

# Using Sensitive Plants as Bioindicators of Ground Level Ozone Pollution



## Implementation Guide

### Authors

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## **About the Authors**

Dr. Irene Ladd has been involved in educational reform both locally and state wide for the past 25 years. She is a retired teacher of 33 years and served on New Hampshire's Science Curriculum Frameworks Committee. She joined the Surface Ozone Measurement for GLOBE (SMOG) Team to develop the surface ozone protocol and field test the instruments used for measuring surface ozone. She has developed educational materials to integrate the study of surface ozone with core curriculum, and the training to implement the program. Dr. Ladd continues to provide training nationally and internationally and providing guidance toward the implementation of inquiry in the classroom through authentic science and student research. The collaboration to incorporate the use of ozone sensitive plants with the Surface Ozone Protocol is a natural extension to taking surface ozone measurements for the GLOBE (Global Learning and Observations to Benefit the Environment) Program. Irene was educated at Keene State College, NH, University of NH, and Vanderbilt University, TN.

Susan Sachs is a National Park Ranger and the education coordinator of the Appalachian Highlands Science Learning Center located in Great Smoky Mountains National Park. A big part of her job involves creating educational opportunities from the research that occurs in the parks of the Appalachian Highlands monitoring network (besides Great Smoky Mountains National Park she covers Big South Fork National River & Recreation Area, Obed Wild and Scenic River and the Blue Ridge Parkway). Her personal education philosophy is that people learn best when they are involved in learning activities that are both meaningful and relevant so many of the education programs at her center involve students, teachers and others in collecting data for actual research projects. The ozone bio-monitoring project is one example of several citizen science projects on going at the Appalachian Highlands Science Learning Center. Susan was educated at the University of Maryland, College Park and has lived and worked in National Parks in Washington, DC, Alaska, Arizona, and California. Susan currently resides in the biologically diverse mountains of North Carolina.

## Acknowledgements

The information in this guide represents contributions from research scientists, professionals monitoring air quality and plant responses in national parks and forests, and other highly regarded sources. Reprinted material is included with permission and the sources are indicated. Resources are identified in the Appendix.

A GLOBE (Global Learning and Observations to Benefit the Environment) Advanced workshop was held at the North Carolina Center for the Advancement of Teaching during August 2004. The basic atmospheric protocols, and advanced surface ozone and aerosols protocols were part of the training. In addition, Susan Sachs, Education coordinator at the Appalachian Highlands Science Learning Center in the Great Smoky Mountains National Park trained the participants to identify and estimate the percentage of ozone induced foliar injury present on the Cut-leaf Coneflower. Monitoring symptoms of ozone induced foliar injury to native plants was a natural extension to taking surface ozone measurements. Through the collaborative efforts of Susan Sachs and Irene Ladd, plans were made to develop an implementation guide, field charts, protocol, and training program. We thank the scientists for their contributions toward the development of this guide.

In addition to developing materials to monitor ozone injury to native plants, we were also interested in identifying an agricultural crop that could be easily integrated with core curriculum in educational and non-educational settings. Bill Jackson and Alice Cohen from the US Forest Service in North Carolina have collaborated with Dr. Kent Burkey, USDA-ARS Plant Science Research Unit in Raleigh, NC, to concurrently field test ozone sensitive and resistant beans. They were using modified guidelines and readily available retail materials to monitor germination, growth, and ozone injury. The results of the parallel study will provide the information needed to develop the protocol and training program for monitoring ozone symptoms on resistant and sensitive beans.

## Contributing Research Scientists

Dr. Art Chappelka is a Professor in the School of Forestry & Wildlife Sciences at Auburn University, Auburn, AL, USA. He received his Ph.D. in plant pathology from Virginia Tech in 1986. Since 1987, Dr. Chappelka has been on the staff within the School investigating the responses of forest trees and associated plant species to air pollutants. Dr. Chappelka has authored or co-authored over 50 peer-reviewed journal articles and 12 book chapters. He is a reviewer for several international journals, and has participated on numerous US EPA peer-review panels. His primary interests are in air pollution and global climate effects to terrestrial ecosystems; native plant community responses (shifts in diversity) to air pollutants and global climate change; plant-stress-air pollution/global climate change interactions; air toxics, and urban forestry.

Dr. Jack Fishman has been studying ozone for more than 30 years. He came to NASA in 1979 where he has developed a way to measure ozone pollution from satellites. Using satellites, he discovered large plumes of ozone pollution coming from industrialized regions of the world such as the eastern United States, Europe, and eastern Asia. Somewhat surprisingly, he also found large amounts of ozone over the South Atlantic Ocean. Such plumes could even be identified in measurements thousands of kilometers from their origin. In 1992, he led a group of scientists to investigate the composition and origin of these elevated ozone concentrations where they flew in NASA's specially instrumented DC-8 airplane over a 5-week period. The source of the pollution was found to be widespread biomass burning in southern Africa and Brazil and a unique meteorological situation that resulted in both plumes being transported over ocean areas adjacent to Angola and Namibia. In 1990, Dr. Fishman co-authored *Global Alert: The Ozone Pollution Crisis*, a book for general audiences that expressed how important the issue of global pollution is and how we can take measures to reduce the detrimental effects of widespread ozone pollution.

Dr. Howie Neufeld received his B.S. in Forestry from Rutgers University in 1975, his M.F. in Forest Sciences from the Yale School of Forestry and Environmental Science in 1977, and his Ph.D. in Botany from the University of Georgia in 1984. He was a post-doctoral fellow at New Mexico State University under Dr. Gary Cunningham from 1984-1985, working on the ecophysiology of range grasses and creosote bush. In 1985 he began an NRC post-doctoral appointment under Drs. Dave Tingey and Bill Hogsett at the EPA Lab in Corvallis, OR. While there, he worked on the effects of ozone on root growth of tree seedlings. After two years, he came back to the University of Georgia as a research coordinator in the Forestry School before accepting a position as Assistant Professor of Biology at Appalachian State University. Currently he is Professor of Biology, Past-President of The Association of Southeastern Biologists (ASB), and President-Elect of the Southern Appalachian Botanical Society.

Dr. Neufeld's research expertise is in the area of plant physiological ecology, and has included work on plants in swamps, deserts, and forest understories. For the past 20 years, he has been active in air pollution effects research, including acidic deposition (rain and fog studies on spruce trees and hardwoods of the eastern United States, and tropospheric ozone on native wildflowers. From 1988-1992 he was the principal investigator of a National Park Service /Environmental Protection Agency (EPA) sponsored research project on the effects of ozone on plants native to Great Smoky Mountains National Park. These results have been published in a variety of journals, and additional papers are in preparation and press.

