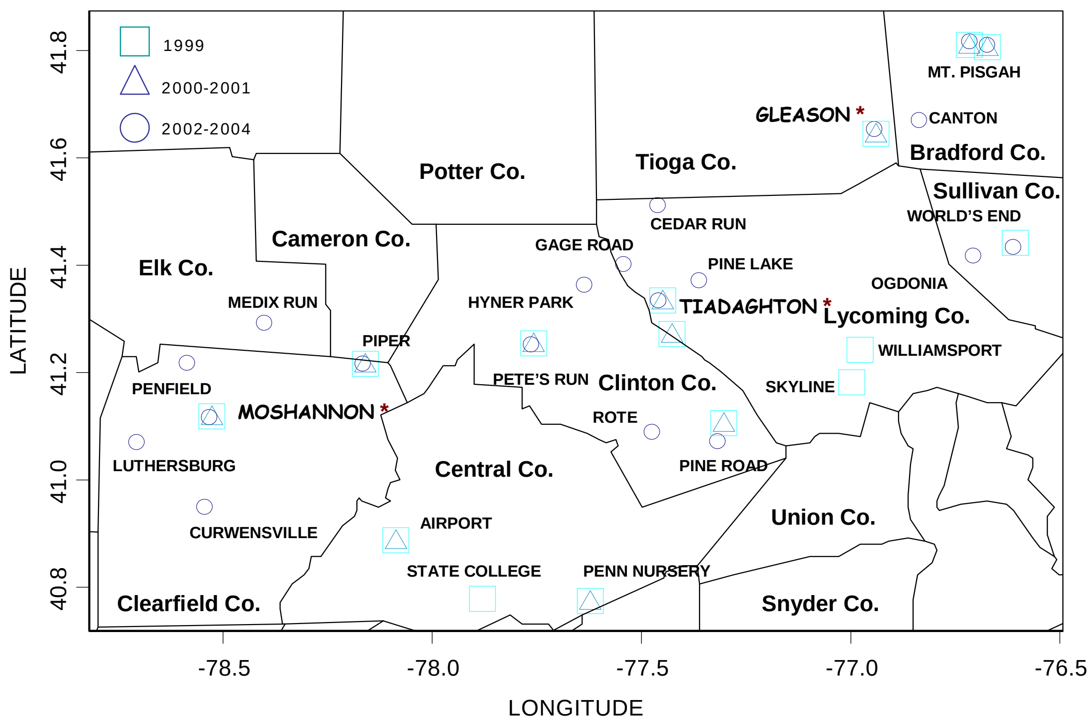
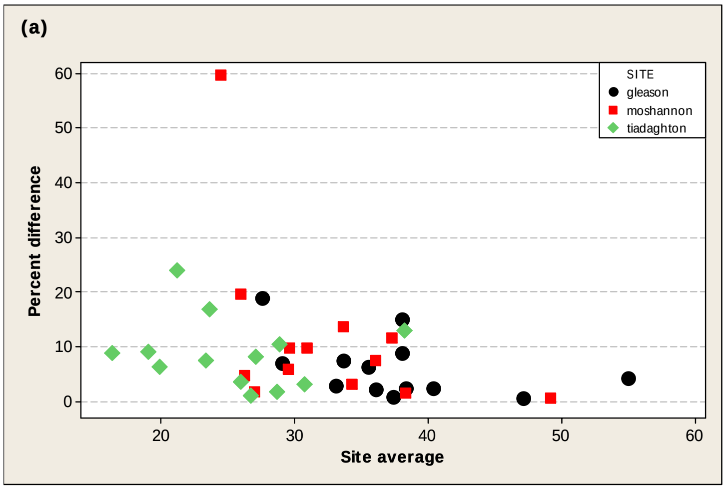


Figure 2. Relationship between percentage difference of replicate samples and average of duplicates at three air quality monitoring sites in north-central Pennsylvania, as monitored during 2003 (a) and 2004 (b).



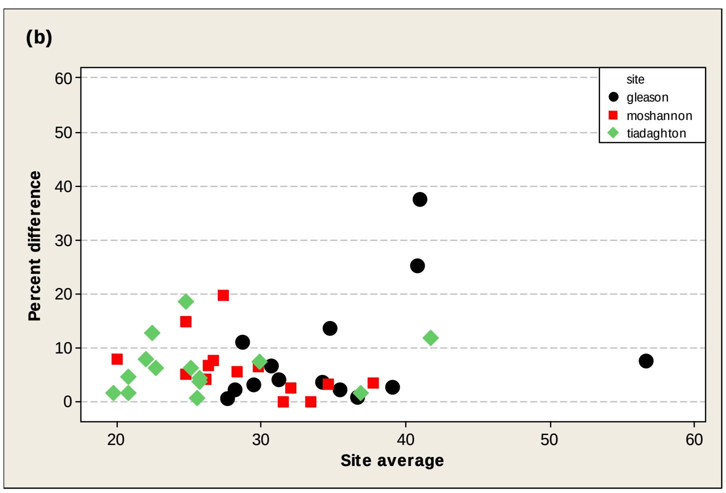


Figure 3. Linear regressions between real-time and passive data for three locations having co-located monitoring systems in north-central Pennsylvania, 2002 (a), 2003 (b), 2004 (c), and 2004 (d) site specific.

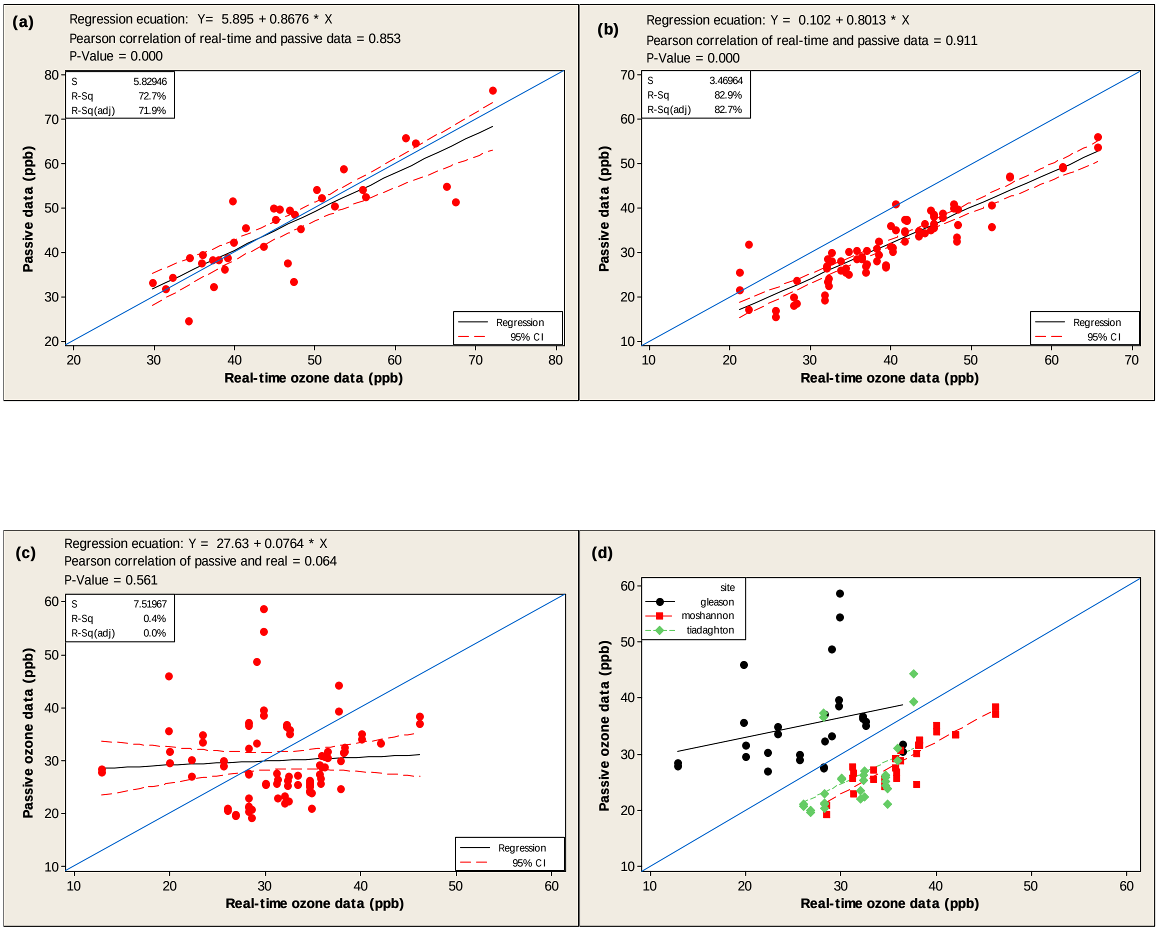


Figure 4. Plot of passive and corrected data versus real-time data at three locations having co-located monitoring systems in north-central Pennsylvania during 2003 (a) and 2004 (b).

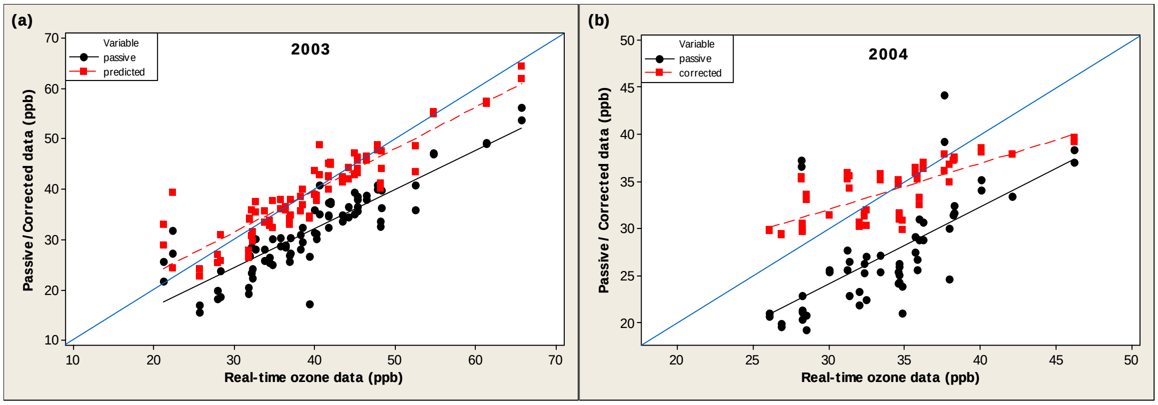


Figure 5. Plot of residuals (measured as real-time minus predicted) ozone data for 2003 (a) and 2004 (b).

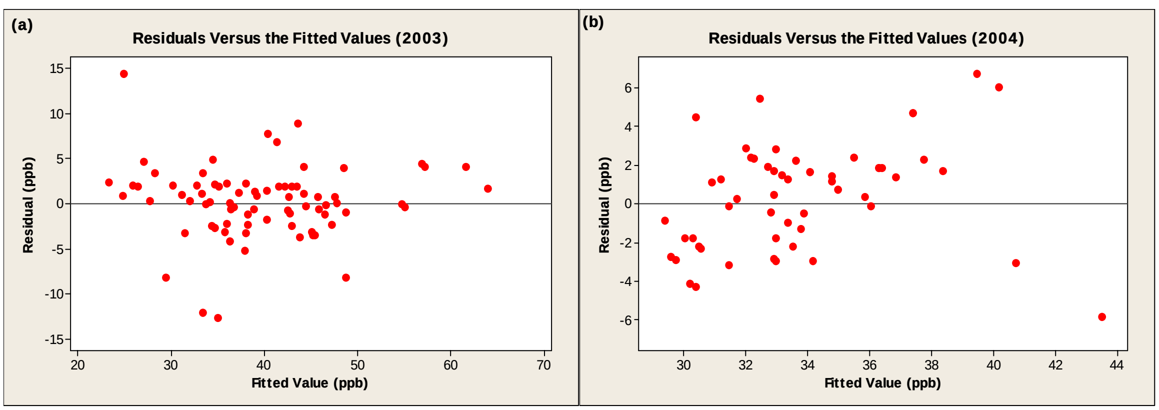


Figure 6. Comparison of seasonal ozone averages between sites (a), and year vs. elevation (b) as measured with Ogawa passive samplers at the 20 monitoring sites in north-central Pennsylvania during 2002-2004, in 2004 the data for Gleason were from the real-time monitor.

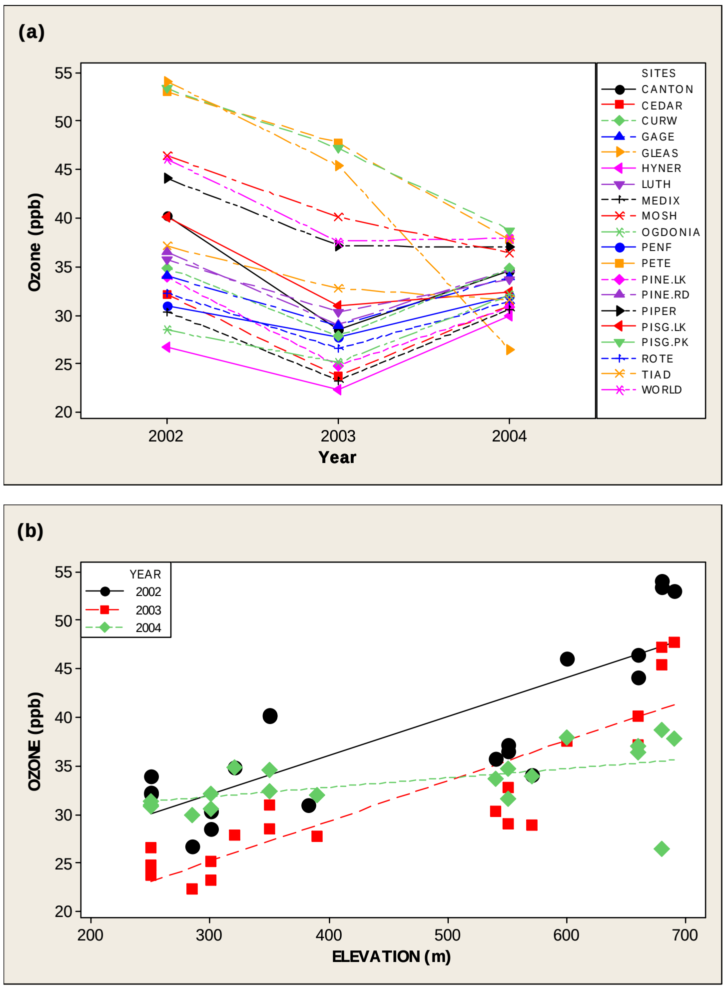


Figure 7. Seasonal averages of temperature, relative humidity and wind speed at three air quality monitoring sites in north-central Pennsylvania during 2002-2004.

