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SCREENING NATIVE PLANTS OF NORTHEASTERN UNITED STATES AND SOUTHERN SPAIN FOR SENSITIVITY TO OZONE.

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Introduction

Tropospheric ozone has been identified as the single most important regional scale air pollutant of concern to the health and productivity of forests in the United States. Similarly recent studies have shown high ozone exposures and effects to vegetation across many areas of Mediterranean Climes in southern Europe.

The long-rang transport of ozone and its photochemical precursors has been well documented across Northeastern United States and much of the European continent. More specifically, patterns of tropospheric ozone have been identified across the sub-Alpine regions of northern Italy and southern Switzerland. In southern Europe, recent studies have shown high ozone exposures in southern Spain, and described physiological - chemical processes which relate the atmospheric circulation at various scales from local, to regional, to sub-continental areas, with behavior of polluted air masses over the Iberian peninsula and the western Mediterranean region. Within both regions as involved in the present study, i.e. northeastern United States and southern Spain, growing-season ozone exposures have been shown to induce distinctive foliar symptoms on several ozone sensitive plant species and on several more plant species, which have yet to be proven due to ozone exposures.

On broadleaf species, visible symptoms resulting from ozone exposure are exhibited as mild chlorosis, adaxial leaf surface pigmentation (stippling) and premature leaf senescence. Stippling has been described as the most definitive symptom of ozone injury.

Recent field surveys of natural vegetation (1999,2000)conducted during the United States Department of Agriculture - Forest Health Monitoring programs in northeastern USA have revealed many more plant species that exhibit ozone-like foliar stipple and mid summer season leaf reddening. In many areas of southern Spain, Sanz and Millan (1998) have described ozone-like patterns of foliar injuries to forest species within native forest and natural plant communities. Several studies which have followed the field surveys have determined ozone exposures to be a cause of symptoms of several species. More recent surveys (1999, 2000) conducted by Centro De Estudios Ambientales Mediterraneo (CEAM) in southern Spain have determined foliar ozone-like injury to be occurring on many more plants species than had been identified in previous surveys (1996-1997).

The objective of this continuing study is to evaluate the response of several newly identified field-symptomatic species to controlled ozone exposure as delivered within a Continuously Stirred Tank Reactor (CSTR) chamber system at Penn State University. The eventual goal of this collaborative project is the development of several native species, which may serve as bioindicators of ambient ozone exposure while plants are growing under natural field conditions.

Materials and Methods

To confirm ozone sensitivity on plant species, a series of experiments under controlled ozone exposures have been conducted within 4 (CSTR) chambers situated within a greenhouse equipped with a charcoal filtration air supply system on the Penn State University Park campus. The greenhouse is also equipped with a cooling-humidification system and thermal blanket for temperature control, supplemental lighting, and exhaust fan system.

Each CSTR chamber has been equipped with an external light source, which produces light quality similar to natural sunlight. Light, temperature, and relative humidity were monitored throughout the exposure treatment periods. Within each CSTR chamber, ozone was delivered via a controllable micro metering system with concentrations monitored with a TECO Model 49 ozone analyzer and data logger/computer recording system.

Figure 1. The CSTR chamber system as situated within a charcoal filtered air-supplied greenhouse on the campus of the Pennsylvania State University. The four chamber delivering 30, 60, 90, 120 ppb ozone are seen in the background, each with its own light source; a Griffin ozone generator is within the building to the left.

Four ozone exposure treatments (30, 60, 90, and 120 ppb) were delivered in square-wave fashion for 7 hrs, 5 days/wk; exposures began at 0900 hr.and ended at 1559 hr.

The study design for the 2000 growing season consisted of one replicate for each ozone treatment but with sufficient number of plants from each plant species investigated to provide a viable test of species sensitivity. Species were placed into the chambers and exposed until typical ozone symptoms developed or for a period of no longer than 6 weeks. Several entries of species were made into the exposures depending upon the availability of the respective species, plant condition, and the rapidity of symptom development for those species already undergoing exposures. Once symptoms were confirmed at the 60 and >60 ppb ozone exposures, that respective species was removed from the 2000 investigation.

Mature seeds from symptomatic plants, were collected during each of the respective surveys. Seeds from sSpain were cleaned, dried and sent to the Pennsylvania State University following proper entrance through the USDA Plant Introduction Station for import into the United States. Seedlings and seed were obtained from several sources for the Northeastern USA species.

During the growing season of 2000 we fumigated: *Acer saccharinum*,L., *Asclepias incarnata,L.*, *Cercis Canadensis*,L., *Campsis radican,L.*, *Helaintus divaricatus*,L., *Prunus americana*, Ehrh., *Rhus copalina*,L, *Viburnum lentago*, L., and *Vernonia altissima*, Schreb. plant species from the USA, and *Collutea arborescens*,L., *Epilobium ssp.* L., *Fraxinus ornus,* L., *Iberis Carnosa*,Willd., and *Ulmus minor*,Mill. plant species from Spain.