## Contributing Research Scientists

Dr. Margaret Pippin is a Research Scientist in Atmospheric Sciences at NASA Langley Research Center. Margaret came to Langley in 2001 after completing post-doctoral research at Western Michigan University, where she specialized in making measurements of organic nitrates at the University of Michigan Biological Station. She comes from a background of both modeling and field measurements, with an emphasis on data analysis. She is interested in the analysis of observational data sets to better understand the chemistry of the atmosphere with a particular interest in the chemistry of biogenic hydrocarbons and their ozone production potential. Margaret has been active in science education for over twenty years and enjoys working with students of all ages.

Dr. Pippin has spent the past several years improving the quality of the GLOBE surface ozone measurements. She has performed extensive laboratory testing to determine the relationship between the absorbance (color change) of the Eco-Badge test cards and the ozone concentration, and the dependence of this color change upon temperature and humidity. Margaret is also involved in GLOBE teacher training for the Surface Ozone Protocol and often visits GLOBE schools to discuss atmospheric science and methods of analysis with the students.

Dr. John M. Skelly is Retired '04, Professor Emeritus Plant Pathology from the Department of Plant Pathology, Pennsylvania State University. BS Forestry '62; MS '64 and Ph.D '68, Plant Pathology Penn State. He taught at Virginia Tech 1968-82; and returned to Penn State 1982-2004. His expertise in forest pathology with specialization in air pollution caused effects to forest trees and native plants within North Temperate regions. John has been involved in writing the Criteria Documents for the US E.P.A. and as a consultant to the U.S. Dept. Of Justice and the United Nations on diagnosing air pollution caused injury to plants. International cooperative projects have been held with colleagues in Canada, Mexico, Switzerland, Spain, Germany, and Italy.

## Organization of this guide

This guide is designed as a resource and field manual to implement the observation and recording of ozone induced foliar injury to Cut-leaf coneflower (*Rudbeckia laciniata*) and Common milkweed (*Asclepias syriaca*).

**Section I** provides background information on the formation of ozone air pollution and the importance of understanding the transport patterns of local, regional and global levels of ozone air pollution and its impact on vegetation as it is transported from urban industrial centers to more rural and forested downwind regions.

**Section II** provides a general introduction to the characteristics of ozone's induced foliar injury to the leaves of broadleaf species and the processes used to identify ozone sensitive plants. The guide also describes the scientific processes used to identify ozone-induced foliar symptoms on plants in the environment and in controlled open top chambers.

**Section III** describes methods to be used in setting up a bioindicator garden for detecting ozone-induced injuries, and the protocol for gathering and submitting data on Cut-leaf coneflower (*Rudbeckia laciniata*), and Common milkweed (*Asclepias syriaca*) to the Hands on the Land Website, host to the interactive database.

# Using Sensitive Plants as Bioindicators of Ozone Air Pollution

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# SECTION I: Formation of Ozone Air Pollution

## Introduction to Ozone Formation

### Quality of Air

The atmosphere is made up of a mixture of gases. The gases include nitrogen, oxygen, carbon dioxide, water vapor, argon, and trace gases. Nitrogen makes up approximately 78% of the atmosphere and oxygen 21 percent. Only about 1% of the atmosphere is made up of a combination of the remaining gases.

The quality of air is affected by the composition of the atmosphere. The addition of pollutants created from various human activities gradually contributes to the deterioration of air quality. The study of surface ozone (ozone produced near the ground where people breathe the air) and using ozone sensitive plants as bioindicators of ozone induced plant injury will help develop an understanding of how human behavior affects the quality of air and an awareness of its environmental impact.

Ozone gas is a form of oxygen. It is a colorless, highly reactive gas that exists from the Earth's surface miles up into the atmosphere. Ozone has three atoms of oxygen (O<sub>3</sub>), whereas the oxygen molecule we need to breathe only has two atoms. It is the third atom that makes ozone a highly reactive gas.

“Good” ozone is located in the level of the atmosphere called the stratosphere. It traps ultraviolet rays and protects the life forms on Earth. This is the ozone layer we hear so much about. In contrast, ozone produced in the troposphere at the level we breathe is considered a pollutant and harmful to plants and animals. Ozone at this level is called surface ozone, or “bad” ozone. It is the main component of smog.

The low concentrations of surface ozone that naturally occur in the troposphere are produced by intense sunlight driven reactions involving several key hydrocarbons given off by plants and nitrogen oxides produced as part of the natural nitrogen cycles of Earth's atmosphere. Under normal conditions, these photochemically driven gases may produce ozone concentrations between 10-40 ppb depending upon the season of the year (Lefohn et al. 1990).

Other natural sources of ozone may include lightning and turbulent weather conditions that transport concentrations of ozone down to Earth's surface from the stratosphere. This phenomenon occurs commonly on mountaintops during thunderstorms and has been linked to symptoms on eastern white pine during late spring and early storms throughout the Appalachian Mountains.

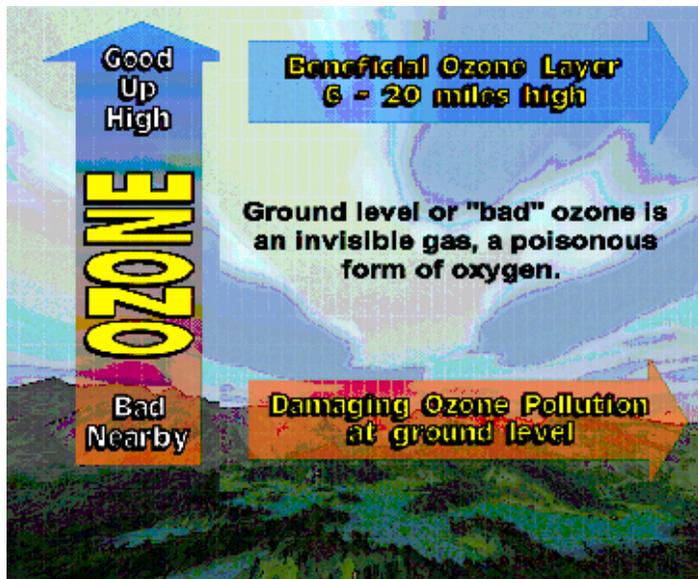


Image courtesy of the National Park Service

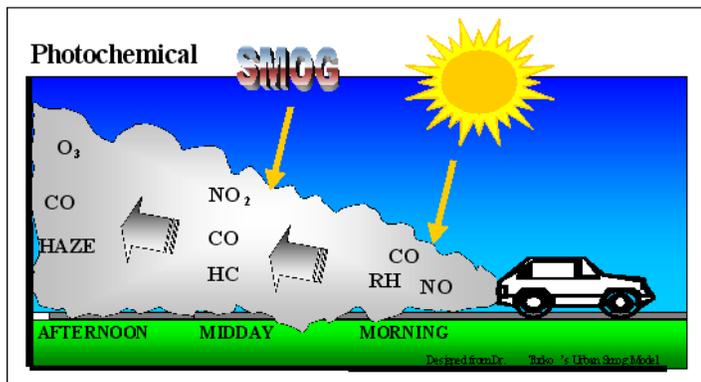
### Ozone Pollution Cycle

Human activity has added new concentrations of pollutants to the air we breathe. The major contributors are vehicles (automobiles, trucks, buses and airplanes) and industrial burning of fossil fuels (as oil and coal burning utility plants). The primary gases that contribute to the production of surface ozone are exhausts from any internal combustion gasoline-fueled engine. The major by-products of the combustion process are: carbon monoxide (CO), nitrogen oxides (NO), and partially burned hydrocarbons called volatile organic compounds (VOCs) that are very reactive.