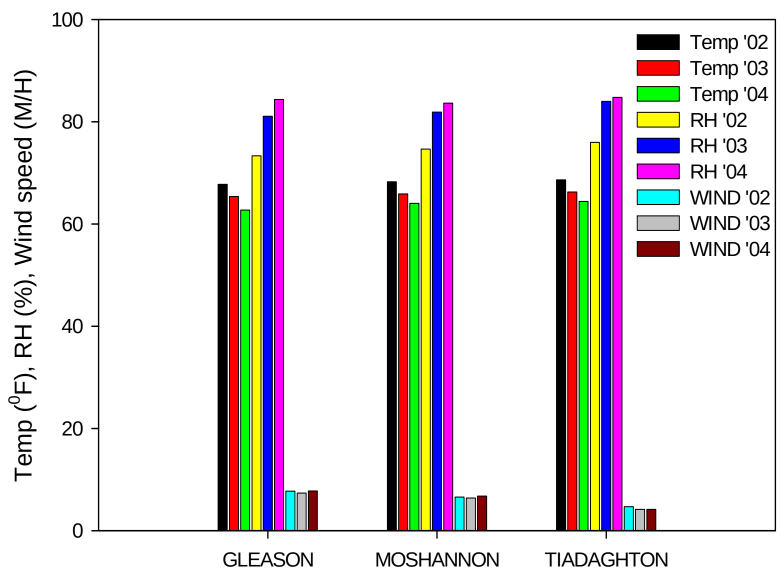


Figure 8. Comparison of seasonal ozone averages between pair of sites (high vs. low elevation) as measured at the 20 monitoring sites in north-central Pennsylvania during 2002-2004.

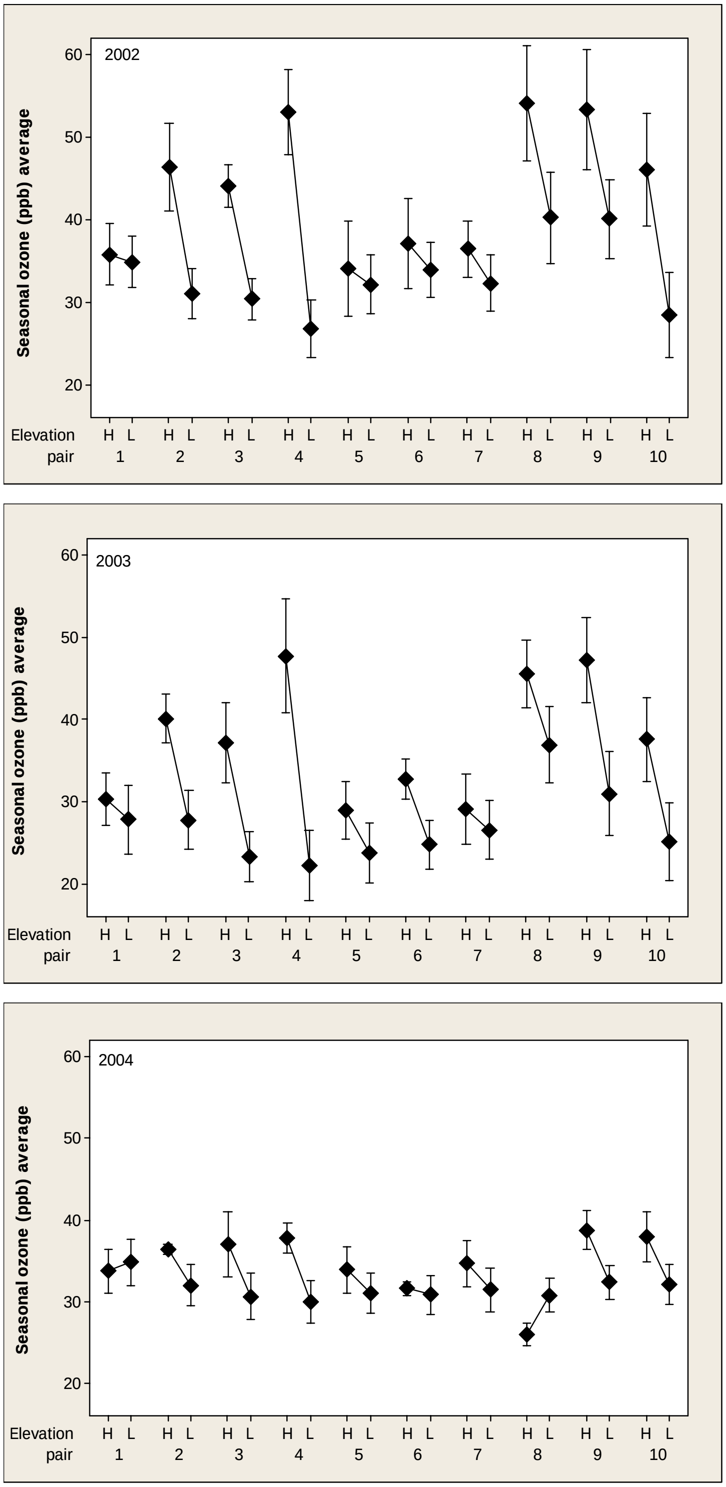


Figure 9. Hourly ozone distributions recorded for one randomly selected week of 2003 (a) and of 2004 (b) at the three air quality monitoring sites in north-central Pennsylvania.

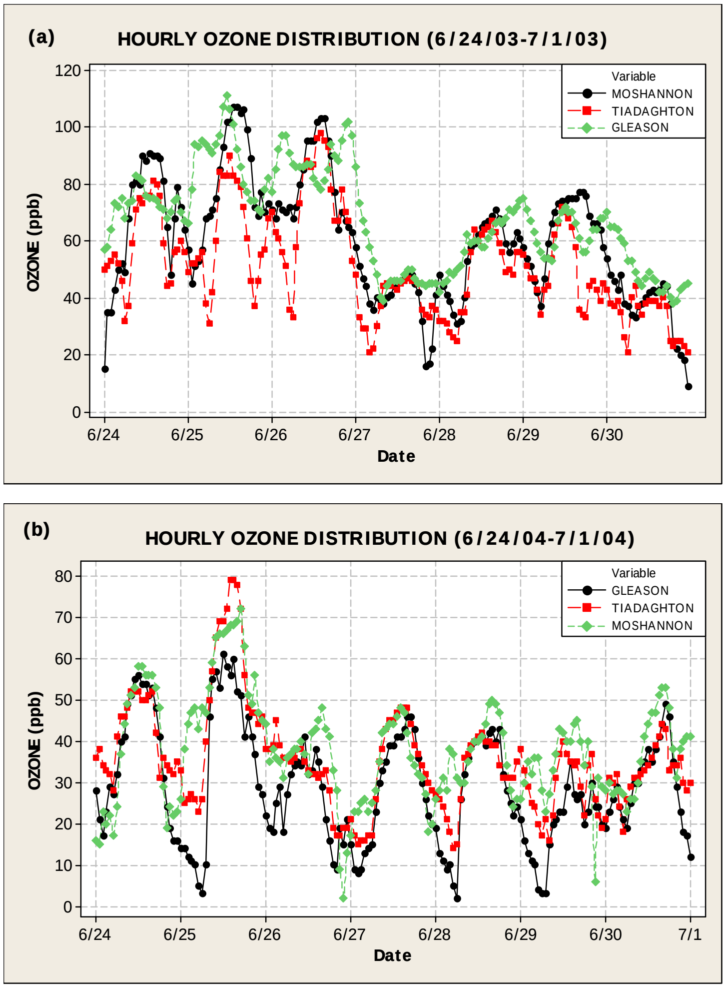


Figure 10. Cumulative Distribution Functions of ozone data at three air quality sites in north-central Pennsylvania during 2002-2004.

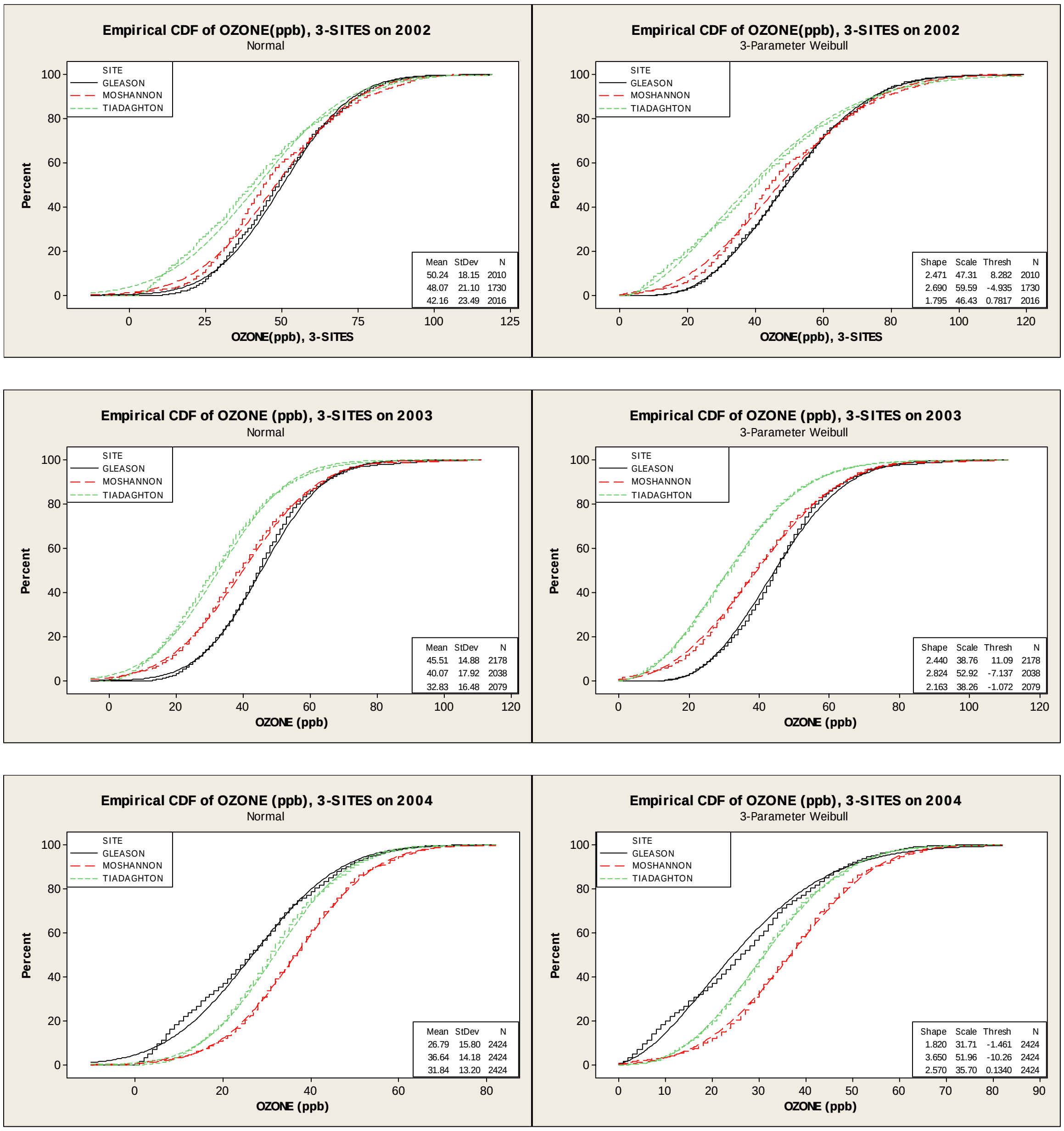


Figure 11. Histogram of the difference between weekly means as measured with passive samplers and weekly means of synthetically generated data at 20 monitoring sites in north-central Pennsylvania during 2002-2004.

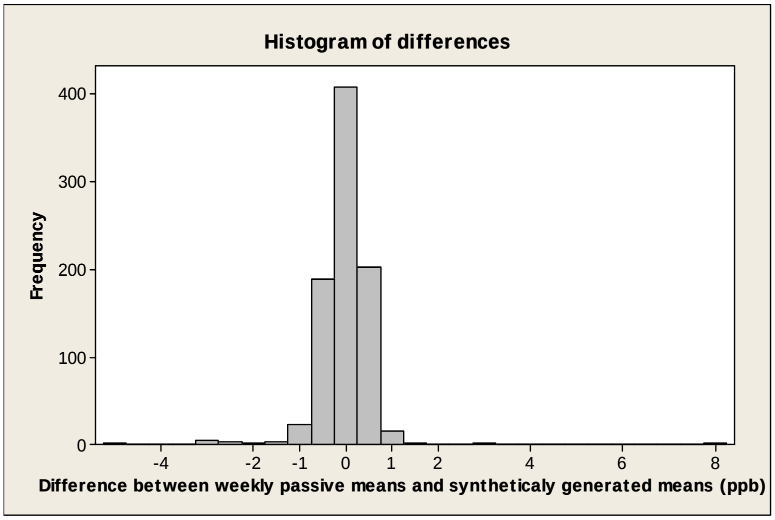
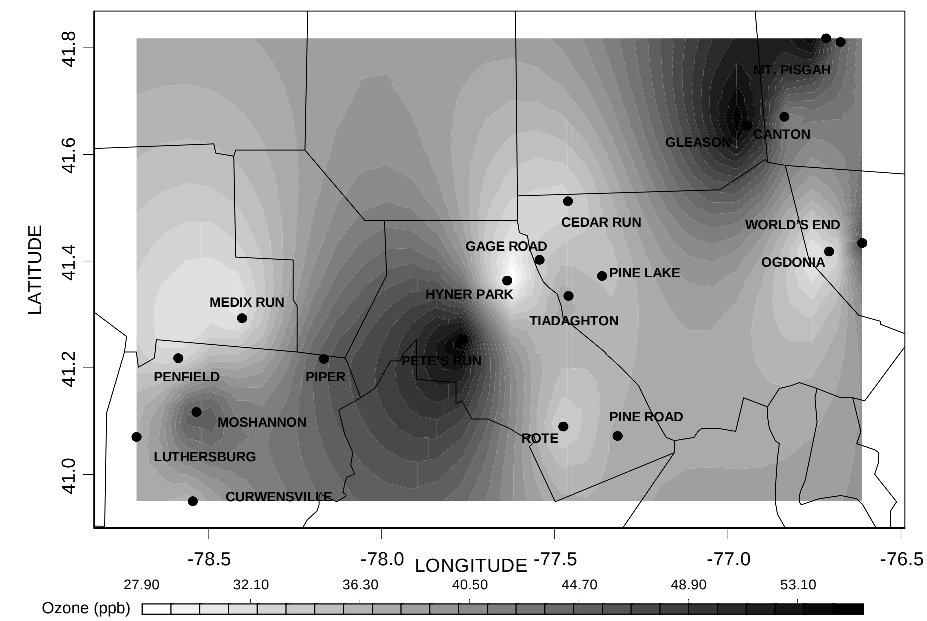


Figure 12. Estimated seasonal average of ambient ozone concentration during 2002 and estimated zones of reliability of ozone predictions, in north-central Pennsylvania.



