The Use of Plants as Bioindicators of Ozone[1]

William J. Manning[2]

Abstract

*A variety of vascular plant species exhibit typical foliar injury symptoms when exposed to ambient ozone, making them useful as bioindicators of relative air quality for a particular location or region. They are quite useful in areas where mechanical ozone monitors are not available. Bioindicators are often introduced plant species known as sentinels. They are known to be sensitive to ozone and will respond rapidly if they are given special care to ensure ozone uptake and injury. Sentinels are usually genetically-uniform, rapid growing herbaceous annuals. Their rapid and well-characterized response to ozone exposure has made them quite useful. Bel-W3 tobacco (*Nicotiana tabacum *L.) is a sentinel plant bioindicator for ozone that is used all over the world. Detector bioindicators are plant species that are found growing naturally in an area and known to be sensitive to ozone only when conditions are appropriate for ozone uptake and plant injury. Detectors are often slow-growing, determinate perennial plants, shrubs, or trees that respond slowly to ozone, with symptoms occurring quite late in the growing season. Populations of detectors are not genetically uniform and only part of a population may show ozone injury symptoms. Black cherry (*Prunus serotina *L.) is a common detector bioindicator for ozone in North America. A comparison of surveys of sentinel and detector bioindicators in the same area often show different results. From an ecological perspective, visible injury on a detector bioindicator is more significant than visible injury on a sentinel bioindicator. When using plants as bioindicators, careful consideration needs to be given to the nature, requirements, and utility of sentinels and detectors in relation to the relevance and utility of the results obtained.*

Introduction

For more than 50 years, certain plant species have been used as bioindicators because they are sensitive to ozone under ambient conditions (Middleton and others 1950, Noble and Wright 1958). Sensitive individuals exhibit typical foliar injury symptoms when exposed to ambient ozone under conditions appropriate for ozone uptake. These symptoms are considered to be diagnostic or typical as they have been verified in exposure/response studies under experimental conditions (Krupa and Manning 1988). These plants are considered to be reliable biological indicators or bioindicators for ambient ozone. The subjective determination of the intensity or extent of foliar injury of bioindicators is used as an index of relative air quality for ozone for a particular location or region. Ozone has become an air pollution problem in most industrialized nations, resulting in an increased interest in using bioindicator plants on a world-wide basis. According to Guderian and others (1985), injury on Bel-W3 tobacco (*Nicotiana tabacum* L.) is usually the first indication a county or region has developed an ozone problem. The history and use of plants as ozone bioindicators has been extensively reviewed elsewhere (Arndt and others 1987, Burton 1986, deBauer 1972, Feder and Manning 1979, Guderian and others 1985, Heck 1966, Hernandez and deBauer 1989, Manning 1991, Manning and Feder 1980, Posthumus 1982, Stuebing and Jager 1982, Tonneijck and Posthumus 1987, Weintstein and Laurence 1989).

This paper discusses the use of introduced species or sentinels and naturally occurring plants or detectors as bioindicators of ozone exposure.

Sentinels and Detectors

From an ecological perspective, Spellerberg (1991) recognizes two types of plant bioindicators useful in studies designed to detect gaseous air pollutants (*table 1*). Sentinels are non-indigenous plant species, consisting of well-defined selections,

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[2] Phytopathologist, Department of Microbiology, University of Massachusetts, Amherst, MA 01003-5730.

cultivars or clones, that exhibit diagnostic reliable foliar symptoms when exposed to ambient ozone. Sentinels are grown in charcoal-filtered air and then introduced into areas, usually for short time periods. They are quite useful in identifying areas where ozone concentrations and exposures are suspected to be high enough and of a long enough duration to cause foliar injury (Ashmore and others 1980, Bytnerowicz and others 1993, deBauer 1972, Heck and Heagle 1970, Hernandez and deBauer 1989, Jacobson and Feder 1974, Kelleher and Feder 1978, Lipa and Votapkova 1995, Noble and Wright 1958, Oshima 1974, Posthumus 1982). Examples of sentinel bioindicators for ozone include Bel-W3 tobacco (Heck and others 1964, Heck and Heagle 1970, Heggestad 1991) and morning glory (*Ipomoea purpurea*) (Manning 1977, Nouchi and Aoki 1979). Detector plants are indigenous to an area that may exhibit typical foliar injury symptoms to ozone exposure *in situ*. They are useful in assessing the long-term or cumulative effects of ozone. Examples include the sensitive individuals in populations of black cherry (*Prunus serotina*) (Davis and others 1982), Ponderosa (*Pinus ponderosa*) and Jeffrey (*P. Jeffreyi*) pines (Stolte and others 1992), and milkweed (*Asclepias syriaca*) (Duchelle and Skelly 1981).