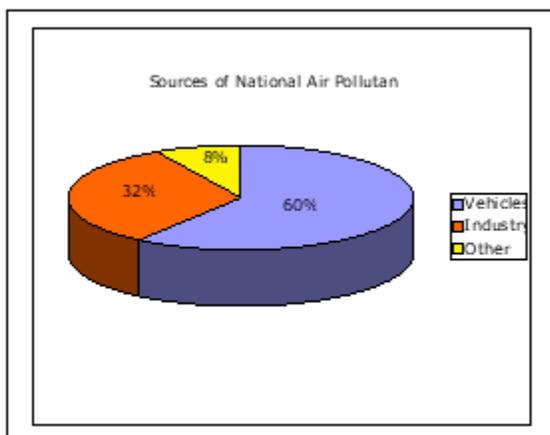
Nitrogen molecules in the atmosphere are non-reactive to the sun's energy (they don't split into atoms and attach to other atoms to form something new), but the intense heat within the combustion chamber

causes the nitrogen molecules to split into two nitrogen atoms. These split nitrogen atoms link up with oxygen atoms and form a byproduct called nitrogen oxide (NO).

The air taken into the engine combines with the gasoline (hydrocarbons) before entering the combustion cylinders. If there were complete combustion, as the hydrocarbon or gas molecules ignite and split and produce energy in the engine, the byproducts would be carbon dioxide (CO<sub>2</sub>) and water vapor. However, complete combustion does not occur and some hydrocarbons remain as VOCs.



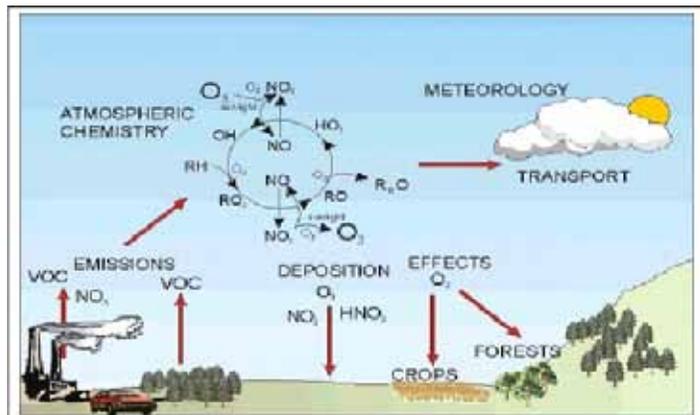
Hydrocarbons are also a byproduct whenever things are burned. For example, a forest fire produces hydrocarbons, which are molecules of carbon and hydrogen. But these are far-less reactive and of lesser importance to the formation ozone than are the VOC's emitted within industrial and auto transportation exhausts. The pie graph below identifies the major sources of primary air pollutants contributing to the production of the secondary pollutant, surface ozone.



Courtesy of the Environmental Protection Agency

These new gases have contributed to the production of higher concentrations of surface ozone, i.e. the formation of ozone air pollution. In general, ozone is a main component of urban smog, and it is commonly

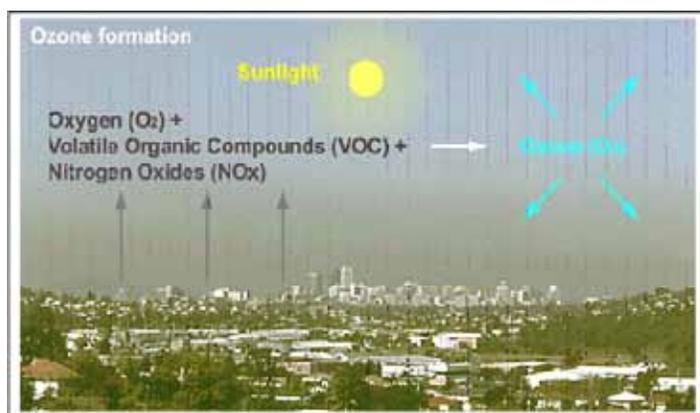
referred to as photochemical smog. The production of surface ozone air pollution usually peaks in the later afternoon at low elevations, but may remain high all day at higher elevations, such as in the mountains.



<http://www.york.ac.uk/inst/sei/APS/a-proj.htm>

The afternoon peak at low elevations occurs because the formation of ozone is dependent on the sun's energy, and it proceeds faster at higher temperatures, so the concentrations tend to build up from morning to the afternoon. At night though, other chemicals in the atmosphere may break down the ozone, which is why it starts off each day at a lower concentration than the preceding afternoon.

During the course of the day, the pollutants react in the presence of the sun's radiant energy and the oxides of nitrogen (NO) produce concentrations of ozone, air pollution. A photochemically formed hazy air mass is formed that has a brown tinge due to combustion in fuel powered engines.



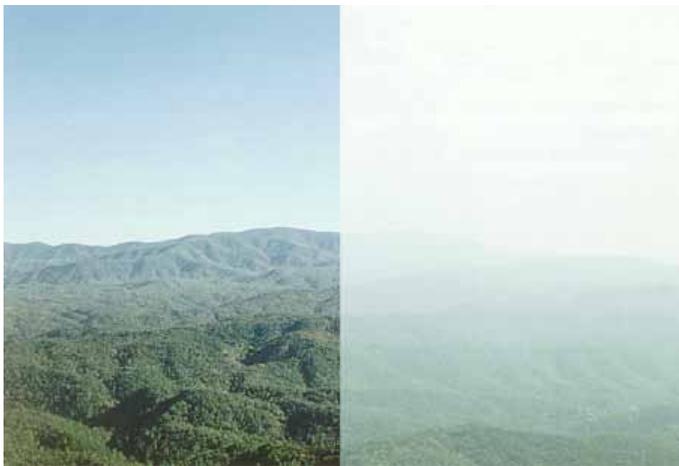
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Whether the primary pollutants are produced through a forest fire or the fire of a combustion engine, the byproducts produced are the precursors needed for the production of surface ozone. These primary

## Influence of Weather and Episodic Events on Ozone Formation

pollutants react with the energy of the sun to produce a secondary pollutant, surface ozone.

