THE FALSE POSITIVE IN BIOINDICATORS OF AIR POLLUTION[1]

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Abstract. Yellow-poplar (*Liriodendron tulipifera*) seedlings were fumigated with a gradient of ozone to determine what effect ozone has on the species. The fumigated seedlings displayed physical symptoms of ozone injury but a large number of the individuals from the charcoal-filtered treatment showed physical symptoms of ozone when indeed no ozone was present. This is an indication that what appears to be ozone damage on yellow-poplar may not be caused by ozone.

There is widespread concern within the scientific community that plants are suffering from the ill effects of a variety of air pollutants. Ozone (O_3), with its widespread distribution, probably has the greatest negative impact on vegetation of all air pollutants (2, 3). Few areas in the United States are free of ozone pollution. Exposure to ozone can result in both acute (symptomatic)and chronic (changes in growth, yield or productivity, and quality) effects (2).

Plant indicators are a good way to monitor air pollution levels without having to invest in expensive equipment or in exhaustive laboratory proceedures. Phytometers have been used since the 1850's in Europe to designate the presence of air pollution. In this century, plant indicators have been used extensively in the United States from determining the suitablity of cultural practices to the presence of pathogens. Yellow-poplar (*Liriodendron tulipifera*) has been identified as a suitable bioindicator of ozone (1, 4).

Plants must possess certain attributes to make good air pollution phytometers. It (they) should be a common species in the area of interest. You should not have to waste a lot of time hunting for a species to determine if pollution is present or absent. The species chosen should be readily identifiable so that misdiagnosis is minimized. Potential bioindicators should exhibit visible symptoms that are easily identifiable. If a species exhibits symptoms that can be determined only by laboratory analyses, the justification for bioindicators is lost. Candidate species for bioindicators of pollution must have foliage during the time of the year pollution is prevalent. If a tree is chosen as a bioindicator of air pollution, seedlings and saplings must occur in the area of interest. If only mature trees exist in a stand, the lowest limb may be too far off the ground to score the individual as symptomatic or asymptomatic. Plants used as bioindicators must give predictable results and have the same symptoms every time, moreover, plants should give reliable results and reliability is what this paper will examine.

Methods and Materials

Yellow-poplar seedlings were fumigated with a gradient of ozone for one growing season at Uplands Research Lab, Great Smoky Mountains National Park. Seedlings from seed collected from the park were grown in individual pots and were placed in one of 15 open-topped growth chambers or three open plots. This allowed for three replications of six treatments. Treatments included 0.5, 1.0, 1.5, 2.0 x ambient, charcoal-filtered, and open-ambient plots. All treatments except for the charcoal-filtered were proportional to ambient conditions so that ozone concentrations can be linked to weather conditions concomitant with ozone production. Ambient concentrations were continuously monitored and treatments were updated every two minutes with the aid of a TECO model 49 ozone analyser. In addition to monitoring ambient ozone concentrations, all meteorological parameters, and CO_2 and NO_x concentrations were analyzed and recorded on a data logger and computer. All monitors were calibrated

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weekly with a NBS tracable standard and were audited quarterly by personnel from the Tennessee Department of Health and Environment. Technicians watered plants manually every day to ensure that all individuals receive enough water for soil saturation and luxury consumption. This was done because drought has been demonstrated to be a mitigating factor to ozone injury (3).

Results and Discussion

Seedlings were scored as being symptomatic or asymptomatic. Symptoms of ozone damage are manifested by general chlorosis and dark stipple in the interveinal areas on the adaxial side of the leaf. This may lead to bifacial necrosis, premature leaf senescence, or death of the individual leaf. Looking at charcoal-filtered (5.8 ppb seasonal 1hr mean) and 2.0 x ambient (46.5 ppb seasonal 1hr mean) treatments only (ozone exposure concentrations are shown in Table 1), no symptoms of ozone damage are expected on the plants from the charcoal-filtered chambers and if a plant is sensitive to ozone, it should exhibit visible symptoms in the 2.0 x ambient treatment. At the end of the 1990 fumigation season, sixty-four (90 percent) of the individuals from the 2.0 x ambient exhibited physical symptoms consistent with ozone injury and seven (10 percent) showed no symptoms. These are reasonable observations. The great majority is expected to be sensitive to ozone in the high treatment and a few to be resistant to the damaging effects of ozone. Thirty-eight (53 percent) seedlings from the charcoal-filtered chambers exhibited no symptoms of ozone damage, which is expected, however 34 (47 percent) seedlings showed symptoms of physical injury (Table 2). The individuals from the 2.0 x ambient treatment that exhibited symptoms are true positives, and the asymptomatic seedlings (resistant to ozone) are false negatives. As mentioned earlier, these results are acceptable. The seedlings from the charcoal-filtered treatment that were asymptomatic are correct rejections (true negatives) but those which exhibited ozone like symptoms are false positives, which is equivalent to Beta in statistical analysis. If yellow-poplar is used as a bioindicator of ozone, it has a high probability that it may indicate high ozone levels when indeed they are not present, a Type II error. This invalidates claims that Southern Appalachian yellow-poplar is a good bioindicator of ozone. When trying to identify species suitable for being bioindicators of ozone or any other pollutant, one should look at the incidence of a high number of false positive readings before making any judgments.

Table 1. Ozone exposure summaries for the 1990 fumigation season.

	Ozone concentrations (ppb)		
	Total ppb-hours	1hr daily mean	
Target	Total dose	Seasonal	Highest
Charcoal-filtered	12192.7	5.8	42.2
Non-filtered	52559.0	24.5	79.0
2.0 x ambient	100011.9	46.5	161.1

Table 2. Incidence of ozone injury on yellow-poplar.

Treatment	Damaged (percent)	Undamaged (percent)
Charcoal filtered	34 (47) [false positive]	38 (53) [true negative]
2.0 x Ambient	64 (90) [true positive]	7 (10) [false negative]

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Résumé. Les semis de tulipier (*Liriodendron tulipifera*) étaient fumigés avec un gradient d'ozone pour déterminer quels étaient les effets, s'il y en a, de l'ozone sur cette espèce. Les semis fumigés exhibaient des symptômes de dommages par l'ozone. De même, un nombre important des individus traités avec un filtre au charbon montrait des dommages par l'ozone lorsqu'il n'y avait vraiment pas d'ozone en présence. Les indicateurs communément acceptés de dommages par l'ozone sur le tulipier ne peuvent être reliés à l'ozone.

Zusammenfassung. Sämlinge des Tulpenbaums (*Liriodendron tulipifera*) wurden mit steigenden Ozonkonzentrationen begast um festzustellen, ob und welche wirkung Ozon auf diese Baumart hat. Die begasteh Sämlinge zeigten Symptome von Ozonschäden auf. Eine große Anzahl von Exemplaren, die Luft durch einen Aktivkohlefilter zugeführt bekamen, zeigten ebenfalls Symptome von Ozonschäden, obwohl kein Ozon vorhanden war. Allgemein akzeptierte Anzeichen für einen Ozonschaden beim Tulpenbaum werden offenbar nicht durch Ozon verursacht.