Table 1 *— Types of indicator plant species used to assess relative air quality for ozone (Spellerberg 1991).*

Sentinels	Well-defined plants known to be sensitive to ozone are introduced into an area to serve as early warning devices or checks on the efficiency of abatement practices. Examples—Bel-W3 tobacco (*Nicotiana tabacum*), Morning glory (*Ipomoea purpurea*)
Detectors	Plants that naturally occur in the area of interest that may exhibit typical foliar responses to ozone. Examples—Black cherry (*Prunus serotina*), Milkweed (*Asclepias syriaca*)

Sentinels are the most commonly used bioindicators of ambient ozone. They are usually well-defined, genetically-uniform, herbaceous annuals that grow rapidly. Their response time to ozone is rapid, serving as an early warning of ozone presence. Rapid response, however, may only occur in the early stages of their growth cycles, necessitating frequent re-introduction of new plants. They must be grown in charcoal-filtered air until they are old enough to move to the field. To minimize edaphic factors, they are usually grown in pots of a uniform artificial growing medium. They require water, fertilizer, shading, and often pesticide applications, on a regular basis. Protection from animals and vandals may also be required. If sentinels do not receive the special care they require, they will not respond well to ozone in a typical or relevant manner.

Detectors are native plants selected *in situ* and usually are not given any special cultural care. Usually they are determinate perennial plants, trees, or shrubs that respond slowly and often fairly late in the growing season. Only the sensitive individuals in a population of a detector bioindicator will respond to ozone and only when they experience appropriate edaphic and tropospheric conditions coupled with ozone exposures and concentrations sufficient to cause foliar injury. The distribution of ozone-sensitive genotypes (where there is little phenotypic variation) in a population of a detector bioindicator is usually not well-known. This adds uncertainty in interpreting results from detectors. When large numbers of individuals are present, confidence coefficients increase (Stolte and others 1992).

Bioindicator Plant Response to Ozone

Sentinels and detectors evaluated in the same area often show different results: sentinels may respond but detectors may not. Results from ozone plant bioindicator studies must be interpreted on the basis of a number of interacting biological, cultural, and physical factors (*table 2*). Foliar ozone injury symptoms are only an indication of previous exposure to ozone, when the concentration was high enough for a certain time period, and environmental conditions were conducive to ozone uptake and cellular injury. The visible response is a relative index of air quality for ozone during a previous exposure period. It is not possible to use this response to quantitatively assess air quality for ozone. This is because we do not yet truly understand the relationship between the occurrence and magnitude of plant response and the environmental conditions and concentrations and durations of ambient ozone present when the plant injury response was initiated. If data from a co-located or nearby mechanical ozone monitor is available, however, periods when monitored ozone concentrations have potential biological significance or meaning can be indicated, as reflected by frequency, duration, and intensity of plant responses.

Table 2 — *Biological, cultural, and physical factors affecting responses to ozone (Manning 1991).*

Biological and Cultural		
Factors		***Responses***
Biological		
	Genetic diversity	Homogeneous plants give uniform responses; species, clones, cultivars, and provenances react differently to O_3
	Stage of plant development	Plant developmental stages and leaf age affect responses to O_3
Cultural		
	General cultural practices	Optimal or usual practices result in "typical" responses to O_3
Growth media		Soilless media allow uniformity and reproductibility, but are less relevant than natural soils.
Nutrients		Optimal levels usually result in optimal injury from O_3.
Pesticides		Variable effects, ranging from none to protection, from injury to reduced tolerance to joint effects with O_3.
Physical		
Factors		***Responses***
Air movement		Must be sufficient to alter boundary layer resistance to allow O_3 uptake.
Light		
	Intensity, photoperiod, and quality	Ideal value varies for each plant; less or more than ideal value for each plant reduces O_3 sensitivity
Temperature		Injury increases in a range from 3 to 30 °C.
Water		
	Relative humidity	Controls stomatal opening exchange; uptake of O_3 and injury should increase as relative humidity increases.
Soil moisture tolerance		Water stress increases O_3 due to stomatal closure