Surface concentrations of ozone air pollution begin to increase seasonally from April through September in the eastern regions of the United States. This is the time period of increased amounts of sunlight, higher temperatures, and commonly occurring stagnating high-pressure systems (Bermuda Highs) over vast regions of the Midwest and Mid-Atlantic States. Under these atmospheric conditions ozone air pollution reaches its highest levels during the hottest and most sunlit months of the year. Depending upon weather patterns, the concentrations of ozone air pollution though usually higher in the summer, can vary year to year. For example, during the hot, dry years of the mid- to late 1990s, ozone reached record levels in the Great Smoky Mountains National Park, but in 2004, a wet and cool year, ozone levels were greatly reduced. The amount of ozone formed each year is highly dependent on weather conditions, and less so on variations in pollutant emission, which are relatively constant from year to year.



The two side by side photos above show the haze problem in Great Smoky Mountains National Park. The weather conditions of hot, humid, stagnant air that cause sulfur particles to combine with water vapor to create haze, are the same conditions that increase the ground level ozone levels.

Initially surface ozone was thought to be a local problem, but the pollution-laden air masses may travel beyond the local areas where they were originally produced. The slow moving air masses pick up pollutants

all day long as they travel over industrial centers; large fossil fuel fired power plants, incinerators, and most importantly over large and even small urban areas with many forms of fossil fuel fired transportation. The collection of pollutant gases in slow-moving air masses quickly reacts in the warm sunlit air. The most important air pollutant formed within these air masses is ozone air pollution.

In the 1950s and 1960s, the Los Angeles area was the region most affected by the production of photochemical smog. After the passage of the Clean Air Act in 1970, many more monitoring stations were set up across the United States and now we commonly see increased pollution well beyond the local areas where they were initially produced. Slow moving air masses allow pollutants to “cook” all day long. As they meander, even more ozone can be formed as they pass over even relatively small urban areas. Complicated chemistry and meteorology can result in the transport of surface ozone pollution to areas that are generally considered rural or even pristine, such as National Parks.

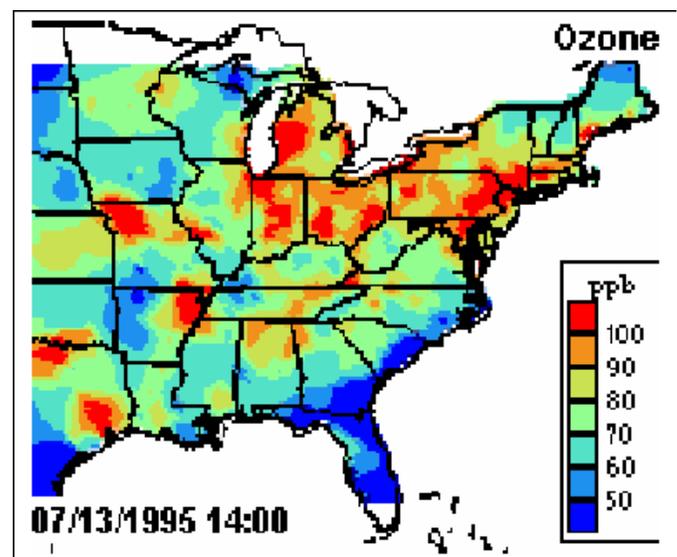
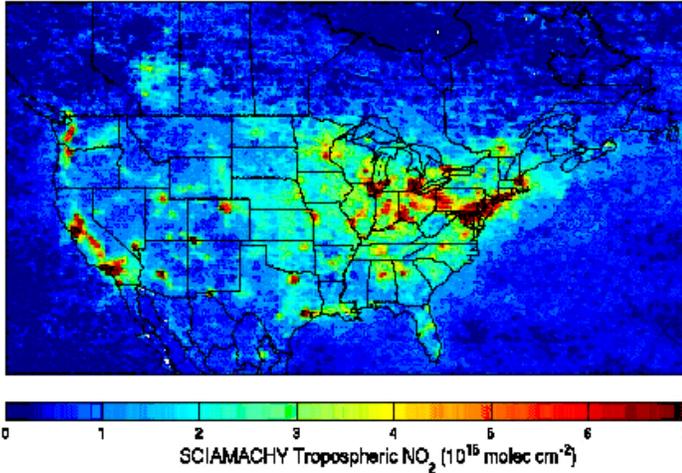


Image courtesy of Environmental Protection Agency's Air Now website.

The above figure for July 13, 1995, shows the sketchy high ozone patterns where detrimental concentrations are found throughout rural areas in Indiana, Ohio and Pennsylvania as well as in densely forested regions in Tennessee, Georgia, and North Carolina. Depending on the meteorological situation, high levels of ozone can remain over an area for a

period of time and plants will be exposed to ozone air pollution.

Slow moving high-pressure air masses are common occurrences in the eastern United States. These air masses transport significant concentrations of ozone air pollution to the plant communities of mountainous, forested, and rural areas downwind of the industrial urban areas.

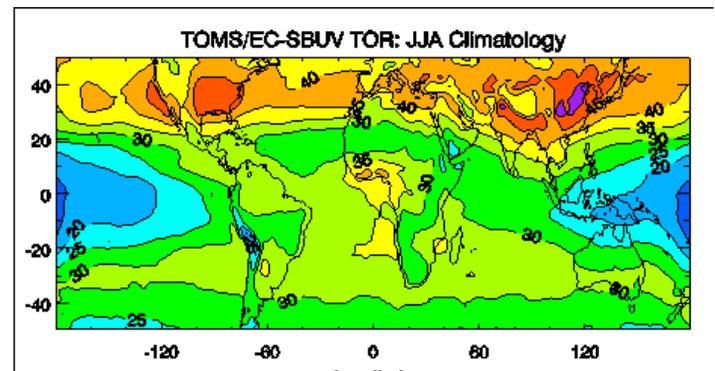


Location of Industrial Emissions in U.S. derived from a satellite. It is the climatology for the year 2003.

The map above is a satellite image that identifies the location of industrial emissions in the U.S.. Due to the continuing photochemically driven processes as the air mass slowly moves downwind, these more remote areas often have greater ozone exposures for longer periods of time. If the high air pressure mass stagnates and remains over an area for a period of time,

the pollution can build up and expose plants to more severe levels of ozone.

Although some controls have been implemented that have helped to decrease the level of primary pollutant gases entering the atmosphere that contribute to the production of ozone air pollution, the large increase in the numbers of vehicles and the increase in the miles driven have offset many of the gains that have been made through the implementation of pollution controls. New satellite instruments can now map ozone pollution and the global depiction above shows that high ozone concentrations are not unique to the eastern U.S. or California. In the summertime, even higher concentrations are now found over China and India, where pollution controls are not as rigid as in the U.S. Furthermore, scientists also believe that emissions from these regions will continue to grow as these countries continue to develop.



