

NE-176: Characterization & Mechanisms of Plant Responses to Ozone in the Northeastern U.S.

Technical Committee Report of Progress and Accomplishments in 1997:1/1/1997 - 12/31/97

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PROGRESS OF THE WORK & PRINCIPAL ACCOMPLISHMENTS, January – December, 1997.

Objective 1. Characterize whole plant response to O₃, including carbon assimilation and allocation, growth and productivity.

Responses of *Populus tremuloides*. We exposed O₃-tolerant and O₃-sensitive aspen clones to 1x, 1.7x, and 3x ambient O₃ in open-top chambers. Injury was visible in days at 3.0x exposure, increased over the 3 months, and lower leaves fell off. Photosynthesis decreased on both clones, by 40% less than ambient at 1.7x and 70% at 3.0x after 3 months. O₃ did not affect stomatal conductance. Elevated O₃ reduced end-of-season above-ground dry weight by 70-80% in the sensitive clone, and ~ 50% in the tolerant clone. Root dry weight was reduced by 27% in tolerant clones and 49% in sensitive clones. O₃ linear effects on foliar injury, growth, and biomass were significant. Neither ascorbic acid nor glutathione correlated with clonal differences nor were their levels stimulated by O₃. (*J. Laurence & Sung-Chul Yun, B.T.I.*)

Response of Blue Wildrye to O₃. Blue wild rye grass shoots from an O₃-polluted site responded more to O₃ exposure than shoots from a less polluted site. Below ground, the pattern was reversed: O₃ reduced mycorrhizal colonization more in grass from the less polluted site than grass from the more polluted site. O₃ exposure also decreased microbial biomass and increased fungal biomass to a greater extent in the soil containing grass from the less polluted site. (*U.S. EPA- C. Andersen*)

Effects of elevated O₃ and CO₂ on growth and physiology of yellow-poplar & eastern white pine. After 5 years of exposure to O₃ in open-top chambers, white pine growth did not respond to either O₃ or O₃ plus 2x ambient CO₂. Yellow-poplar bole and diameter growth was stimulated by elevated O₃ plus 2x CO₂ but biomass was not. (*J. Rebbeck, USDA Forest Service, Ohio*)

Short-term effects of elevated O₃ and CO₂ on growth & physiology of black cherry, green ash, & yellow-poplar seedlings. Exposure to 2x ambient CO₂ stimulated net photosynthesis and biomass production in all 3 species but had no effect on net photosynthesis. O₃ effects on biomass varied with species. Few significant interactive effects of O₃ and CO₂ were observed. Elevated CO₂ may ameliorate O₃ effects on biomass. (*J. Rebbeck, USDA For. Serv., Ohio & K. Loats, Denison U.*)

O₃ effects on snap beans. Snap beans grown on Long island in open-top chambers with filtered air were exposed to less O₃ (≥20 ppb lower when ambient O₃ was ≥60 ppb). Compared to biomass under filtered air, ambient O₃ reduced stem and leaf weight by 32% and reduced pod weight of sensitive *Oregon91* by 39% but did not affect *Strike*, the main LI variety. Ambient O₃ reduced growth of the sensitive clover clone (NC-S) in mid-July. The forage dry weight ratios (NC-S/NC-R) were 1.01, 1.03, 0.74, and 0.78 for harvests on 6/17, 7/18, 8/17, and 9/12. (*M. McGrath, Cornell, LI-NY*)

O₃ effects on loblolly pine. Loblolly pine competing with other woody and herbaceous species grew slower with subambient O₃. Increased O₃ reduced species numbers, % cover and the rate of blackberry broomsedge litter decomposition. Several native plant species in Class I Southern Wilderness areas (Blackberry, yellow-poplar, black cherry, etc.) did not respond visibly to ambient O₃. (*A. Chappelka, School of Forestry, Auburn*)

Other responses. *Tall milkweed* was inventoried for O₃ sensitivity for 5 years in Great Smoky Mountains National Park. Visible injury of large *yellow-poplar* trees correlated negatively with growth. Flower numbers and duration of flowering of *butterfly bush* was very sensitive to O₃. (*A. Chappelka, School of Forestry, Auburn*)

Objective 2. Identify and delineate primary factors, both biotic and environmental, that determine plant response to O₃

Correlation of atmospheric conditions that promote O₃ with conditions that promote plant uptake of O₃. The correlation of O₃ concentration and O₃ uptake rate was computed using data on concentration, air temperature, air humidity, wind velocity and radiation, from representative U.S. and German sites. We used the PLant-ATmosphere INteraction model to relate the meteorological variables to O₃ uptake. Atmospheric conditions that elevate O₃ are poorly correlated with conditions that promote uptake. This observation is consistent with the daily patterns of atmospheric CO₂ flux. (*S. Krupa – U. Minn.*)