Previous research has determined some well-defined bioindicators for ambient ozone (*table 3*). Each of these bioindicators will exhibit typical ozone injury symptoms (*table 4*). Tobacco cultivar Bel-W3 is the best-defined, best-described, and most widely-used bioindicator for ambient ozone (Heggestad 1991). It has become the standard sentinel bioindicator for ozone and has been used all over the world to detect ambient ozone in areas ranging from local sites to whole countries and continents. Heck and Heagle (1970) used Bel-W3 tobacco to determine incidence of phytotoxic concentrations of ozone around Cincinnati, Ohio. Bel-W3 tobacco plants were used by Manning to demonstrate ozone in Warsaw, Krakow, and forested areas in southern Poland (Bytnerowicz and others 1993). Jacobson and Feder (1974) reported the results of a network of Bel-W3 tobacco plantings in the northeastern United States. Long-range transport of ozone from metropolitan New York to Nantucket Island was demonstrated by response of Bel-W3 tobacco plants on Nantucket (Kelleher and Feder 1978). Ashmore and others (1980) mapped phytotoxic ozone in the British Isles by using networks of Bel-W3 tobacco plants. Schoolchildren in many European countries use Bel-W3 tobacco to assess relative air quality for ozone (Lipa and Votapkova 1995).

Table 3 *— Selected bioindicators of ozone.*[1]

Plant	Latin Name	References
Sentinels		
Blue grass	*Poa annua*	Noble and Wright 1958
Bean	*Phaseolus vulgaris*	Oshima 1974, Sanders and others 1992
Clover	*Trifolium repens*	Heagle and others 1992
	T. subterraneum	Sanders and others 1992
Morning glory	*Ipomoea purpurea*	Manning 1977, Nouchi and Aoki 1979
Spinach	*Spinacea oleraceae*	Posthumus 1982
Tobacco	*Nicotiana tabacum*	Heck and Heagle 1970,
	Bel-W3 (sensitive)	Heaggestad 1991
	Bel-B (tolerant)	
Detectors		
Blackberry	*Rubus* spp.	Chappelka and others 1986, Manning 1991
Black cherry	*Prunus serotina*	Chappelka and others 1992, Davis and others 1982
Green ash	*Fraxinus pennsylvatica*	Davis and Wilhour 1976
Milkweed	*Asdepias sytiaca*	Duchelle and Skelly 1981
Quaking aspen	*Populas tremuloides*	Karnosky 1976, Keller 1988
Sassafras	*Sassafras albidum*	Chappelka 1992
Tulip poplar	*Liriodendron tulipifera*	Davis and Chappelka 1986, 1992; Davis and Wilhour 1976
White ash	*Fraxinus americana*	Chappelka 1992, Davis and Wilhour 1976

[1] Not all varieties or individuals in a species will be ozone-sensitive.

Several plant species have cultivars or clones that differ in their response to ambient ozone. The best defined incidence of this are the tobacco cultivars Bel-W3 (ozone-sensitive) and Bel-B (ozone-tolerant). Bel-W3 tobacco responds to ambient ozone at lower concentrations than does Bel-B. When grown together in an area, their comparative responses to ozone can provide a better description of relative air quality for ozone. If both cultivars do not respond, then the air is relatively clean and ozone concentrations are probably low. If Bel-W3 responds, but Bel-B does not, then ozone concentrations are intermediate. If both cultivars respond, then ozone concentrations are higher (Manning and Feder 1980).

Detectors are being used to assess relative long-term air quality in forested and wilderness areas. Study areas are selected and certain species are examined on an annual basis for incidence and severity of foliar ozone injury. In wilderness areas of New Hampshire and Vermont, Manning and others (1991) have surveyed black cherry, white ash, and milkweed. Chappelka and others (1986, 1992) have used black cherry, sassafras, white ash, yellow poplar, and blackberry in the southeastern United States. The USDA Forest Service's Forest Health Monitoring Program uses detector bioindicators in its forest surveys in the U.S. (Conkling and Byers 1993). Detector bioindicators are used in forested regions of the Carpathian Mountains in Poland, Ukraine, Czech Republic, Slovakia, and Rumania (Manning and others, unpublished paper).

EDU (ethylenediurea) is a chemical known to protect plants from ozone injury (Carnahan and others 1978). It can be used to verify the response of an ozone bioindicator in the field. This is especially useful if an ozone monitor is not readily available or there are questions about the nature of the response of the bioindicator. This approach was used in Poland (Bytnerowicz and others 1993). Bel-W3 tobacco plants were outplanted in a number of locations. Half of the plants were treated with EDU before outplanting. The treated plants did not develop any symptoms of foliar injury while the non-treated plants developed varying degrees of typical ozone injury symptoms, verifying that ozone was the cause.