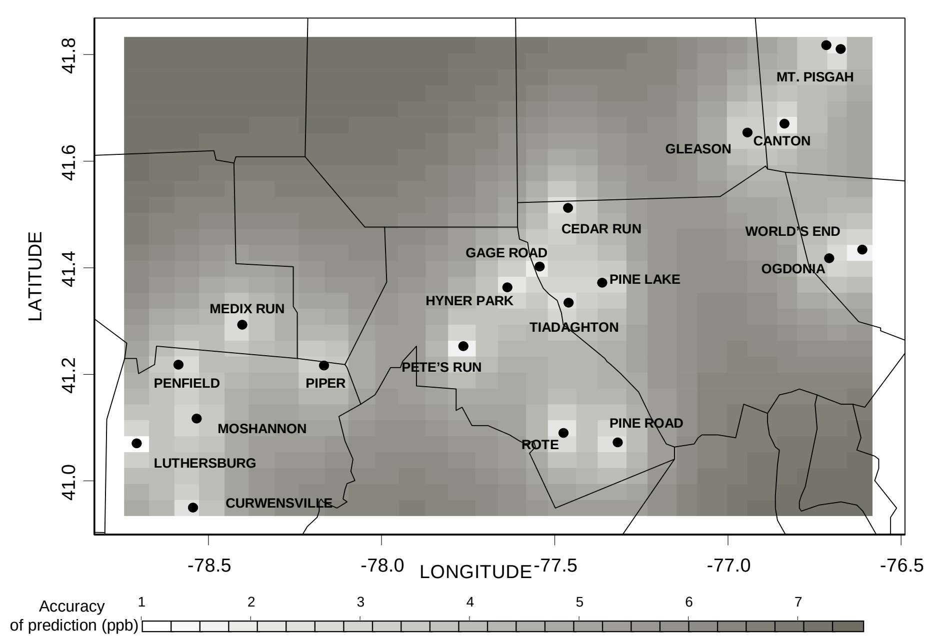


Figure 13. Estimated weekly average of ambient ozone concentrations during 2002 in north-central Pennsylvania.

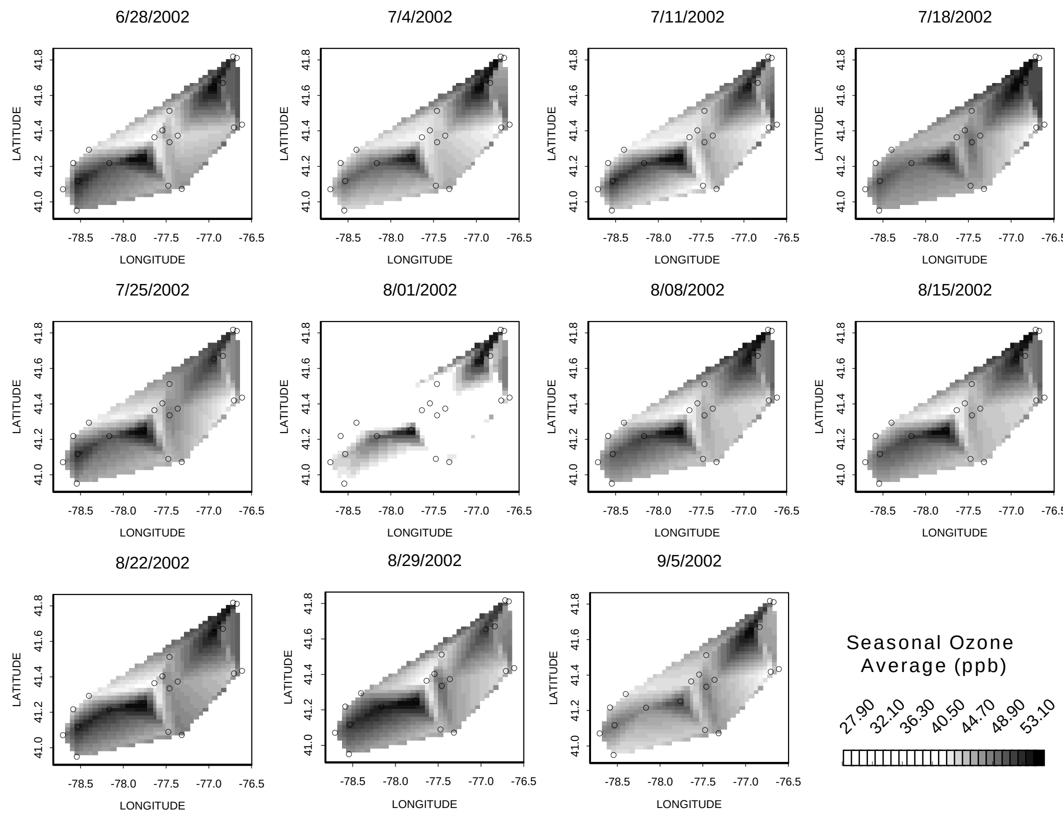


Figure 14. Estimated seasonal average of ambient ozone concentration during 2003 and estimated zones of reliability of ozone predictions, in north-central Pennsylvania.

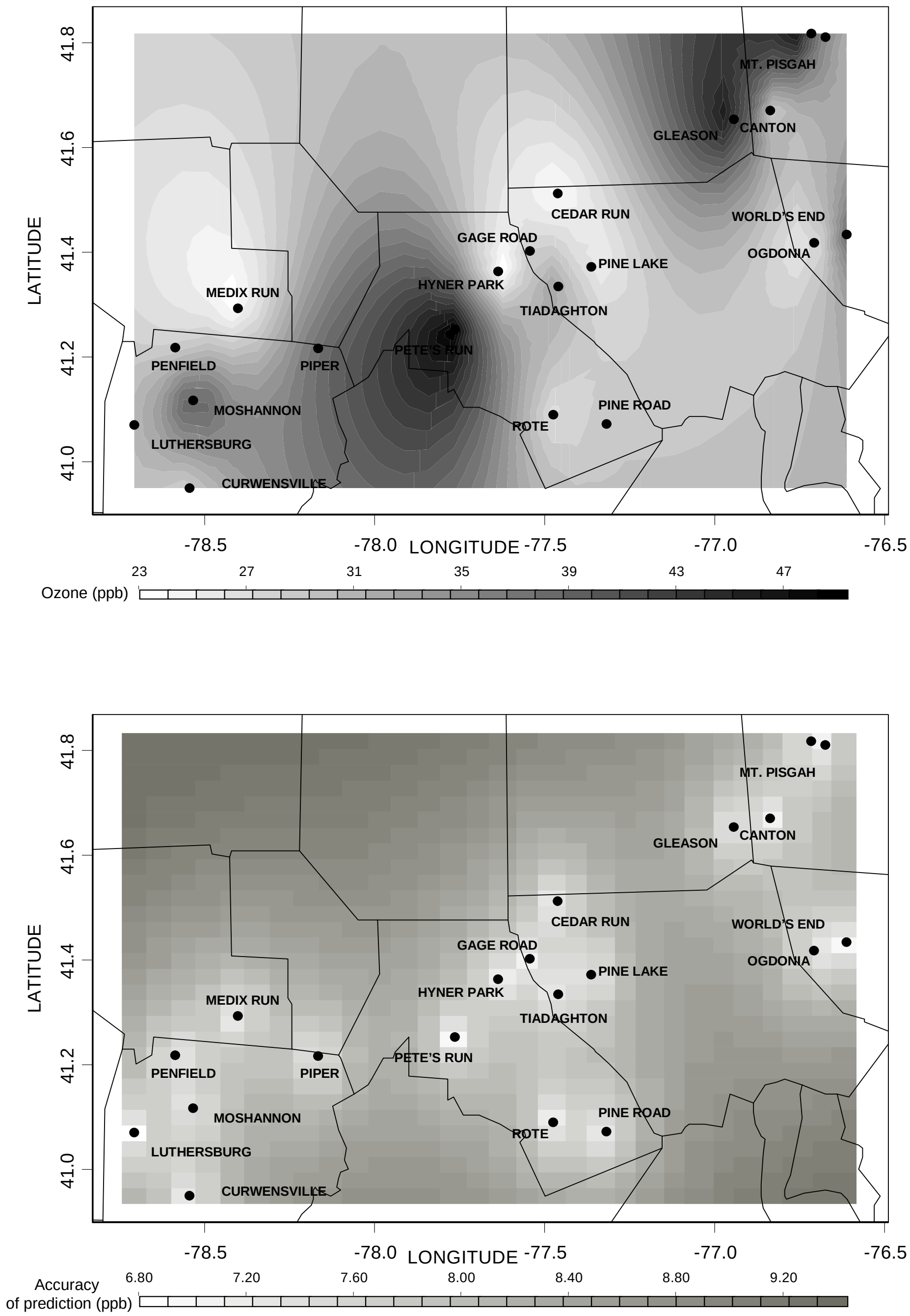


Figure 15. Estimated weekly average of ambient ozone concentrations during 2003 in north-central Pennsylvania.

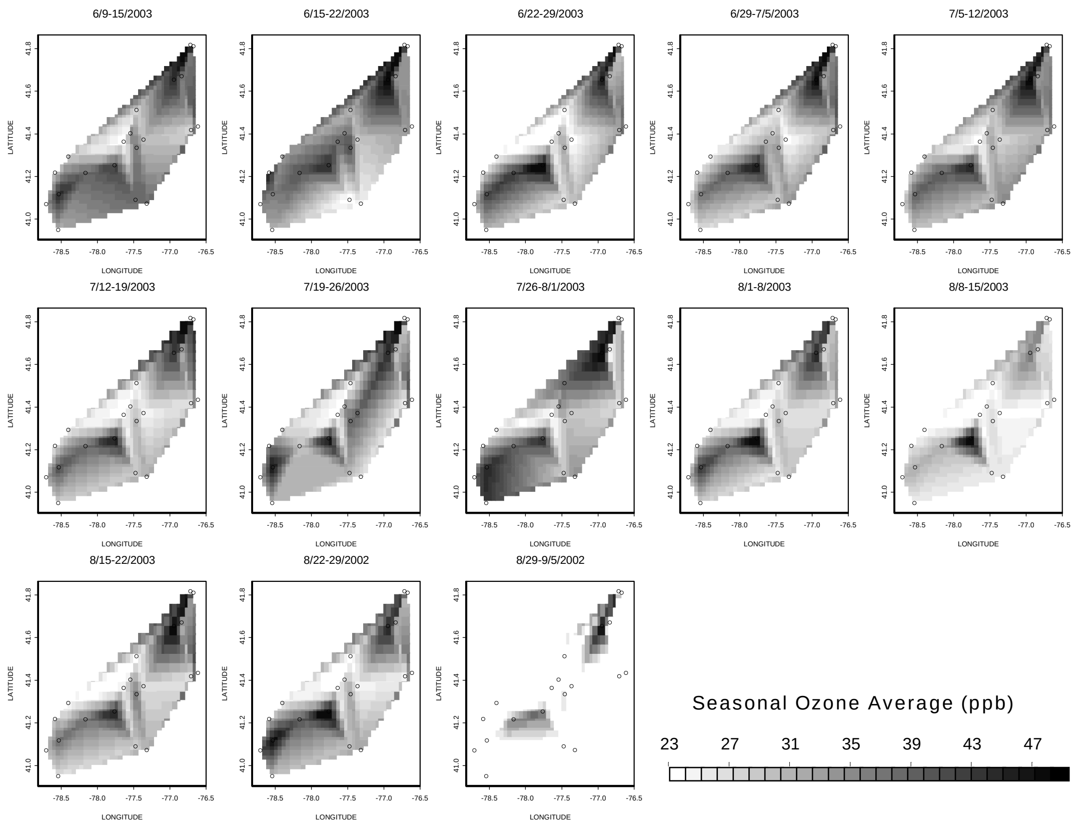
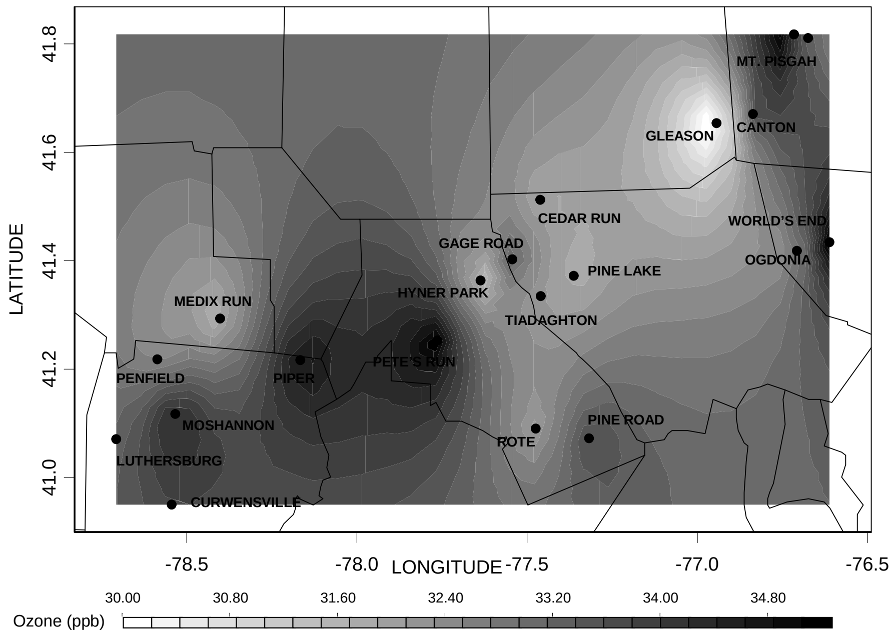


Figure 16. Estimated seasonal average of ambient ozone concentration during 2004 and estimated zones of reliability of ozone predictions, in north-central Pennsylvania.