Interactive effects of O₃ and CO₂ on cotton yield. Cotton in open-top field chambers was exposed to elevated CO₂ and O₃. Urea provided low, medium, and high N levels. Increased O₃ concentrations injured cotton plants and suppressed yield. Positive effects of CO₂ enrichment on cotton yield were greater for plants stressed by O₃ than for unstressed plants. The nature of the response was similar at the three N levels. (*USDA-ARS, NC - Joe Miller*)

Interactive effects of O₃ and CO₂ on soybean yield. Yields of soybeans exposed to 12 or 24h daily CO₂ enrichment were evaluated in pots and in field plantings. Neither exposure nor potting affected yield. O₃

flux through stomata was reduced by 45% by elevated CO₂. There was no significant difference in pod weight per plant or in levels of ascorbic acid or glutathione between plants that received equal flux densities of O₃ at ambient and 2x ambient CO₂. Yield decreased by 20% in plants treated with higher concentrations of O₃ at ambient CO₂. Neither antioxidant levels nor yield were affected by elevated CO₂ for plants grown in clean air. Stomatal closure may explain why elevated CO₂ reduces yield losses attributable to O₃. (USDA-ARS, NC - Joe Miller)

Role of competition from Blue Wildrye grass to Ponderosa Pine O₃ sensitivity. O₃ level and grass competition interact to affect ponderosa pine height, diameter, and final seedling biomass. O₃ increased rates of CO₂ flux from soil during only 1 of 3 years studied. After 3 years, the soil fungal/bacterial biomass ratio decreased with the presence of grass competition, an response consistent with bacterially-dominant foodwebs characteristic of grassland systems. O₃ reduced fungal/bacterial biomass ratios in all treatments and did not change with grass competition. (U.S. EPA- C. Andersen)

Objective 3. Determine mechanisms of O₃ action and plant defense systems, using cultivars and genotypes characterized in whole plant experiments.

The role of flavonoids in soybean sensitivity to O₃. We compared yield and physiological response to O₃ in open-top field chambers of soybean isolines lacking flavonoids or containing different combinations of kaempferol glycosides. Data from 1995, 1996, and 1997 were consistent. Plants with high levels of leaf K9 have lower photosynthesis, and lower chlorophyll and specific leaf weights. (USDA CSL, MD- S. Britz, E. Lee, & J. M. Robinson)

The role of foliar antioxidants in sensitivity to O₃. We varied N supplied to spinach and soybeans to vary carbohydrate level and corresponding levels of Vitamin C (ascorbate [ASC] + dehydroascorbate [DHA]) in leaves. Lower N was correlated with increased carbohydrate in the light, but Vitamin C levels were not consistently correlated with glucose or other potential carbohydrate precursors of Vitamin C. However, N-limitation in both soybean and spinach was correlated with higher ratios of ASC to DHA in leaves in the light. Leaves of soybeans exposed to O₃ season-long had significantly greater fluorescence intensity at both 680 and 740 nm at both ambient and elevated atmospheric CO₂. Intensity was greater in O₃-sensitive cultivar *Forrest* than in O₃-tolerant *Essex*. Varied content of specific flavonol glycosides in soybeans was not correlated with increased resistance to O₃ damage. (USDA CSL, MD- S. Britz, E. Lee, & J. M. Robinson)

Apoplast antioxidants against O₃ reactive byproducts. We tested an hypothesis that transgenic plants engineered to express high levels of antioxidant enzymes in the apoplast might be protected against oxidative damage by O₃'s reactive byproducts. Chimeric constructs were made which have the proteinase inhibitor II signal sequence fused in frame with the coding sequences for pea cytosolic Cu/Zn-superoxide dismutase (Cu/Zn-SOD), ascorbate peroxidase (APX), and monodehydroascorbate reductase (MDAR) to enable secretion of the passenger protein to the apoplast. Expression of the transgene was driven by the CaMV35S promoter. Transgenic tobacco plants (Samsun NN) were obtained by *Agrobacterium tumefaciens* mediated transformation. Cu/Zn-SOD and APX were successfully secreted into the apoplast while MDAR was not. Most of the preliminary transgenic lines which expressed APX or Cu/Zn-SOD in the apoplast did not show tolerance to acute O₃ exposure. (B. Zelinskas, Rutgers)

Antioxidant Enzymes in Wheat Cultivars in Response to Atmospheric O₃, CO₂, and Moisture. Wheat cultivars *Susquehanna* and *Gore* were exposed to O₃ at 20 ± 5, 40 ± 5, and 60 ± 5 nLL, to CO₂ at 355 and 500 μLL⁻¹, and to two soil moisture regimes (sheltered vs. well-watered) in open-top chambers. Leaf photosynthesis, stomatal conductance, leaf area, chlorophyll fluorescence, and pigment concentrations were measured several times from late April through June. Leaf tissue collected at pre-flowering, anthesis, and grain-fill growth stages were stored in liquid N₂ and are being maintained at -80°C for lipid peroxidation and analyses for monodehydro-ascorbate reductase, ascorbate peroxidase, glutathione reductase and superoxide dismutase. At physiological maturity we measured total biomass, straw and grain yields. (C. Mulchi, U.Md.)