***Table 4** — Common symptoms of foliar ozone injury (Krupa and Manning 1988).*

Acute injury	
Flecking	Small necrotic areas due to death of palisade cells, metallic or brown, fading to grey or white.
Stippling	Tiny punctate spots where a few palisade cells are dead or injured, may be white, black-red, or red-purple.
Chronic injury	
Pigmentation (Bronzing)	Leaves turn red-brown to brown as phenolic tan pigments accumulate.
Chlorosis	May result from pigmentation or may occur alone as chlorophyll breaks down.
Premature senescence	Early loss of leaves or fruit.

Methodology for Assessing Bioindicator Plants

Great care must be taken in assessing the response of bioindicator plants to ambient ozone. In most cases, assessment of bioindicators involves the subjective determination of the intensity or extent of acute ozone injury symptoms (*table 4*). Leaf injury evaluations should be made at regular intervals, often weekly, and by the same person. The use of a set of color photographs illustrating degrees or categories of severity of injury can help to standardize the evaluation process (Heck 1966; Heck and others 1966, 1969; Oshima 1976). If plants are left in the field for more than 1 week, then new injury on both older and new leaves needs to be estimated and recorded.

The observer should look at each leaf and visually integrate the injured areas of each leaf and then determine the percentage of the total leaf area that has been injured. Depending on the type of plant, extent of injury, and purpose of the study, the evaluation system can be quite simple and uncomplicated (*table 5*). With only six indices, this is a simple system to use. Where more precise information is required, an expanded system is more appropriate (*table 6*).

***Table 5** — System for evaluation of ozone injury for bean (*Phaseolus vulgaris *L.) (Manning and Feder 1980).*

	Rating Systems	
Injury rating	**Injury severity index**	**Percent leaf injury**
None	0	0
Slight	1	1-25
Moderate	2	26-50
Moderate-severe	3	51-75
Severe	4	76-99
Complete	5	100

***Table 6** — The Horsfall-Barratt Scale for assessing foliar injury (Horsfall and Barratt 1945, Horsfall and Cowling 1978).*

(Percent severity or class incidence)	
0	0
1	0-3
2	3-6
3	6-12
4	12-25
5	25-50
6	50-75
7	75-88
8	88-94
9	94-97
10	97-100
11	100

Data can be depicted graphically. Plotting weekly injury scores, or number of leaves injured, on a weekly basis against elapsed time gives a cumulative injury curve over time. If data are available from a nearby mechanical ozone monitor, ozone injury can be plotted against cumulative ozone or related to episodes in which thresholds are exceeded (Manning and Feder 1980).

As an alternative to using subjective determination of the intensity or extent of foliar injury as a measure of ozone effects, other investigators have developed bioindicator systems that use shoot biomass response from sequential harvests as a measure of ozone effects. Oshima and others (1976) developed a standardized pot culture system with alfalfa (*Medicago sativa* L.) along an ozone gradient in southern California. Heagle and others (1994) have developed an ozone bioindicator system by using sensitive (NCS) and resistant (NCR) clones of ladino clover (*Trifolium repens* L.) NCS and NCR plants are grown in standardized pot culture. Shoot biomass is removed at 28-day intervals and dry weights are obtained. Ozone impact is determined by obtaining dry weights of harvested shoot biomass and calculating the ratios of NCS to NCR. A ratio of less than one indicates that ambient ozone has had an adverse effect on foliar biomass of NCS.

Summary

Bioindicator plants for ozone can be extremely useful in assessing relative air quality, especially in areas where ozone monitors are not available. If they are not used properly, however, poor quality or misleading results will be obtained. Common factors that produce poor results are poor plant culture for sentinels, failure to adhere to regular evaluation schedules, careless and inaccurate evaluation of symptoms, and the use of more than one person to evaluate plant responses.

Care should be taken in interpreting responses of sentinel bioindicators. As they are well-watered and fertilized, they respond much more frequently than do detectors. Their response indicates the occurrence of periods of ozone exposure when detectors might respond if they were also growing under conditions of sufficient soil moisture and fertility. From an ecological perspective, response of detector bioindicators to ozone is more important than a response by a sentinel bioindicator. A response by a sentinel indicates what could happen under ideal conditions, while a response by a detector indicates what did happen under more realistic conditions.

There is an unfortunate tendency to confuse a bioindicator with a biomonitor. A bioindicator indicates that the system has been affected. A biomonitor should also indicate how much of the causal factor was present and caused the observed effect. Currently, bioindicators of ambient ozone cannot be used as biomonitors. It is not possible to make quantitative inferences about air quality for ozone on the basis of plant symptom expression alone.

With a clear understanding of the strengths and weaknesses of the use of plants as bioindicators of ozone exposure, they can be quite useful in assessing relative air quality, especially in programs like Forest Health Monitoring, in remote areas, and in emerging nations, where technology is lacking.

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