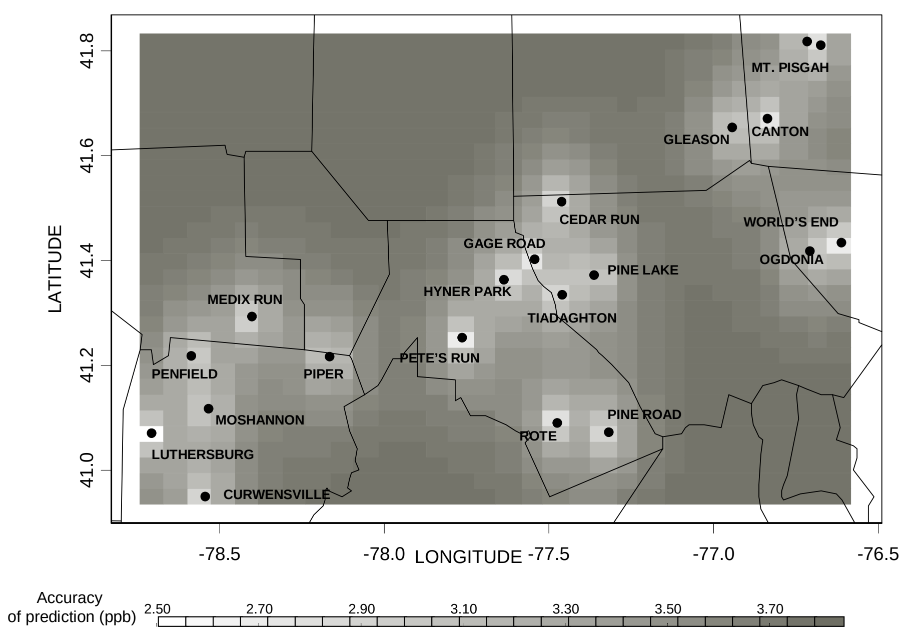


Figure 17. Estimated weekly average of ambient ozone concentrations during 2004 in north-central Pennsylvania.

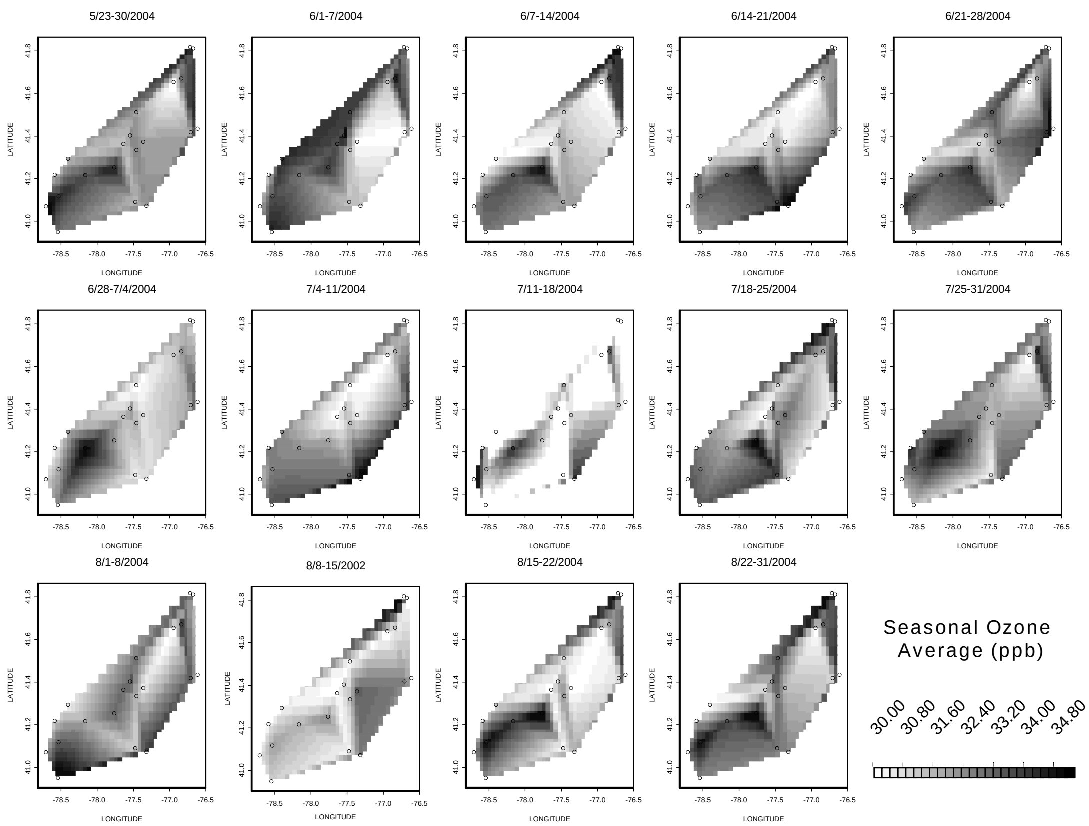
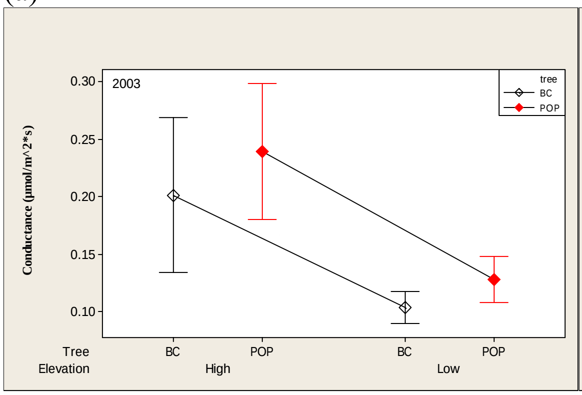


Figure 18. Stomatal conductance for seedlings of black cherry (BC) and hybrid poplar (POP) averaged over the season of 2003 (a), and 2004 (b). Data were collected at 12 open plot sites within north-central Pennsylvania. Data are elevation means and 95% confidence interval for the means.

(a)



(b)

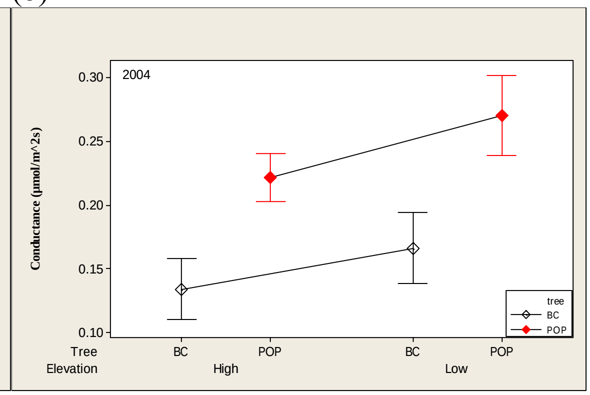
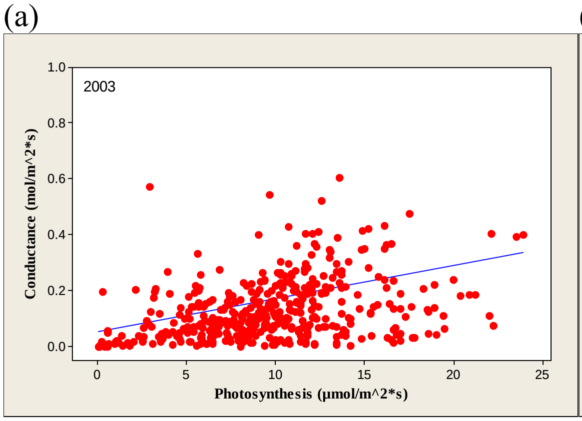


Figure 19. Relationship between stomatal conductance and photosynthesis for the data collected through 2003 (a) and 2004 (b) for seedlings of black cherry and hybrid poplar at the 12 open plot sites within north-central Pennsylvania.

(a)



(b)

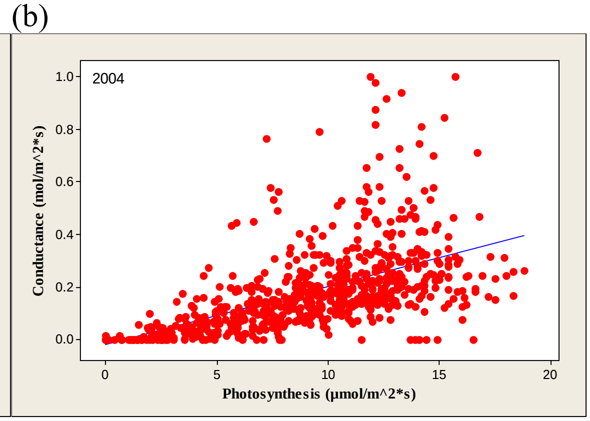
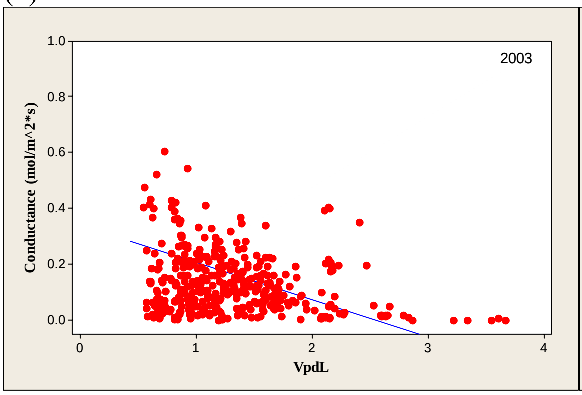


Figure 20. Relationship between stomatal conductance and vapor pressure deficit for the data collected during 2003 (a) and 2004 (b) for seedlings of black cherry and hybrid poplar at the 12 open plot sites within north-central Pennsylvania.

(a)



(b)

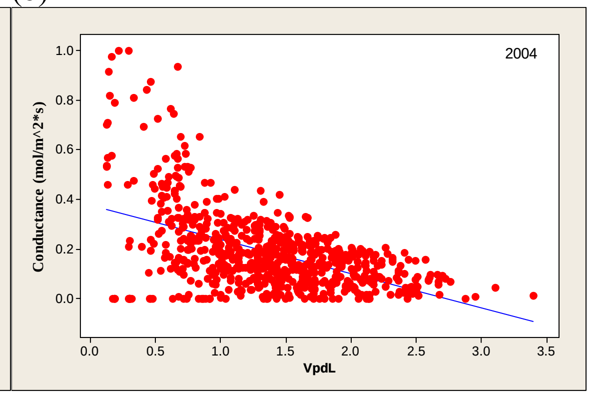
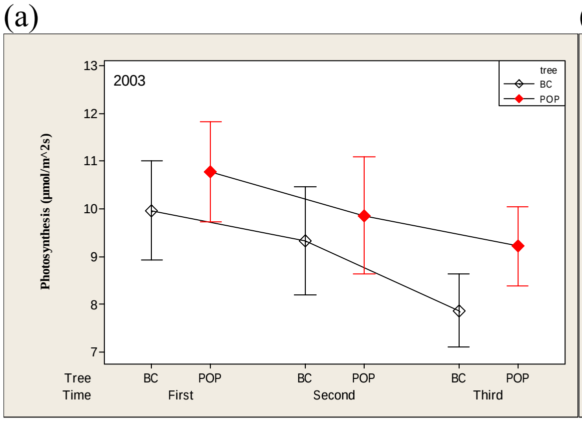


Figure 21. Photosynthesis rates of seedlings of black cherry (BC) and hybrid poplar (POP) averaged over the season of 2003 (a) and 2004 (b). Data were collected at 12 open plot sites within north-central Pennsylvania. Data are means over the time of the measurement and 95% confidence interval for the means.

(a)



(b)

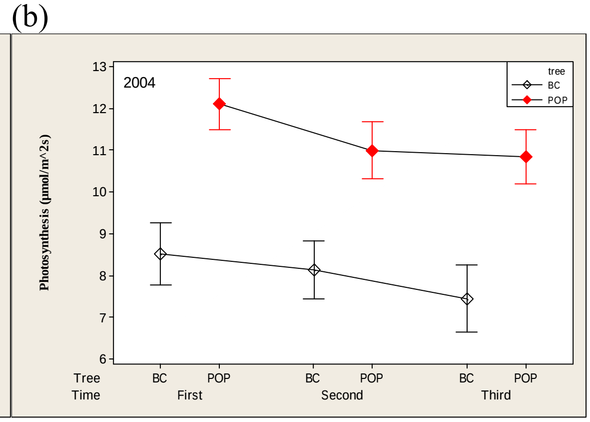
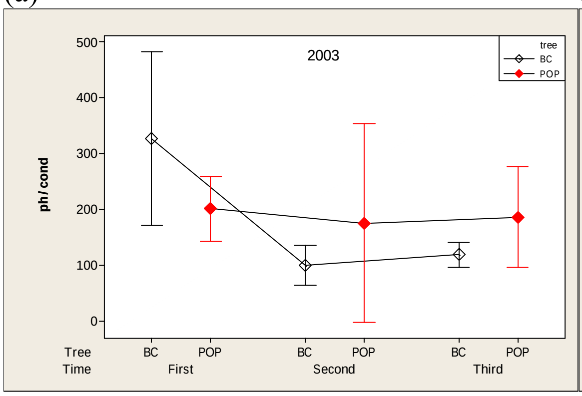


Figure 22. The P_n/g_s ratio for seedlings of black cherry (BC) and hybrid poplar (POP) averaged over the season of 2003 (a) and 2004 (b). Data were collected at 12 open plot sites within north-central Pennsylvania. Data are means over the time of the measurement and 95% confidence interval for the means.

(a)



(b)

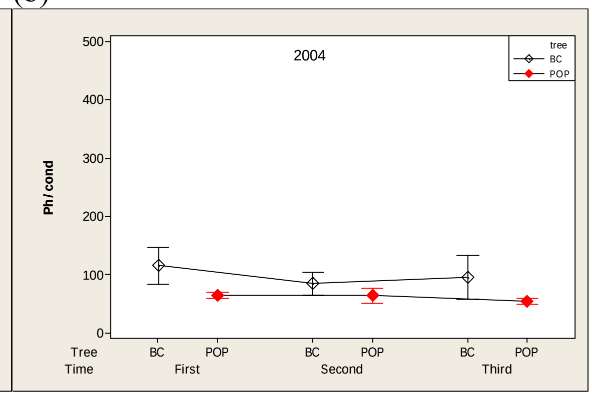
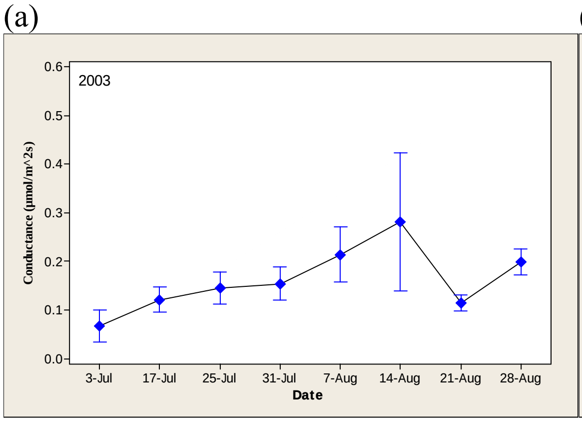


Figure 23. Stomatal conductance of seedlings of black cherry and hybrid poplar averaged over the measurement date of 2003 (a) and 2004 (b). Data were collected at 12 open plot sites within north-central Pennsylvania. Data are site means and 95% confidence interval for the means.