Experiment-long temperature and relative humidity averaged 82, 80, 79, and 80° F, and 73, 76, 76, and 78% for each treatment chamber. Foliar injury ratings of all seedlings within all treatments were recorded daily; visual assessments of ozone-induced injury to foliage have been done using the Horsfall-Barratt rating system.

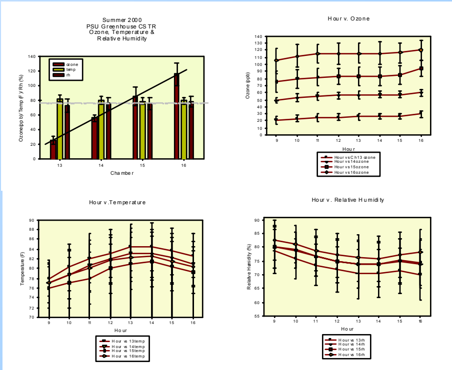

Figure 2. The Ozone concentrations (ppb), Temperature (F) and Relative Humidity (%), averages for the 2000 summer.

Results and Discussion

Of the nine species from the United States thus far tested for ozone sensitivity within the CSTR chambers, all have proven to be symptomatic to ozone exposure under moderate ozone concentrations, and following relatively short duration of exposures. Typical ozone induced upper surface stipple, chlorotic spotting, and pre-mature leaf senescence were noted on all species.

Thus far, the ranking of plant sensitivities as based upon the severity of visual symptoms would follow as (most sensitive) Rhus copalina > Asclepias incarnata > Acer saccharinum > Heliantus divaricatus > Cercis canadensis >Vernonia altissima = Viburnum lentago = Prunus americana = Campsis radicans

Figure 3. (*Acer saccharinum*,L.) visual symptoms were observed as a white colored stipple, no matter the length of exposure or treatment applied. At 120 ppb the first symptom appeared after 14 hr of treatment and after 25 days some of the plants shown symptoms of early senescence. The lowest level of treatment at which plants developed visual injuries was 60 ppb. None of the plants kept in the charcoal filtered air supplied greenhouse for control or those exposed to 30 ppb ozone treatment showed the stipple or any other ozone-like injuries.

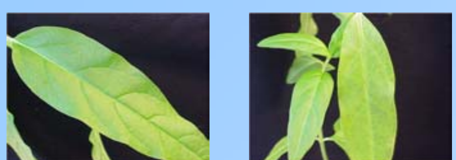

Figure 4. (*Asclepias incarnata,L.)* Visual symptoms were initially observed as an upper leaf surface reddening and brown stipple; after prolonged exposure early leaf senescence also developed. The first symptoms were noted after 7 hr of exposure within the 120 ppb treatment chamber. No ozone-like symptoms were observed on plants within the 30 ppb treatment chamber. Instead a strong difference in growth between plants exposed to 120 ppb and 90 ppb and those exposed to 60 ppb and 30 ppb ozone concentrations was noted. Plants kept in the charcoal filtered air supplied greenhouse as controls shown no ozone injury.

Figure 5. (*Cercis Canadensis*,L.) After 5 weeks of fumigations all plants exposed within the 90 and 120 ppb ozone treatments developed symptoms, and only half of those exposed within 60 ppb ozone treatment chamber were found with symptoms. The symptoms appeared as brown to black colored stipple with early senescence of older leaves. The first symptoms developed at 120 ppb ozone exposure after the first week of treatment.

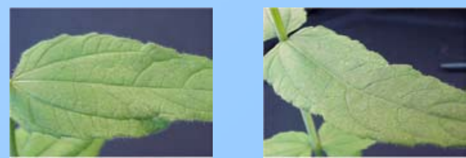

Figure 6. *(Helaintus divaricatus*,L.) This species has proven to be sensitive to 120 ppb, 90 ppb and 60 ppb ozone exposures. After 5 weeks of fumigations, plants exposed to 90 and 120 ppb ozone treatment developed symptoms as upper surface stipple; plants exposed to 60 ppb ozone treatment developed symptoms as bleached tissues. The first visual symptom appeared at the end of the second week of exposure within the 120 ppb chamber; at the end of 5 weeks of exposure all plants exposed to concentrations > 30 ppb showed symptoms of early leaf senescence.

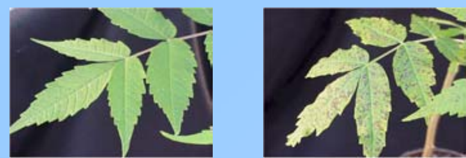

Figure 7. (*Rhus copalina*,L) Thus far, this species has been the most sensitive among the native plants of the USA included in this study. After 5 weeks of exposure to ozone treatments, all plants exposed to ozone showed symptoms. Visual symptoms developed as stipple, followed by flecking, early leaf senescence and premature leaf drop. The first symptom appeared at the end of first day of exposure within the 120 ppb ozone treatment chamber. We also recorded a difference in growth between plants exposed to the four ozone treatments and those specimens retained in the charcoal filtered air supplied greenhouse as controls.