## SECTION II:

# Investigation of Ozone Injury Induced Foliar Injury to Plants

## Surface Ozone is Harmful

### Ozone Air Pollution is Harmful to Humans

High concentrations of ozone air pollution are harmful to both animals and plants. Concern for air quality is reflected most during hot summer days as “ozone alert days” or some other phrase is used to warn children and the elderly to restrict outside activity due to the likelihood of higher exposures to ozone. Why? Because exposure to elevated concentrations of surface ozone over extended periods of time causes health problems. It damages the immune system’s defenses making one susceptible to lung infections. Ozone causes acute respiratory irritation, breathing problems, and aggravates asthma. The pollutant decreases lung capacity by 15% to over 20%. This is because ozone “sunburns” your lungs. Ozone damages the cells that line the air spaces in the lung.

Within a few days, the damaged cells are replaced and the old cells are shed-much in the way that skin peels after a sunburn. If this kind of damage occurs repeatedly, the lung may change permanently in a way that could cause long-term health effects and a lower quality of life. Children are at greatest risk from surface ozone exposure, because they spend more time outside involved in vigorous activities and have a greater demand for intake of air. Their respiratory systems are developing and are most susceptible to permanent damage. The elderly are also more sensitive to ozone because their immune system is not as good as it used to be. For more information, visit the EPA website and view their publication called “What You Need to Know About Ozone and Your Health” at <http://www.airnow.gov/index.cfm?action=health2.smog1#3>

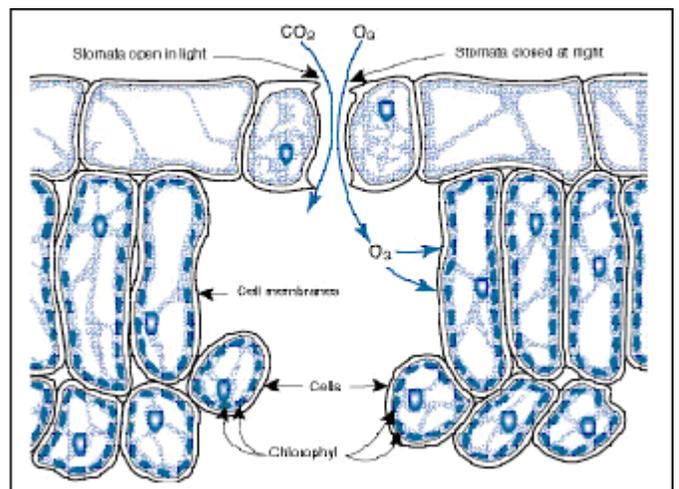


The photos above show a healthy lung air way (left) and an inflamed lung air way (right). Ozone can inflame the lung’s lining, and repeated episodes of inflammation may cause permanent changes in the lung. (EPA photos from “What You Need to Know About Ozone and Your Health”)

### Ozone Air Pollution is Harmful to Plants.

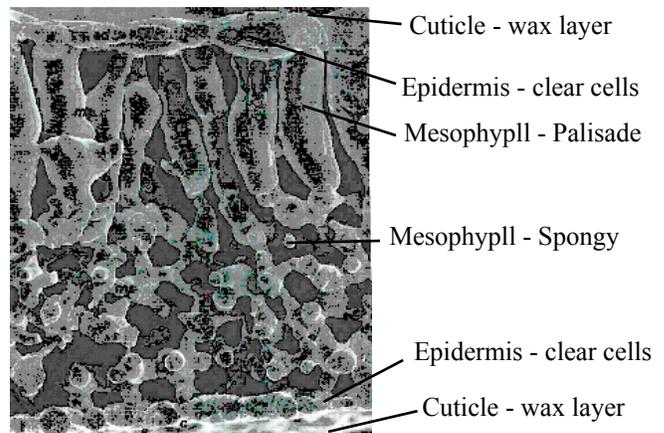
Plants are not unlike humans. They need to take in carbon dioxide for photosynthesis, the process they use to produce their own food, and give off oxygen as a byproduct. The healthy plants, like young children, are actively ...”taking”... in the air.

Ozone enters leaves through their stomata. Stomata are small pores in the leaf that allow gases to enter or leave. When they are open, carbon dioxide can enter, which the plant uses to make its food, such as sugars and starches. At the same time, water exits the leaf, through the process known as transpiration. If ozone is present in the air, it too will enter the leaf through the open stomata, following much the same path as the carbon dioxide.



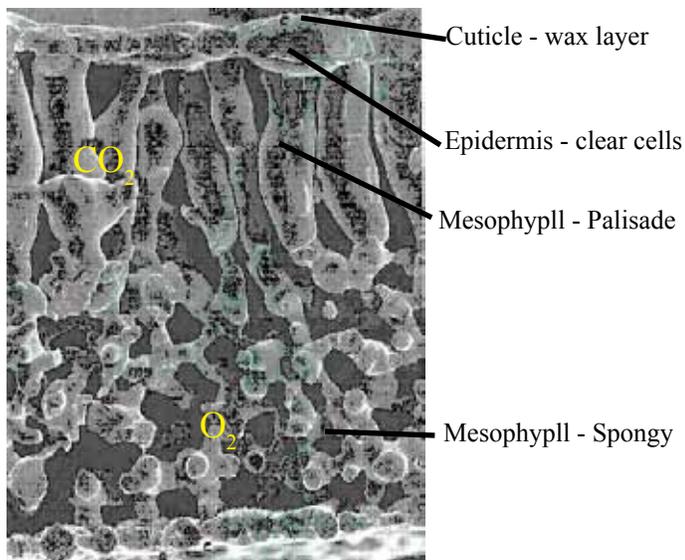
[http://res2.agr.gc.ca/publications/ha/3d\\_e.htm](http://res2.agr.gc.ca/publications/ha/3d_e.htm)

Ozone, however, is a highly reactive molecule, and once it enters the leaf, it will find its way through the leaf interior to the cells responsible for photosynthesis, particularly the palisade and spongy mesophyll tissues.



Cross section view of a leaf, source US Forest Service

The surface layer of cells in a leaf are called the epidermis. The long cells below are the palisade mesophyll cells, where much of the photosynthesis occurs in leaves. The irregular cells further down are spongy mesophyll cells, and they also participate in photosynthesis. At the bottom is the lower epidermal layer, and the curved cells represent guard cells for stomata where the ozone enters on the under side of the leaf. As soon as the ozone enters, it most likely reacts with molecules in the cell wall that end up triggering production of the ROS molecules, which damage the cell. Ozone itself rarely gets far into a cell to cause damage. Note the enhanced airspaces between the spongy mesophyll cells compared to that in the palisade layer. This is where photosynthesis occurs in the leaf.