(a)



(b)

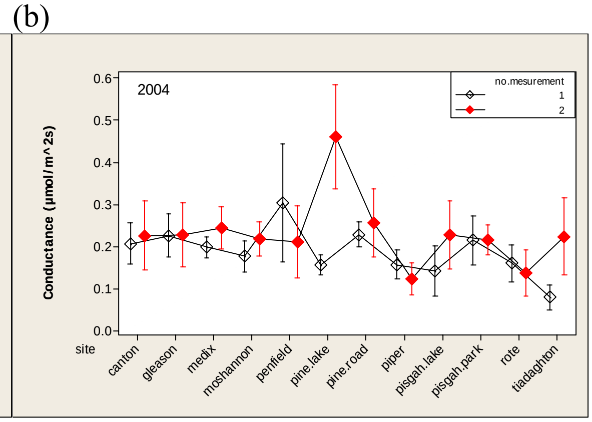


Figure 24. Photosynthesis for seedlings of black cherry (BC) and hybrid poplar (POP) averaged over the measurement date of 2003 (a) and 2004 (b). Data were collected at 12 open plot sites within north-central Pennsylvania. Data are site means and 95% confidence interval for the means.

(a) (b)

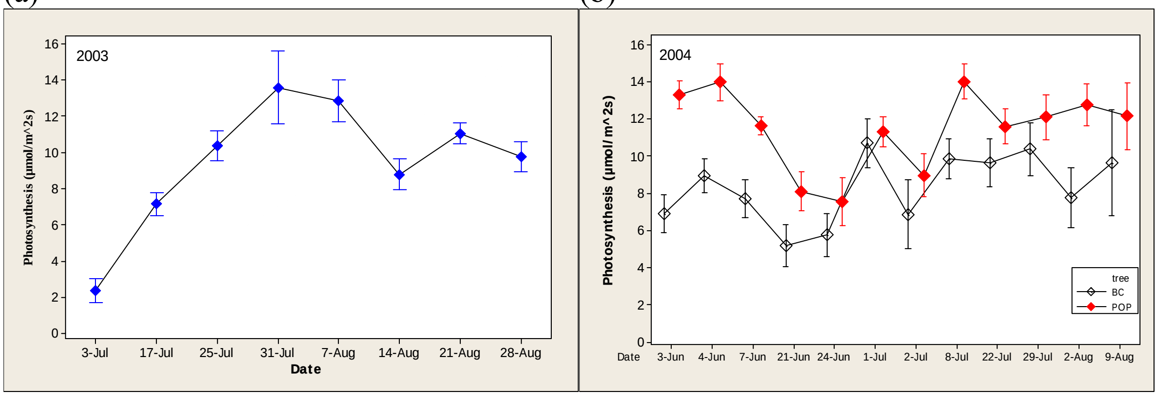


Figure 25. Stomatal conductance for seedlings of black cherry (BC) and hybrid poplar (POP) averaged over the season of 2003 (a) and 2004 (b). Data were collected at 12 open plot sites within north-central Pennsylvania. Data are time of the measurement means and 95% confidence interval for the means.

(a) (b)

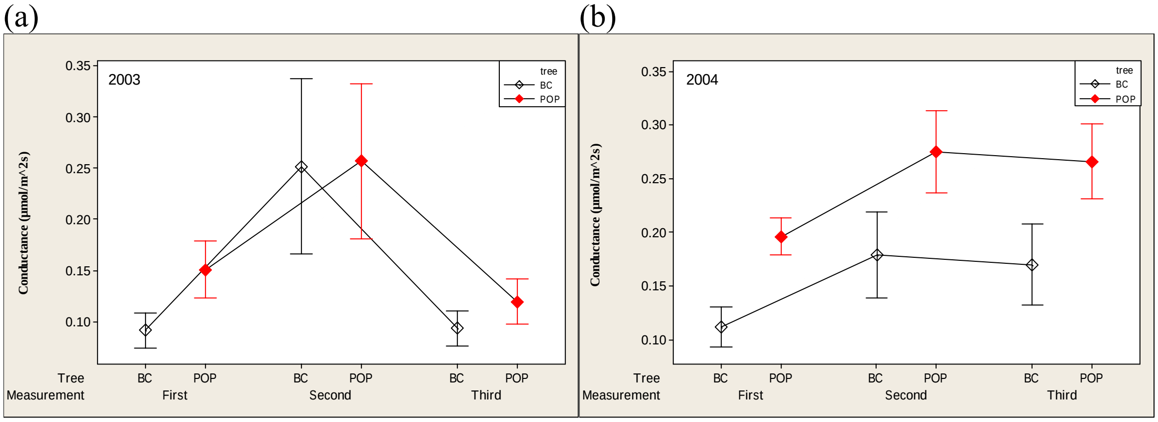


Figure 26. Stomatal conductance (a), and photosynthesis (b) averaged over the season of 2003 and 2004. Data were collected at 12 open plot sites within north-central Pennsylvania. Data are monthly means and 95% confidence interval for the means.

(a) (b)

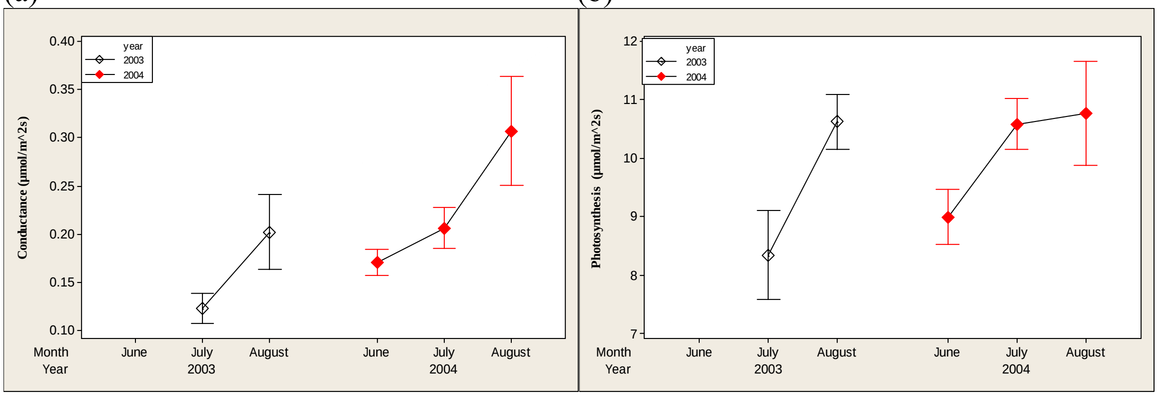


Figure 27. Intensity map of the cumulative ozone distribution (SUM40) in north-central Pennsylvania for 2003, ozone was monitored from June 9th to September 9th.

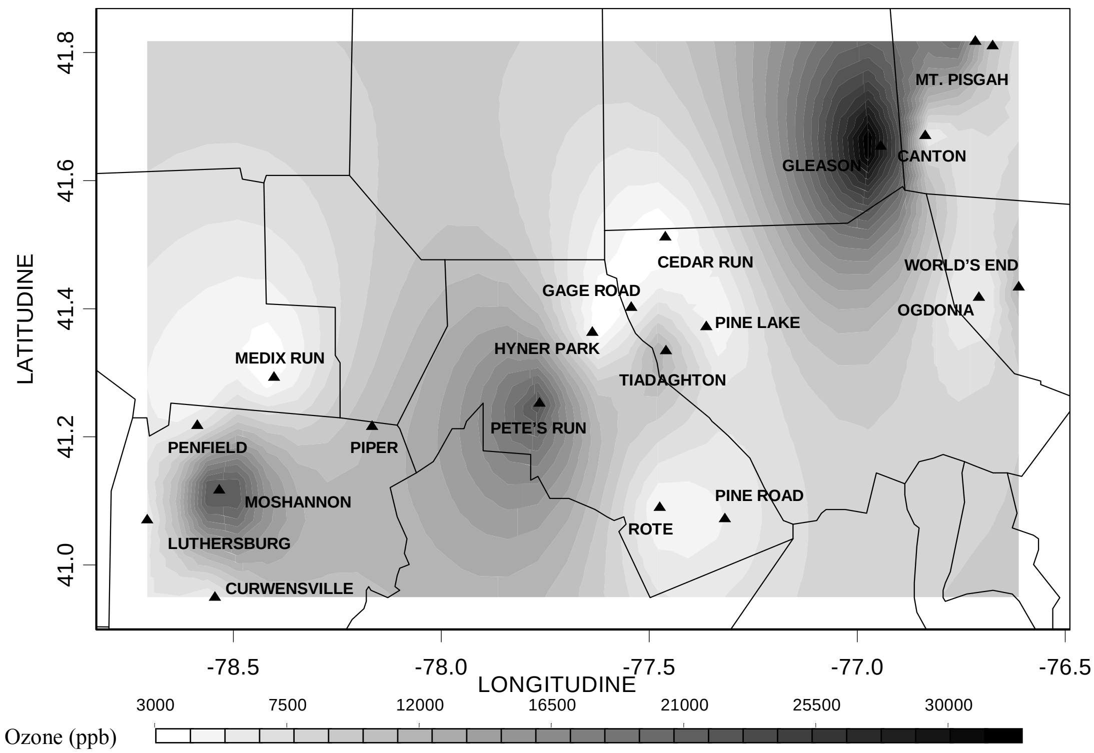


Figure 28. Intensity map of the ozone uptake for black cherry seedlings in north-central Pennsylvania for 2003, ozone was monitored from June 9th to September 9th, ozone uptake was calculated using cumulative SUM0 ozone index.

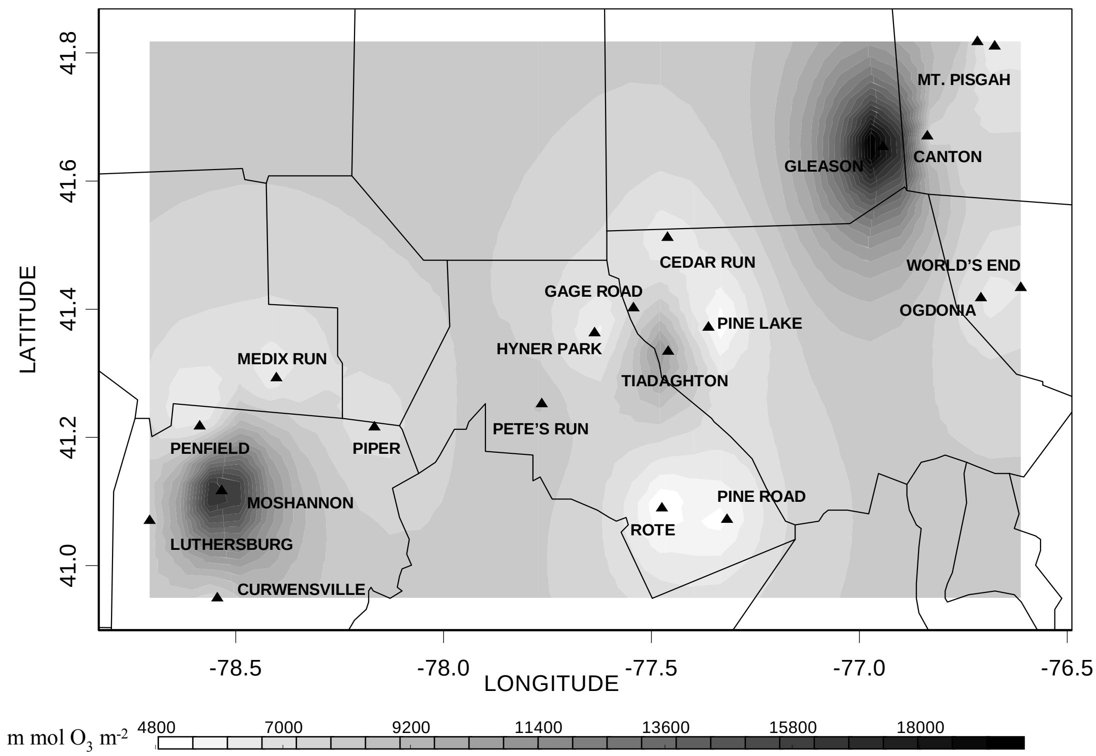


Figure 29. Intensity map of the ozone uptake for hybrid poplar seedlings in north-central Pennsylvania for 2003, ozone was monitored from June 9th to September 9th, ozone uptake was calculated using cumulative SUM0 ozone index.

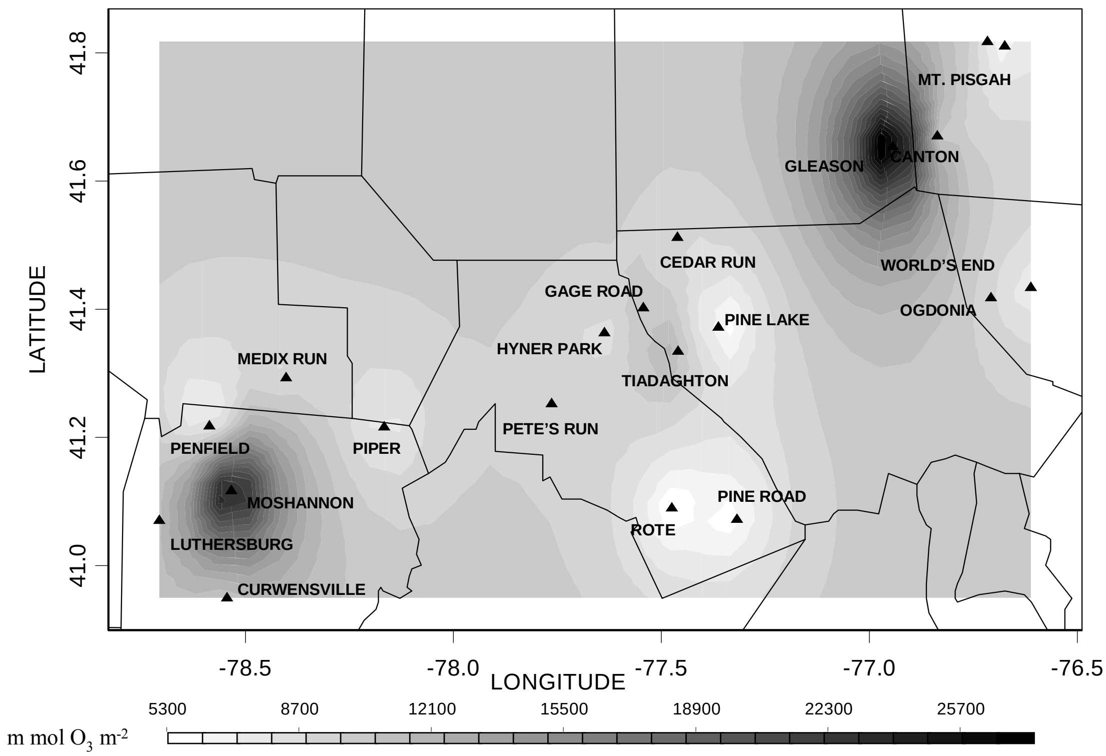


Figure 30. Intensity map of the cumulative ozone distribution (SUM40) in north-central Pennsylvania for 2004, ozone was monitored from May 23rd to September 2nd.