Of the five species from Spain thus far tested for ozone sensitivity within the CSTR chambers, four have proven to be symptomatic to ozone exposure under moderate ozone concentration and following short duration of exposures. Typical ozone induced upper surface stipple, chlorotic spotting, and pre-mature leaf senescence were noted on four of five species. Thus far, the ranking of plant sensitivities based upon the severity of visual symptoms would follow as (most sensitive) *Epilobium ssp. > Collutea arborescens > Ulmus minor > Iberis carnosa.*

Figure 8. (*Collutea arborescens*,L.) After 25 days of fumigations all plants exposed to ozone treatments developed symptoms, C. arborescens appears to be extremely sensitive to ozone exposure, showing symptoms as foliar bleaching, white to brown stipple and premature leaf senescence. Initial symptom developed at the end of the first day of fumigations as stipple within the 120 ppb ozone treatment chamber.

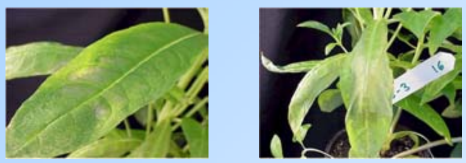

Figure 9. (*Epilobium ssp.*L.) Following 25 days of exposure to ozone treatment every plant became symptomatic. The symptoms developed as foliar bleaching, brown stipple, bifacial flecking and premature leaf senescence. Initial symptoms of bleaching were observed at all plants fumigated within the 120 ppb treatment chamber at the end of the first day of fumigation.

Figure 10. (*Ulmus minor*,Mill.) This species was shown to be sensitive to 60, 90, and 120 ppb ozone exposure. Visual symptoms appeared as white colored upper surface stipple and after prolonged exposure premature leaf senescence developed. The first symptoms were observed after three days of fumigations within the 120 ppb ozone treatment chamber.

Many factors are involved in controlling ozone ingress into the foliage of plants. However, ozone uptake into the leaf is largely regulated by the stomatal conductance at the time of ozone exposure and thus those factors, which control stomatal opening and closing, are of paramount importance. An environment surrounding an ozone-sensitive plant, which favor gas exchange also favors ozone uptake. Obvious controlling factor include: water, sunlight, nutrition and competition. Unfortunately, healthy plants, which are growing well and under favorable conditions are more likely to have the greatest amount of ozone uptake and thus may express more injury. Under controlled ozone exposure it is somewhat more possible to eliminate the external factors and control several abiotic stressors that may affect ozone uptake into the plant, and therefore determine a more exact exposure/response relationship.The results from our 2000 study suggest that ozone was the cause of the visible foliar injury observed on plants species undergoing ozone exposure. Symptoms of ozone-induced foliar injury were observed on all plants species included in this study. Symptoms were comparable to typical symptoms observed for the respective species under natural conditions and ambient ozone exposure. It is important to point out that these results are independent of the material of the study for the plants indigenous to northeastern USA; the plants used were obtained from different nurseries located in the Northeast. For plants native of southern Spain the results are dependent on the material used within the study; all plants were obtained from seeds of symptomatic plants found and collected during the field surveys. Given the within-species variability in sensitivity that has been encountered with *Prunus serotina,* it is likely that a different set of seeds sources would have produced different results. Once plants began to show foliar injury, the percent of plant foliage injured increased through the time of exposure within 60 ppb, 90 ppb and 120 ppb ozone concentrations, comparably, plants within the 30 ppb chamber displayed no or very low percent injury by the end of the ozone exposures.

Observations of foliar injury on native plants of northern US and southern Spain plants species have revealed that among plants under investigations *Rhus copalina, Collutea Arborescens and Epilobium sp.* are the most sensitive to ozone injury and further research may qualify them as bioindicators for ozone exposure. On the other hand plants as *Iberis carnosa* and *Fraxinus ornus* have proven to be tolerant to ozone exposure even if they have been reported to be sensitive by observations made in field surveys. Confirmation of ozone sensitivity remains to be completed for all species observed as symptomatic in field surveys in further research.

Also the results clearly indicate the influence of ozone on the development of foliar injury on plant species. High ozone concentrations were observed to cause a very rapid response with an increasing rate of injury over time. Thus far within this study, our focus has been in determining the response of newly identified plants to ozone exposures; i.e. confirming ozone sensitivity. Our further research will explore the development of foliar injury in response to cumulative ozone exposures.

Surveying and identifying air pollutants impact to forest ecosystems is a complex task and extreme caution must be exercised in the diagnosis of air pollution injury to plants. Visible foliar injury resulting from exposure to air pollution identified in field surveys must be confirmed (for minimizing errors in identifying the actual causes of the symptom) by investigations made under controlled conditions. This present research confirms the foliar response of numerous new plant species to be due to ambient ozone exposures.

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