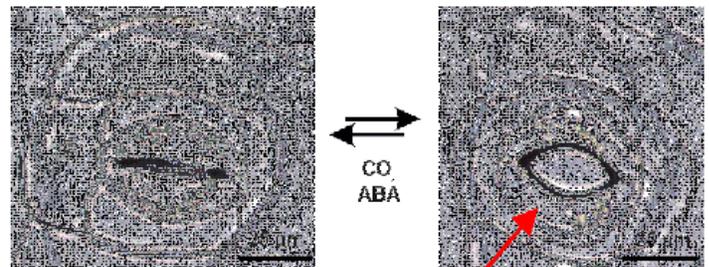


It is of interest that even though ozone primarily comes through the lower epidermal stomata, it is the upper epidermal layer, and eventually the palisade cells that suffer injury first, due to their interaction with light, in reactions not yet fully understood. It has been noticed that a leaf partially shaded by the leaf above may only show injury in the area of the leaf fully exposed, the shaded part of the leaf shows no injury.

Very little ozone can get into a leaf through the cuticle, a waxy layer that covers most of the surfaces of leaves and stems. This waxy layer is nearly impermeable to water, and also to most gases, including both carbon dioxide and ozone. Therefore, the uptake of ozone depends nearly entirely on whether the stomata are open or closed.

Stomata open in response to certain environmental

stimuli. These include light, high humidity, and high temperatures. At night, stomata tend to close because of the lack of light. Thus ozone uptake is greatly reduced at this time of the day. If the humidity should go down, this causes excessive water loss from the cells surrounding the stomatal pore, and the stomata close. With respect to temperature, stomata generally open more the warmer it gets. Finally, high carbon dioxide levels will also close stomata.



A stomate is comprised of two guard cells, and some accessory cells that assist in opening and closing. When the guard cells fill with water, they pull away from each other, and the stomatal pore is opened. When they lose water, the two guard cells collapse against each other, closing the pore. The figure above shows a closed pore on the left, and an open one on the right.

All plant cells are surrounded by a cell wall, composed mainly of cellulose and lignins. These fairly rigid molecules provide support for the plant. In order for ozone to damage a cell, it must go through the cell wall first, before reaching the cell's membrane. The cell wall may contain anti-oxidants, which are molecules that can react with, and detoxify, the ozone. This is one way for the plant to protect itself from pathogens and to avoid problems from ozone.

One such anti-oxidant is Vitamin C (also known as ascorbic acid) which is produced by plants. If there is a lot of Vitamin C in the cell wall, it can destroy the ozone before it reaches the cell membrane. If not, then the ozone can reach the cell membrane, where it can cause damage, eventually killing the cell. symptoms than well-watered plants.

If a plant is suffering from drought stress, it will close its stomata to save on water. While this may prevent the plant from drying out, it also lowers photosynthesis, since the carbon dioxide cannot get into the leaf. However, one benefit is that ozone also cannot

get into the leaf. Thus, plants under drought stress often show fewer symptoms than well-watered plants. When ozone is taken into a leaf, it can interfere with a plant's ability to produce and store food. It weakens the plant making it less resistant to disease and insect infestations. In some sensitive agricultural crops such as varieties of soybeans and snap bean, exposure to ozone air pollution also affects the plant's ability to reproduce, thus decreasing crop yield. Some plants are more sensitive to surface ozone than others, and show visible symptoms within days or weeks of their exposure under field and forest conditions.

Ozone causes very specific and unique symptoms on broadleaf plants in the field. The most common symptom is stippling (also called purpling) on the upper side of the leaf's surface with the lower leaf surface absent of symptoms. Stippling consists of very small spots much like someone shook fine pepper onto the leaf surface. The color of the stippling depends on the species of plant. The amount of stippling can

vary depending upon the environment (nutrients in the soil, amount of water, amount of sunlight that are local weather conditions), and the physiological conditions or make up of the plant. The stippling may occur only within certain areas of the leaf's surface, but does not involve any of the large or small veins in the leaf. That is, with ozone-induced stippling, the veins are free of any symptoms whereas many insects while feeding and certain fungus pathogens cause direct injuries to the veins.

In general, the lower older leaves on the plant exhibit more stippling than the newer leaves on the plant. Plants grow from the tips of their stems and hence the new leaves that were produced first in the spring are at the base of the new shoot and are therefore exposed to the ozone air pollution as it occurs throughout the entire spring and summer season. New leaves produced later in the summer "see" far less ozone pollution because they have not been present all season long.



## Studies of Ozone-Sensitive Plants

Many studies and recently published reports in the United States have documented ozone induced foliar injury on ozone sensitive plant species. The injuries are easily observed on sensitive forest tree species such as black cherry (*Prunus serotina*, Ehrh.), white ash (*Fraxinus americana*, L), and Yellow-poplar (*Liriodendron tulipifera*,L.) (Chappelka et. al., 1992; Skelly et al., 1996; Neufeld et al., 1992; Hildebrande et al., 1996). Long-term investigations of selected plants have been conducted in open-top chambers in The Great Smoky Mountains National Park of Tennessee and North Carolina, the Shenandoah National Park in Virginia, and the Allegheny Mountains of north-central Pennsylvania. A few samples of ozone sensitive native species are listed below.

1. *Acer rubrum* (Red maple)
2. *Aesculus octandra* (Yellow buckeye)
3. *Apocynum cannabinum* ( Indian hemp)
4. *Asclepias exaltata* (Tall milkweed)
5. *Aster acuminata* (Whorled-wood aster)
6. *Aster divaricatus* (White-wood aster)
7. *Aster puniceus* (Purple-stemmed aster)
8. *Betula lutea* (Yellow birch)
9. *Cacalia rugelii* (Rugel's ragwort)
10. *Cercis canadensis* (Eastern redbud)
11. *Cornus florida* (Flowering dogwood)
12. *Eupatorium rugosum* (White snakeroot)
13. *Glyceria nubigena* (Smoky Mtn. manna grass)
14. *Krigia montana* (Dwarf dandelion)
15. *Liquidambar styraciflua* (Sweetgum)
16. *Liriodendron tulipifera* (Yellow poplar or Tuliptree)
17. *Lobelia cardinalis* (Cardinal flower)
18. *Magnolia tripetal* (Umbrella-leaf magnolia)
19. *Oxydendron arboretum* (Sourwood)
20. *Pinus pungens* (Pitch pine)
21. *Pinus virginiana* (Virginia pine)
22. *Platanus occidentalis* (American sycamore)
23. *Prunus serotina* (Black cherry)
24. *Rhus copallina* (Winged sumac)
25. *Robinia pseudoacacia* (Black locust)
26. *Rudbeckia hirta* (Black-eyed Susan)
27. *Rudbeckia laciniata* (Cut-leaf coneflower)
28. *Rubus canadensis* (Thornless blackberry)
29. *Rubus idaeus* (Red raspberry)
30. *Sassafras albidum* (Sassafras)
31. *Vernonia noveboracensis* (New York ironweed)

## Using Ozone Sensitive Plants as Bioindicators of Ozone Injury

The use of biological indicators to detect the presence of ozone injury to plants is a longstanding and effective methodology. A bioindicator can be defined as a vascular or non-vascular plant exhibiting a typical and verifiable response when exposed to a plant stress such as ozone. To be considered a good indicator species plants must: 1) exhibit a distinct, verified response, 2) have few or no confounding disease or pest problems, and 3) exhibit genetic stability.