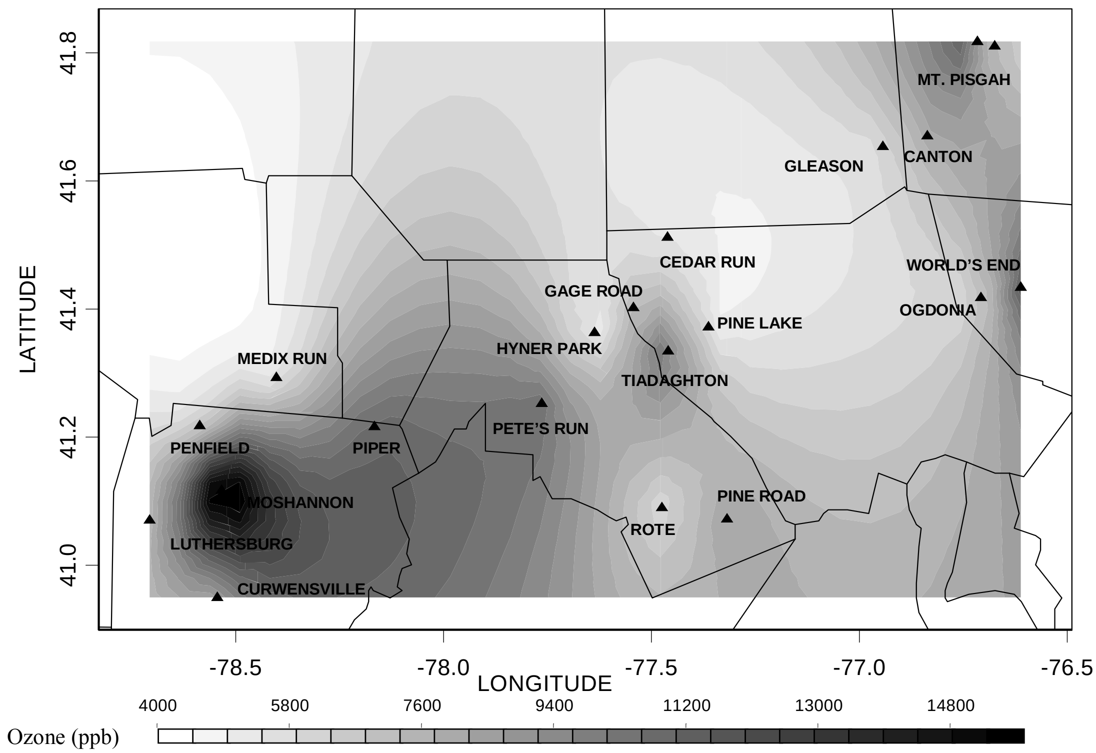


Figure 31. Intensity map of the ozone uptake for black cherry seedlings in north-central Pennsylvania for 2004, ozone was monitored from May 23rd to September 2nd, ozone uptake was calculated using cumulative SUM0 ozone index.

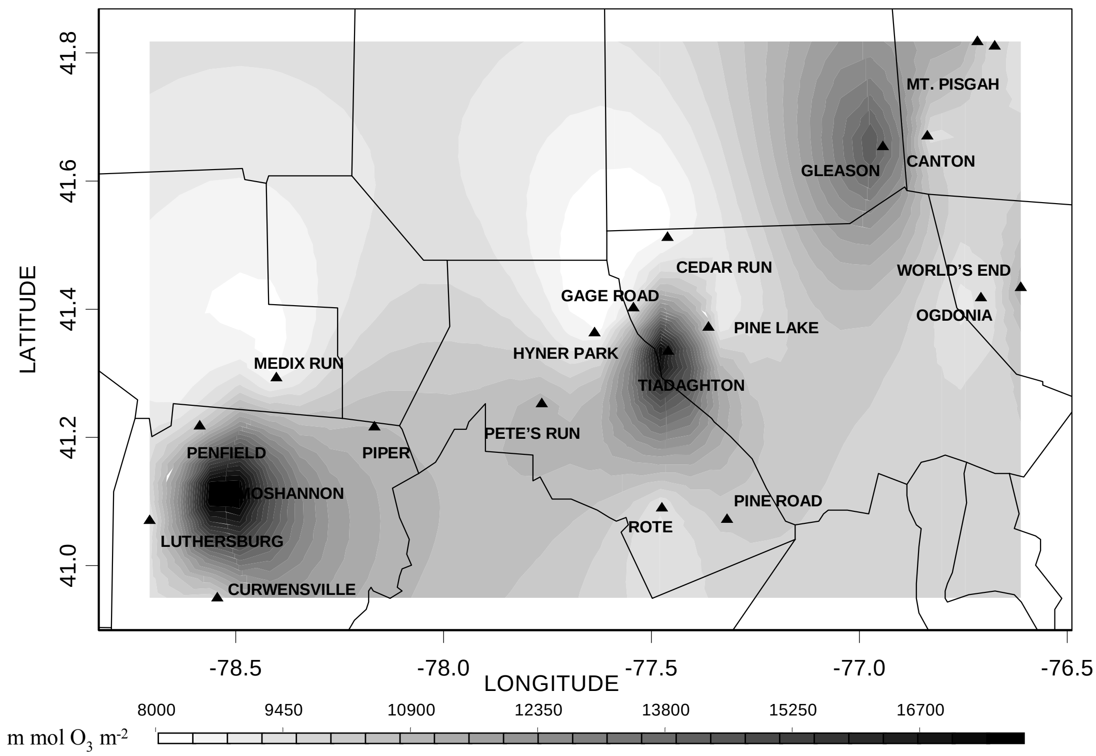


Figure 32. Intensity map of the ozone uptake for hybrid poplar seedlings in north-central Pennsylvania for 2004, ozone was monitored from May 23rd to September 2nd, ozone uptake was calculated using cumulative SUM0 ozone index.

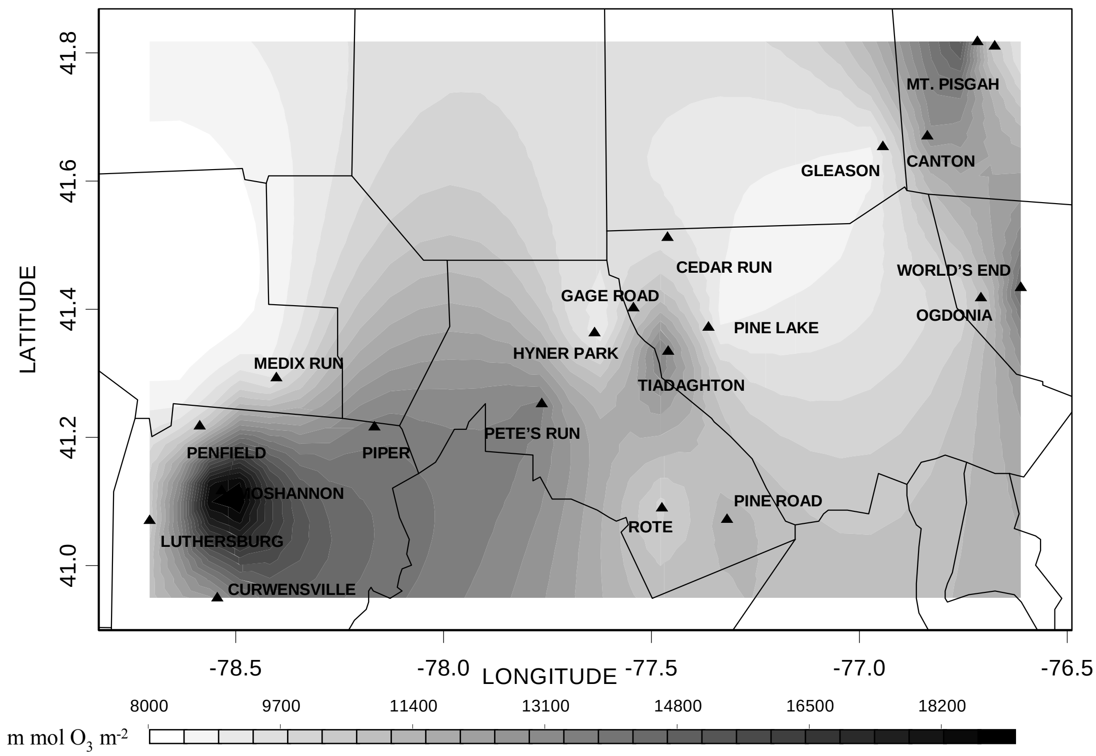


Figure 33. Annual means of ozone concentrations and std. dev. as measured at the 20 sites in the north-central Pennsylvania during 2003 and 2004.

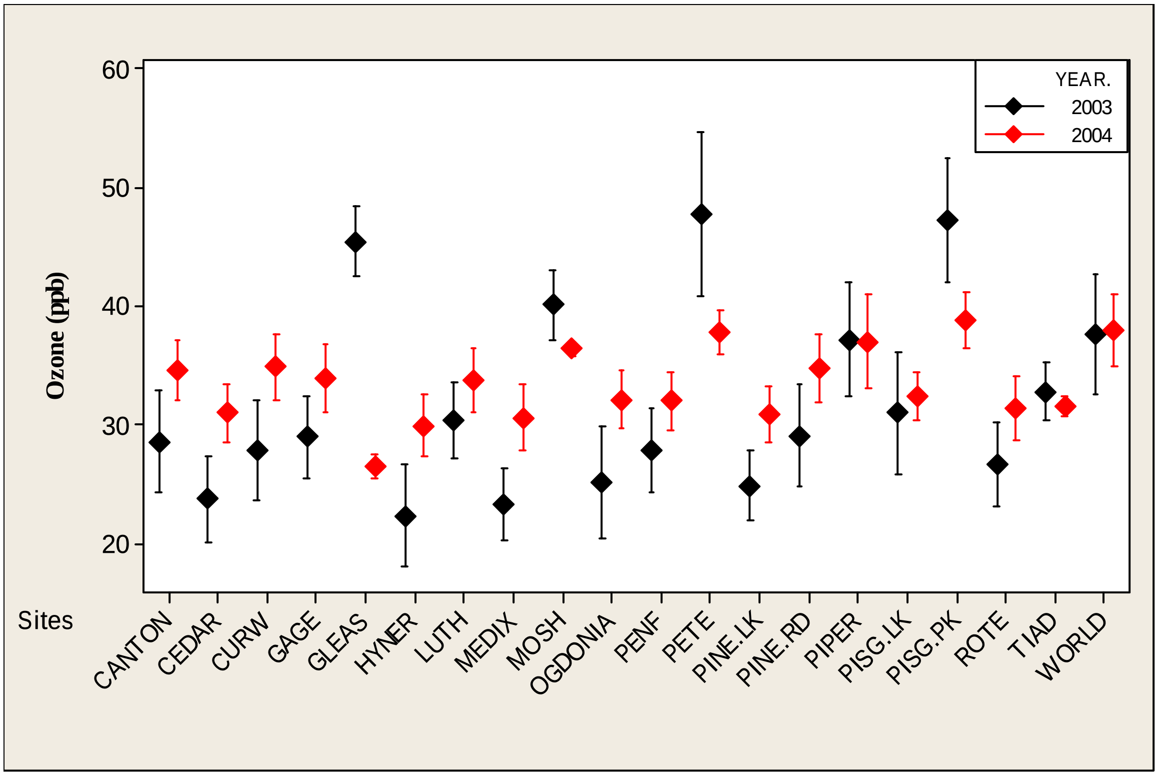


Figure 34. Seasonal ozone average spatial distribution during 2003 in north-central Pennsylvania, ambient ozone was monitored with Ogawa passive samplers from June 9th to September 9th, map shows county borders and study sites.

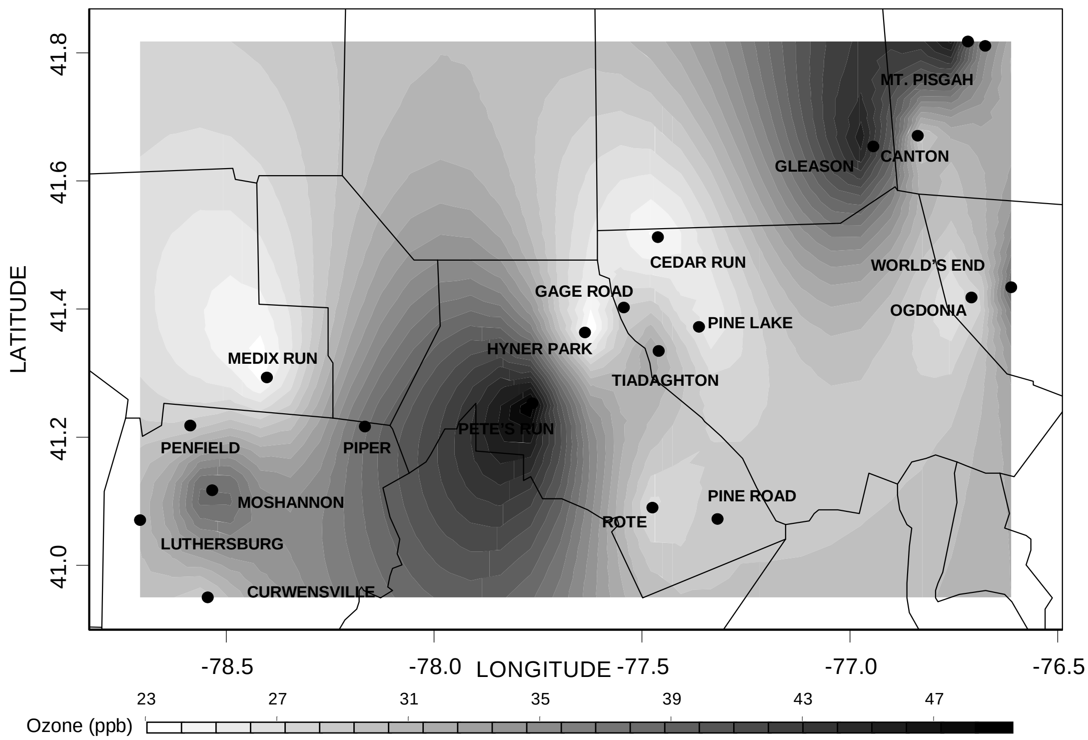


Figure 35. Seasonal ozone average spatial distribution during 2004 in north-central Pennsylvania, ambient ozone was monitored with Ogawa passive samplers from May 23rd to September 2nd, map shows county borders and study sites.