Antioxidant Enzyme Activities in Soybean Cultivars In Response to Atmospheric O₃ and CO₂. Soybean cultivars *Essex* and *Forrest* were grown in open-top chambers under well-watered conditions and exposed to two O₃ levels (27.6 vs. 72.0 nLL⁻¹) and two CO₂ levels (355 vs. 500 μLL⁻¹). Elevated O₃ decreased leaf growth and accelerated chlorophyll degradation. *Forrest* exhibited greater injury from O₃ exposure than *Essex*. Elevated CO₂ reduced the negative effects of O₃ in both cultivars. Growth rates were reduced by O₃ at ambient CO₂ but were unaffected by O₃ at high CO₂. Stomatal conductance was reduced by elevated O₃ at both CO₂ levels. O₃ flux rates into the leaves were increased by O₃ at both CO₂ levels. Ascorbate peroxidase was more active under elevated O₃ at both CO₂ levels with greater response at ambient CO₂. Superoxide dismutase was stimulated by O₃ in *Essex* under ambient CO₂ but not at elevated CO₂. Glutathione reductase was stimulated by O₃ in *Essex* under low CO₂ but not under high CO₂ for *Forrest*. Catalase activities were higher under elevated O₃ and CO₂ for *Essex* but not for *Forrest*. Differential tolerance to O₃ appeared related to enzyme activities in *Essex* rather than stomatal effects. (C. Mulchi, U. Md.)

Nitrogen and O₃ effects on senescence in Poplar. We related nitrogen supply and O₃ impact on foliar senescence and remobilization of soluble N-based biochemicals in ramets of hybrid poplar. Decreasing N supply exacerbates O₃-induced acceleration of leaf senescence. (E. Pell, PA)

O₃ and senescence genes in *Arabidopsis*. *Arabidopsis* plants were treated with 0.15 ppm O₃ for 6h/d for 8 days. Senescence associated genes (SAG) 13 and 14 were expressed after 2 days; SAGs 15, 20 and 21 after 4 days and SAGs 18 and 22 after 8 days. SAG 15 appeared in controls after 6 days; SAG 13 and 20 appeared after 8 days and the others were not detected during the experiment. SAG 13, 17, 19 and *AtGS2* and *AtLOX2* were not detected in response to O₃. (E. Pell, PA)

O₃-induced ACC synthase genes in potato. Potatoes were transformed with antisense for O₃-induced ACC synthase genes *ACS4* and *ACS5*. One transformed line to *ACS4* has been produced and many putative lines are currently being tested to determine success of transformation to *ACS4* and *ACS5*. (E. Pell, Penn. St. U.)

O₃ effects on protoplast K⁺ currents in *Vicia faba*. Patch clamp analysis showed that O₃ induced a reduction in average inward potassium (K⁺) currents in the plasma membrane of protoplasts of *Vicia faba*, but had no effect on outward K⁺ currents. Oxygen-treated and non-treated protoplasts did not exhibit this response. This is the first report of O₃-induced changes in ion channel activity utilizing patch-clamp technology. (E. Pell, PA)

O₃ induced proteins in tolerant white clover. White clover (cvar *Regal*) clones —O₃ tolerant (NC-R) and O₃ sensitive (NC-S) — were used to investigate biochemistry of oxidative tolerance. We have previously identified 2 - 4 small mass (21 to 23.5 kD) polypeptides that are induced by O₃ to a greater extent in NC-R than NC-S at exposure concentrations that do not cause visible foliar injury. These proteins are in sufficiently low concentrations in plants grown in charcoal-filtered air that they are not apparent on 2-dimensional polyacrylamide gels stained with silver. Compared to plants exposed for 1 day, in plants treated for 3 days, O₃-responsive proteins (ORPs) increased 20x in NC-R plants and 5x in NC-S plants. ORP's were acidic, with isoelectric points between pH 4.1 and 4.3, which is distinctly lower than other O₃-induced proteins, making ORP's unique. We estimate a molecular mass of <100 kD, (i.e., less than the family of small heat-shock proteins, which mass at 240 to 468 kD). In field-grown clover, high concentrations of the ORPs were observed in NC-R and correlated with longer retention of soluble foliar protein, compared to NC-S. Efforts are underway to purify sufficient protein for amino acid analyses. {B. Chevone, Virginia Tech.}

USEFULNESS OF FINDINGS: That the effects of elevated CO₂ are mediated by O₃. This has important implications for our understanding of plant productivity responses to elevated CO₂ and for our ability to predict and adapt to future global atmospheric changes. Fluorescence (FL) imaging to detect early responses of soybean to O₃. provides a new rapid, nondestructive remote-sensing techniques that can be used to alert growers to incipient environmental stress and to help breeders select for stress-tolerant lines.

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APPROVED:

Chair, Technical Committee (Date)

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