These sensitive plants can be used to detect the presence of ozone at a specific location or region, and provide unique information regarding ambient air quality in a particular area. Plant indicators of ozone injury can be either introduced (Bel W3 tobacco for example) or native (Common milkweed and Cut-leaf coneflower). Indicator species are most effective when related to ambient ozone conditions.

### Fumigation Experiments

#### Common milkweed (*Asclepias syriaca*)

Common milkweed is a native perennial found throughout the eastern half of the United States, except along some areas of the Gulf Coast. The species spreads naturally by seed and from long underground rhizomes. During the ozone season in the Blue Ridge Mountains of Virginia, Common milkweeds were observed to develop purple stippling (a discrete and very fine purple colored spots of the upper leaf surfaces of the older leaves) and eventual overall chlorosis (the yellowing of the leaf caused by the loss of chlorophyll needed for photosynthesis). The question raised was: Is surface ozone responsible for the foliar (plant) injury? A study was developed to determine whether long-range transported ozone air pollution into to the Blue Ridge Mountains and the Shenandoah National Park was responsible for the observed symptoms. Eleven-week-old milkweed plants were randomly placed into chambers and exposed to one of three following ozone levels: 0.00ppb, 50 ppb, and 150 ppb for six hours a day for seven days. Within the fumigation chambers the temperature ranged from 20 o -27o C and the relative humidity between 70%-80%. Foliar symptoms developed on the upper leaf surfaces of milkweed plants in the chambers within 2-5 days after they were fumigated with (exposed to) the different levels of ozone.



Ozone chamber used in Great Smoky Mountains National Park

The milkweed plants in the chambers with the higher ozone exposures demonstrated increased stippling on the leaves. By mid-June, stippling and chlorosis were observed on milkweed plants growing in no filtered air in open-top chambers, as well as on the milkweed growing in open plots in the field. (Duchelle and Skelly, et al.1981). Milkweeds growing in charcoal-filtered air supplied chambers were free of symptoms and considerably larger and greener than those in the open plots and non-filtered air supplied chambers. These two initial studies confirmed that Common milkweed was very sensitive to ozone air pollution within the polluted air masses being carried from long-distances (the Midwest and Ohio Valley, USA) into the Blue Ridge Mountains of Virginia. The following pictures show milkweed plants that are healthy and injured as a result of exposure to ground-level ozone. (NPS Photos)



Tall milkweed with no ozone injury.



Heavily injured Tall milkweed

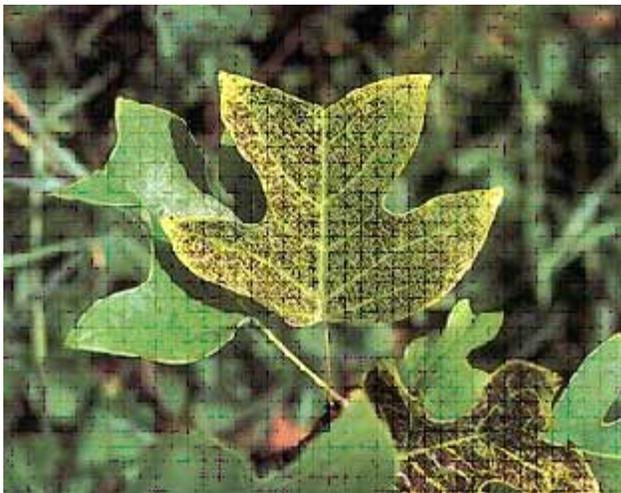
The chlorotic symptoms (the yellow) in the leaves are a stress response to the plant's long-term exposure to ozone air pollution. As the chlorotic symptoms increase the closer the leaf is to senescence or death.

### Open-top Chamber Studies in Great Smoky Mountains National Park

In addition to milkweed, scientists did experiments to find other plants sensitive to exposure to surface ozone. In 1987, a study was set up in the open-top chambers at the Twin Creeks Research facility in Great Smoky Mountains National Park. From 1988 to 1992, nearly 40 different species were fumigated with ozone of differing concentrations, ranging from charcoal-filtered chambers (which scrub out most of the ozone) to ambient (same as in the air outside the chambers), to 50% and 100% above ambient levels (Neufeld et al. 1992).

A variety of species, including both trees and wildflowers, were exposed to ozone over the course of a season. Some perennials, and trees, were exposed to ozone for two growing seasons. The aim of these studies was to verify that the ozone-like symptoms seen in the field on these species was, indeed, due to ozone. Of the 30 species that were able to be grown and fumigated in the chambers, 27 showed ozone-like symptoms after being exposed to elevated ozone. Thus, the researchers were more confident that the symptoms they were identifying in the field were due to ozone and not other factors.

For most species that were sensitive to ozone, growth was reduced whenever the ozone was elevated above ambient (the 50% and 100% treatments). Cut-leaf coneflowers were exposed over two consecutive



Yellow poplar with ozone injury, photo courtesy of US Forest Service.

growing seasons. After the first season, there were no effects on either leaf or flower weights, but ozone-induced foliar stipple was greatly accentuated in the higher treatments. Although the researchers could not detect any growth effects, it was apparent that the leaves were highly sensitive to elevated ozone. For other species, such as Black cherry, Yellow poplar, and Whorled-wood aster, there were significant growth reductions in the elevated ozone treatments.

### Cut-Leaf Coneflower (*Rudbeckia laciniata*)

Cut-leaf coneflower (*Rudbeckia laciniata*) is a native perennial found throughout most of the United States. It grows naturally by seed and from long spreading rhizomes. Cut-leaf coneflower is ever-present in the Great Smoky Mountains National Park, found elevations ranging from 2,000 to 6,600 feet. It grows well within the environment of the open-top chambers. As part of a long-term investigation of the ecology in the Great Smoky Mountains National Park, Cut-leaf coneflower has been evaluated for ozone-induced foliar injury.