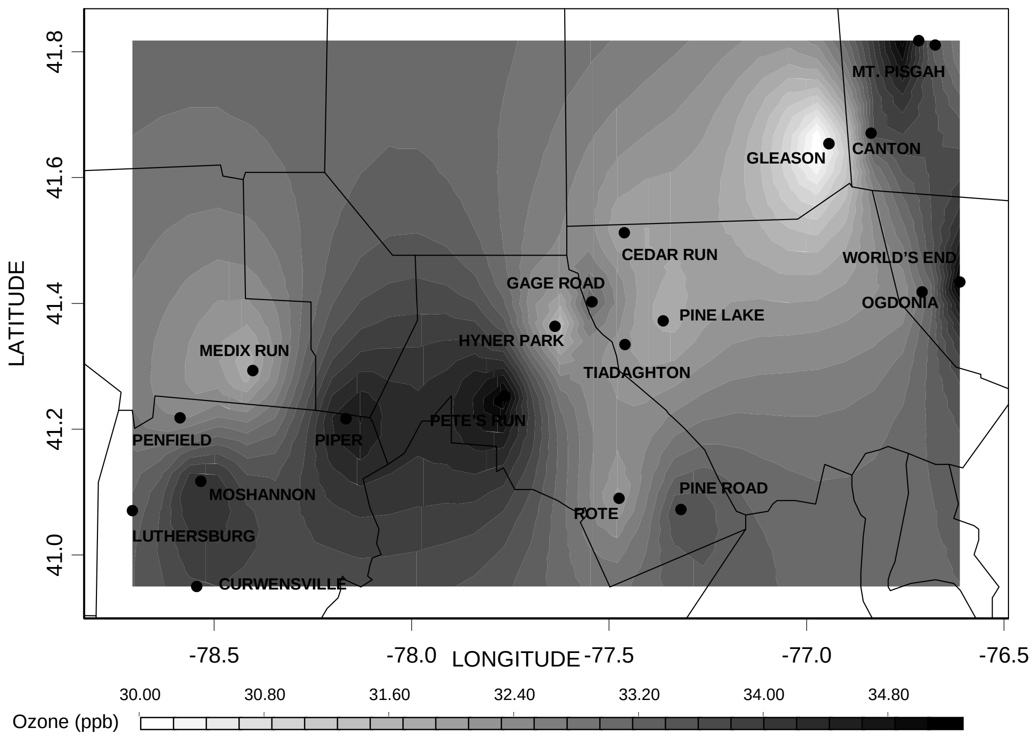


Figure 36. Temporal progress of ozone-induced leaf injury (% INJ) for black cherry and hybrid poplar as recorded at 20 study sites in north-central Pennsylvania during 2003 and 2004. Data are site means.

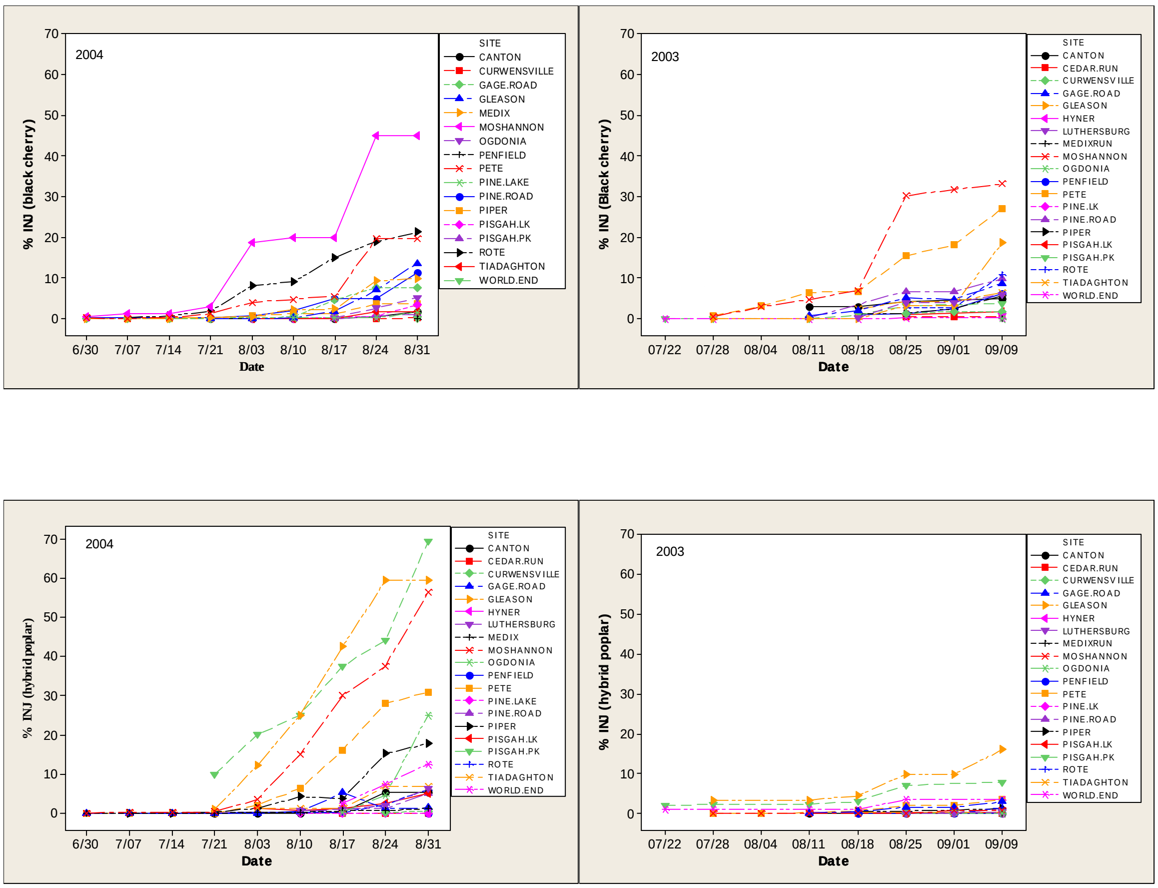
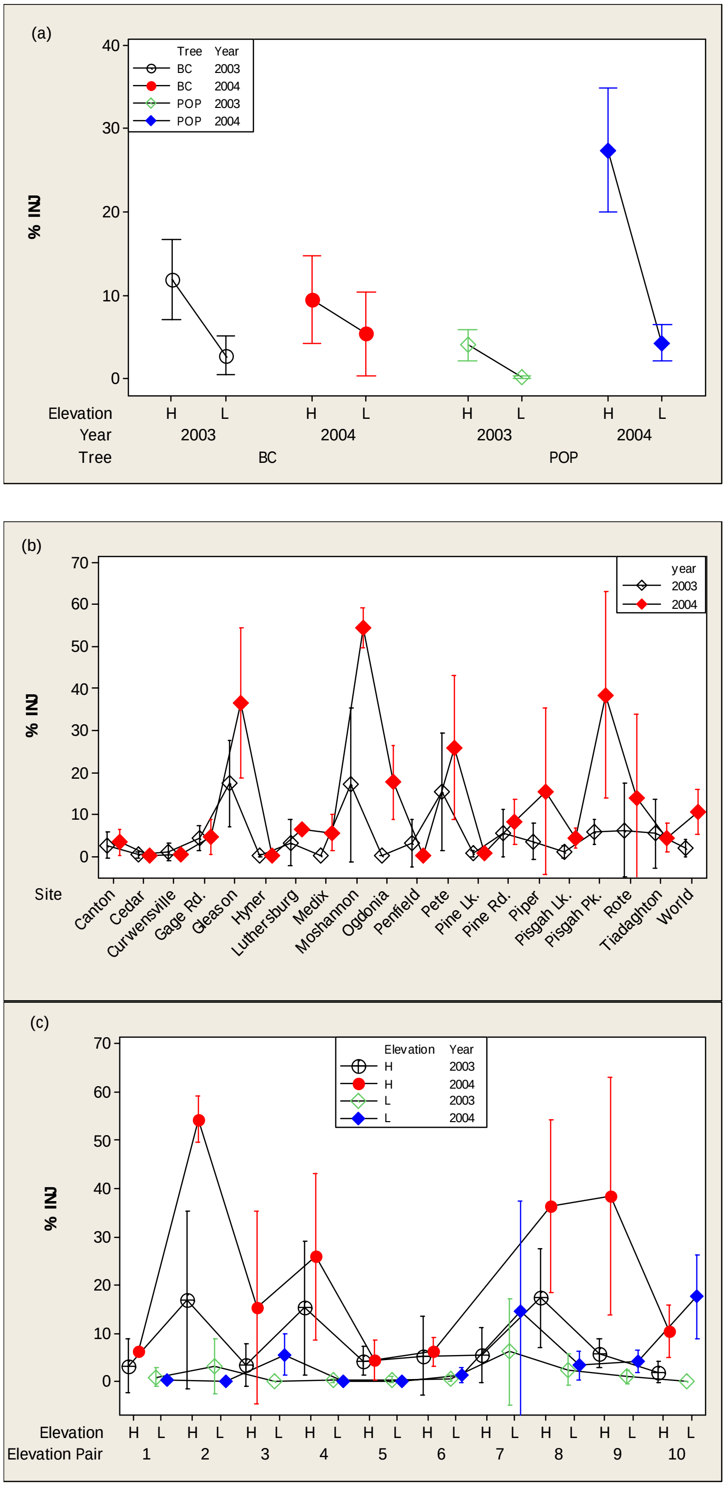


Figure 37. Average injury (%INJ) at high (H) and low (L) elevation sites for black cherry (BC) and hybrid poplar (POP) (a), %INJ per site (b), and %INJ at elevation pairs (c) in the north-central Pennsylvania for 2003 and 2004.



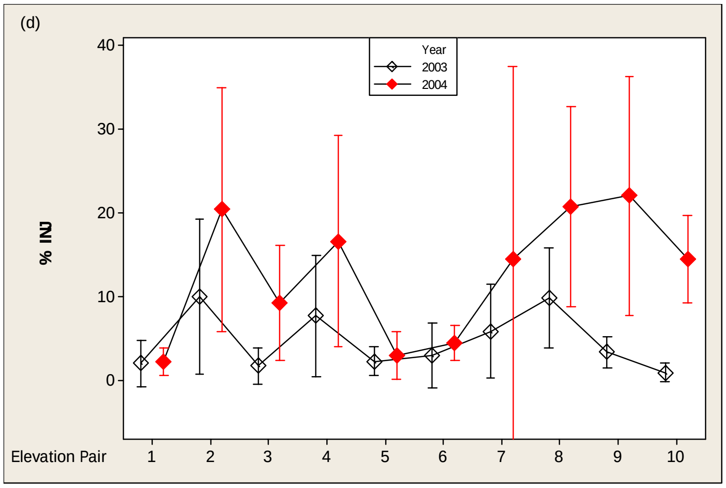
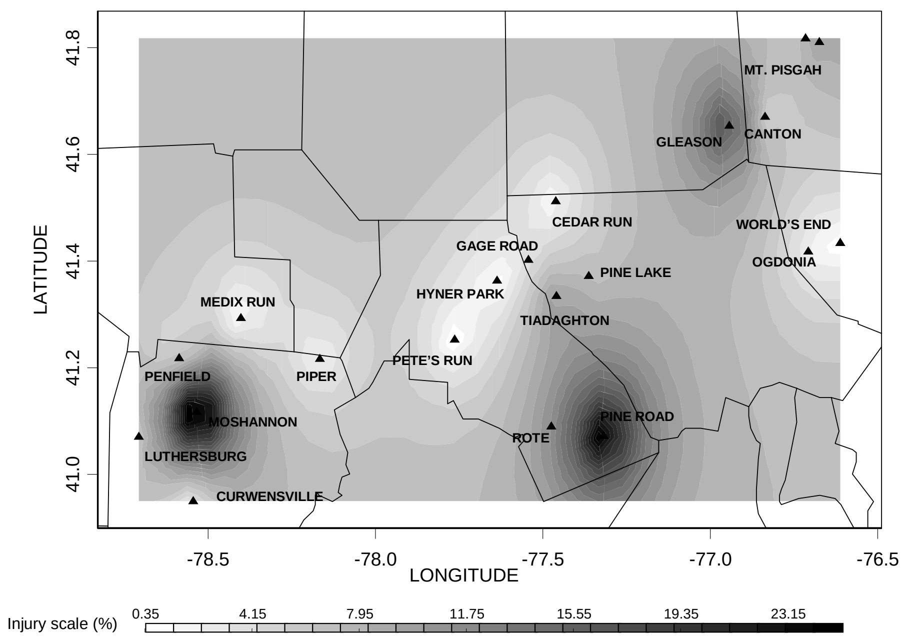

(d)
Year
2003
2004
% INJ
40
30
20
10
0
Elevation Pair
1
2
3
4
5
6
7
8
9
10

Figure 38. Estimated ozone injury for black cherry (%INJ) (a) and ozone uptake by black cherry seedlings (m mol O_3 m^{-2}) (b), during 2003 study season.

(a) Injury



(b) Uptake

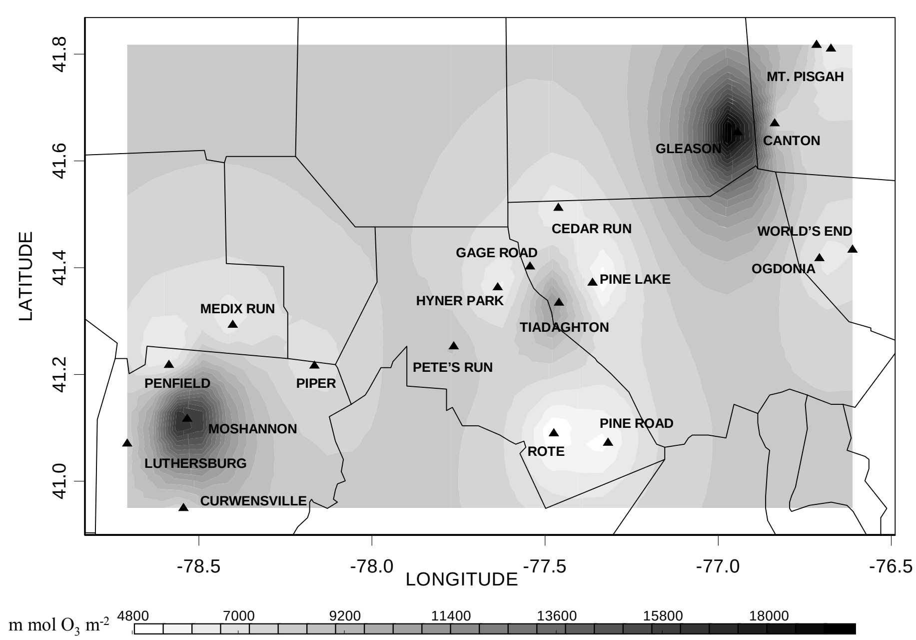
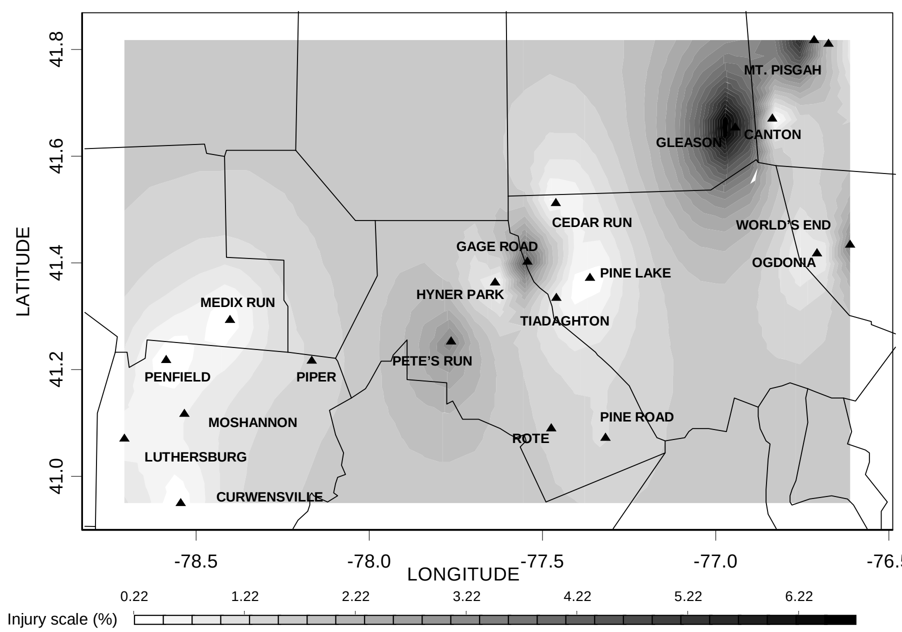


Figure 39. Estimated ozone injury for hybrid poplar (%INJ) (a) and ozone uptake by hybrid poplar cuttings (m mol O_3 m^{-2}) (b), during 2003 study season.

(a) Injury



(b) Uptake

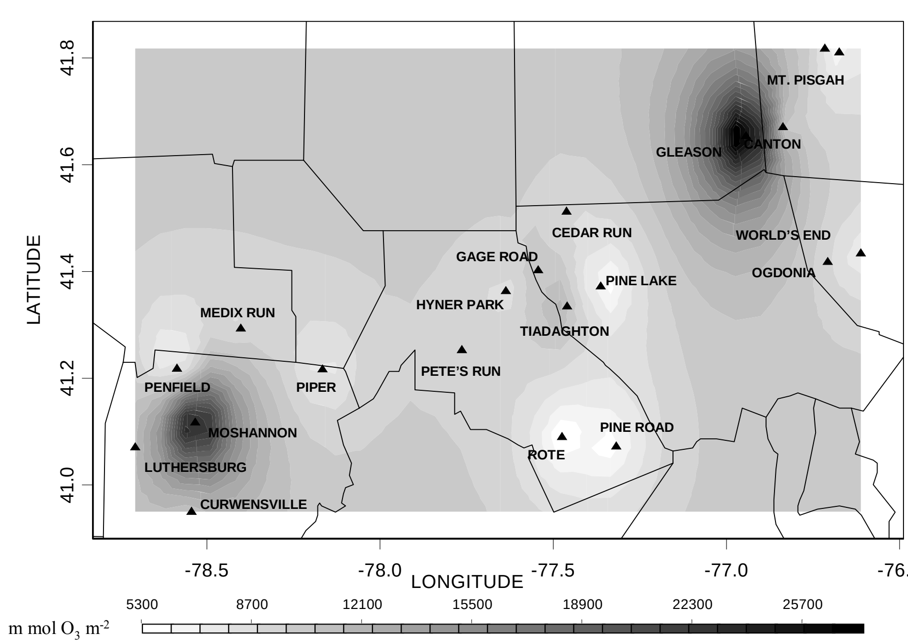
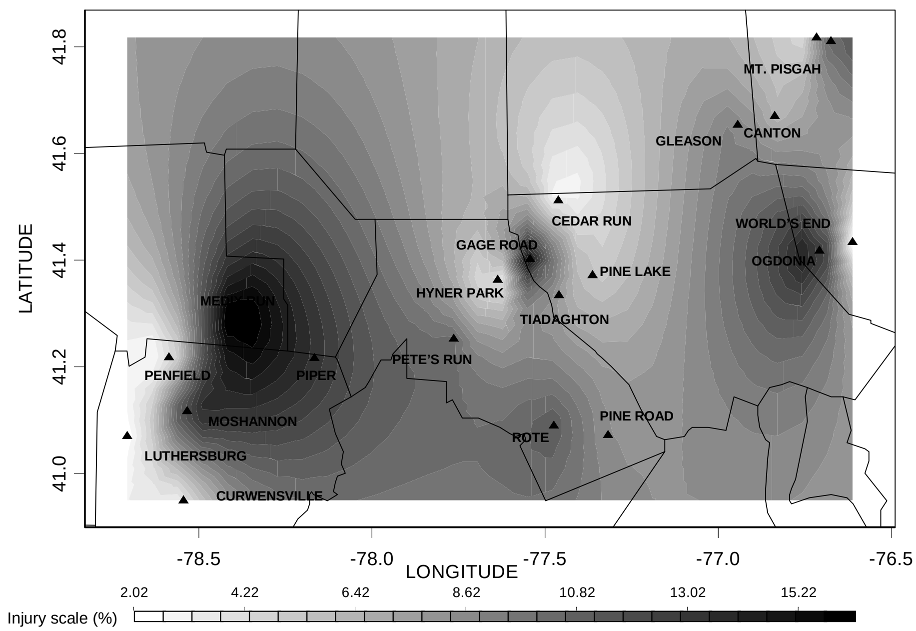


Figure 40. Estimated ozone injury for black cherry (%INJ) (a) and ozone uptake by black cherry seedlings (m mol O_3 m^{-2}) (b), during 2004 study season.

(a) Injury



(b) Uptake

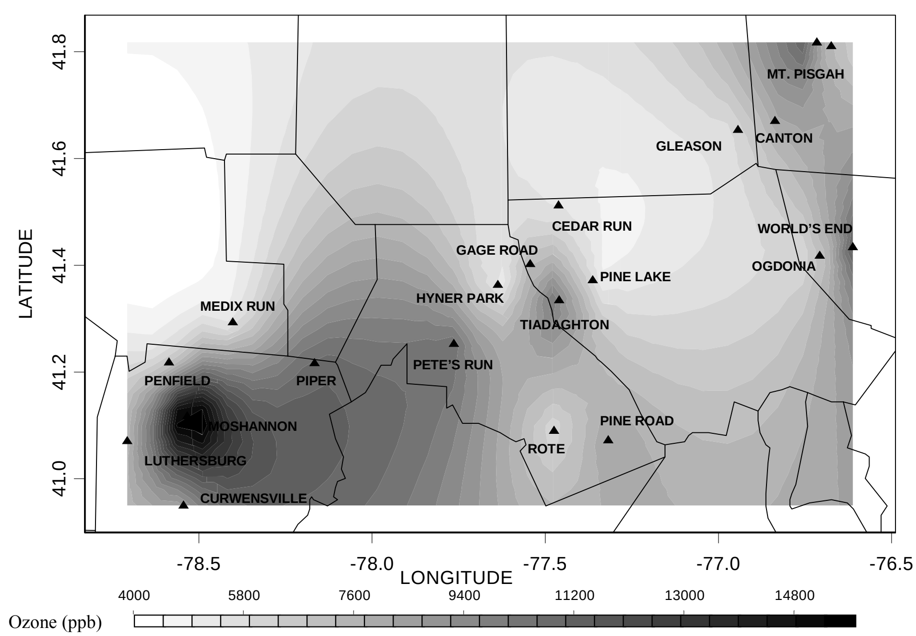
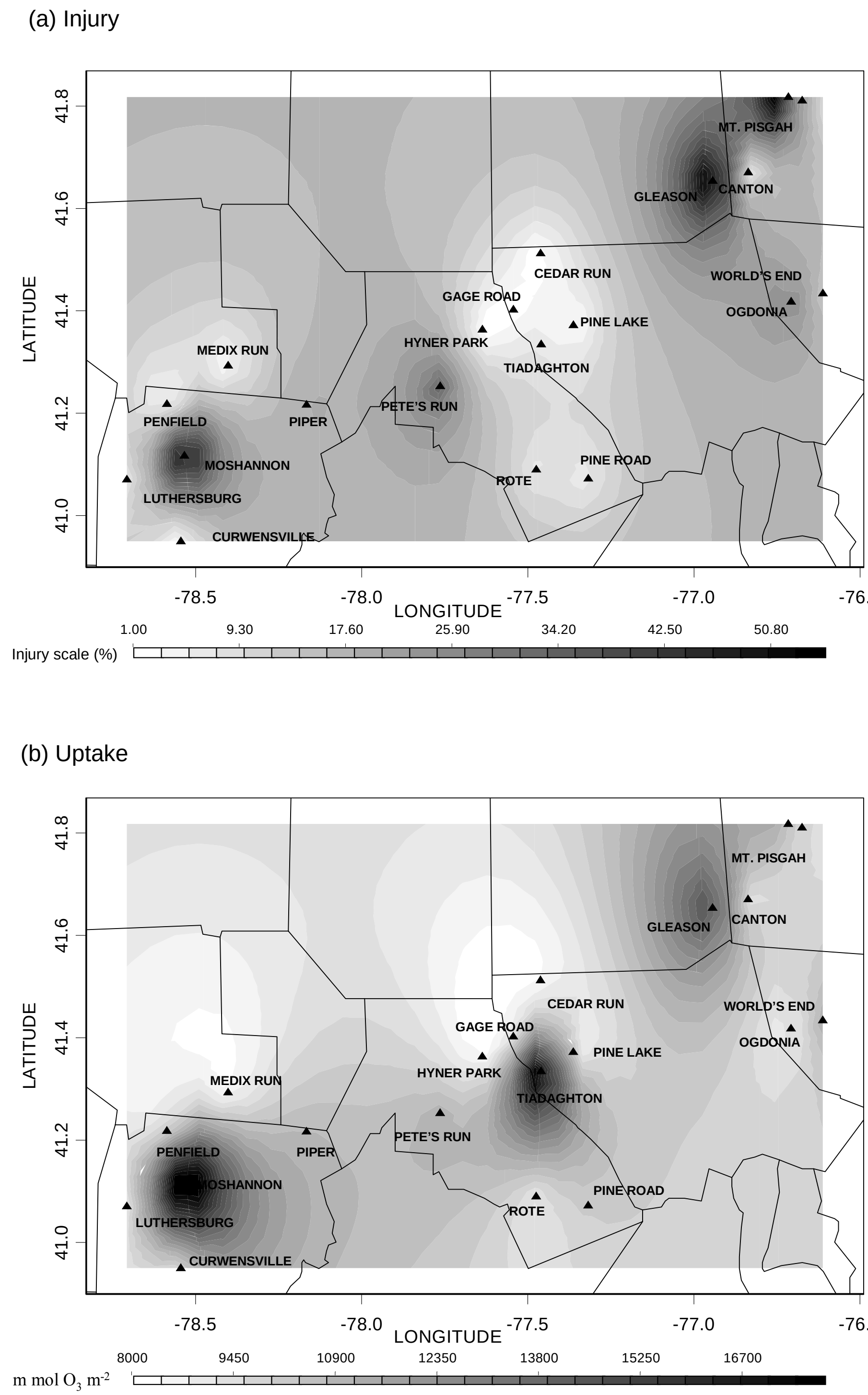


Figure 41. Estimated ozone injury to hybrid poplar (%INJ) (a) and ozone uptake by hybrid poplar cuttings (m mol O_3 m^{-2}) (b), during 2004 study season.



VITA

Teodora Orendovici

Teodora Orendovici was born on March 12, 1964, in Hunedoara, Romania. She is the daughter of Florica and Teodor Tanasescu. She graduated from high school “Electrical High School” in Deva, Roamania in 1983. Ms. Orendovici attended “The West University of Timisoara” Romania from 1984 to 1987, and majored in mathematics. After graduation she worked as a high school teacher from 1987 to 1999 in Timisoara, Romania. In January 2000 Ms Orendovici was appointed to a Graduate Research Assistantship and she enrolled in a Master of Science program at The Pennsylvania State University in the Environmental Pollution Control under the guidance of Prof. John M. Skelly. As a graduate student at Penn State she focused her research in determining the response of native plants of north-eastern USA to ozone exposures. She was conferred a Master of Science degree in August 2002. In August 2002 Ms Orendovici enrolled into the PhD program in Ecology under the guidance of Prof. John M. Skelly and Don D. Davis.

During her graduate programs Ms. Orendovici attended from 32^{nd} to 36^{th} National Air Pollution workshops in the United Stats.