Cut-leaf Coneflower showing ozone damage.  
NPS photo

Field evaluations were done in plots located in the Great Smoky Mountains National Park, which is in the southern Appalachian Mountains of western North Carolina and eastern Tennessee. Cut-leaf coneflower was evaluated on Clingmans Dome trail, Tennessee, from June to September and at Purchase Knob near Waynesville, NC during July. The plants sampled were about 1 meter from each other, and categorized as near and off-trail plants. Near trail plants were lo-

cated less than 5 meters from the trail. Off trail plants were more than 5 meters from the trail. There were four sampling periods for ozone injury at approximately 3-5 week intervals from June to September during 2000. Cut-leaf coneflowers were also examined on and off-trail at Purchase Knob in the Great Smoky Mountains near Waynesville, NC during July. The level of ozone-induced injury was assessed using two methods: the number of injured leaves and the percentage of leaf area injured. The findings of the study at Clingmans Dome trail identified 50% of the overall population of the plants studied had ozone air pollution injury. Plants growing near the trail had significantly greater injury and 3.5 times greater leaf area injury than those growing off-trail. The leaves on the lower half of the plant had 95% of the leaf injury. The pattern of injury was similar for plants near and off-trail. The results with on and off-trail at Purchase Knob showed no differences in the level of injury between the plants. However, ozone injury was greater for the Clingmans Dome plants than those at Purchase Knob. (Chappelka, Neufeld, Davison, Somers, and Renfro et al. 2003).

Ground-level ozone has been identified as the major pollutant causing foliar injury to plants. The two native ozone-sensitive plants that can easily be incorporated into a “Bioindicator Garden for the Detection of Ozone Air Pollution” and used as indicators of the presence of ozone are Cut-leaf coneflower (*Rudbeckia laciniata*) and Common milkweed (*Asclepias syriaca*). They are perennials that are relatively easy to transplant and maintain. Organizing an ozone bioindicator site using these plants will provide a way to observe ozone-induced foliar injury and to observe and measure its impact on plant growth over time. The involvement of different geographic sites in a long-term investigation will provide quantitative data relative to the sensitivity of these two species to ambient air containing ozone air pollution. In addition, the observations by the students involved within this unique project will develop and increase their awareness of the effects of ozone air pollution on sensitive plants within our natural plant communities as an important part of our National Parks.



A student checks Crownbeard plants in the “Ozone Garden” at Purchase Knob, GSMNP, NC.



Dr. Howie Neufeld measures the rate of photosynthesis on ozone injured Cut-leaf coneflower leaves in Great Smoky Mountains National Park.

# SECTION III: The Ozone Bioindicator Garden

## Background Information

### Ozone Measurements and Plant Observations

Participants in educational and non-educational settings have been trained to take surface ozone measurements with a hand-held optical scanner and to gather cloud cover and type, humidity, wind direction and current temperature. The surface ozone and meteorological data are submitted to the GLOBE Student Data Server ([www.globe.gov](http://www.globe.gov)) at Colorado State University, Fort Collins, CO. The surface ozone data can be retrieved in raw form or in graphic visualizations enabling the citizen scientist to analyze their own data for patterns and/or compare their findings to other sites collecting ozone data. Organizing an Ozone Bioindicator Garden containing ozone-sensitive plant species is a natural extension to gathering surface ozone data and enables the observer to develop an awareness of how surface ozone affects vegetation.

An Ozone Bioindicator Garden is a way for educators, students, and citizen scientists from educational and non-educational settings to take part in an important study to determine the effects of ozone air pollution on plant populations. The study of ozone and ozone-induced foliar injury will aid in understanding the relationship between foliar injury, air quality, and plant growth. Ozone Bioindicator Garden data are submitted to the “Hands on the Land” website.

<http://www.handsontheland.org/environmental-monitoring/ozone-bio-monitoring.html>



The layout of the garden at the US Forest Service's Cradle of Forestry in America is lined with space between the beds so data can be easily collected without trampling plants.



The garden at Purchase Knob in Great Smoky Mountains National Park is arranged in rows with 2 feet between each plant and 3 feet between each row.

It is important to plant Cut-leaf coneflower and Common milkweed in the natural soils of the area with enough walkway area to prevent damaging the plants. The garden must be located away from any areas where pesticides, herbicides, fertilizers, etc might be used. The objective is to maintain a natural habitat for each of the ozone sensitive plants. Do NOT use any of the above chemicals in your garden plot as they may affect the results. For example, some fungicides are potent anti-oxidants, and will keep plants from developing any ozone symptoms.

## No Garden Area? Use Pots

If your school is in an area unsuitable for a garden plot, you can use pots. It is important to use as large a pot as possible to accommodate the large root system of the plants and to find a “breathable” material like terra cotta or wood. Black plastic pots will cause the plants to dry out too quickly in the sunlight so they aren’t recommended. It is not advisable to put more than one plant in each pot. Place the pots in an area where they can get as much sunlight as possible. You will need to check them daily for watering if you live in an area that gets very warm.

## Planting the Cut-leaf Coneflower Rhizomes

If you can’t put your plants into the ground or pots immediately, they will store in a refrigerator for about one month if wrapped in moist paper towels. The plants do best if they are planted in the early fall so they have a chance to establish themselves before overwintering. Coneflower overwinter with a rosette of basal leaves that remains green. In the spring, the plant will add new basal leaves and then send up a main stalk, this is the part of the plant you will collect data on, not the basal rosette.

Your roots have been collected directly off of plants from an existing garden. This ensures that all of the plants have genetic material originating with the garden at Purchase Knob in Great Smoky Mountains National Park. The rhizome should still have a bit of green plant material, when planting; this area should be at the top and just barely covered by soil. Dig a hole in the ground that is slightly deeper than the length of any root hairs hanging down when the green part of the plant is the top.



## Organizing the Plants for Study

Once the design of the garden is established, each plant will need to have an ID number using CF for Cut-leaf coneflower and MW for Common milkweed. Also consider a different numbering pattern for each plant within the species. For example, if you have 4 Cut-leaf coneflower plants, tag one as CF01, CF02, CF03 and CF04. This will help prevent confusing data from one Coneflower plant with another, because the numbers are not too similar. The plant ID number and leaf labeling pattern developed during the initial set up of the garden needs to be the same each year measurements are taken.

## Measuring the Plants

After the plant ID is established, the plant height needs to be recorded using a meter stick.

- The measurement has to be recorded in centimeters (cm) on the data sheet. If the plant is not fully grown, only measure up to the base of the top-most leaf. The leaves stand erect when they aren’t fully open and if you measure to the top of the leaf, the plant may appear to shrink once the leaf opens fully and drops down.
- Count the total number of leaves coming off of the main stem of the entire plant and record observations (in flower, in bud, node with no leaf, etc). Do NOT count all of the leaves on the plant, just those directly coming off the main stem.
- It is important to recognize that leaves may fall off the plant but they still should be counted and included in data collection. To determine if a leaf is missing, feel along the stem of the plant for a small knob, this is a leaf node and indicates that a leaf used to be there and was lost. Work from the bottom of the plant towards the top, running your fingers up the stem.
- Data collection for each plant will start at the base of the plant and will work up towards the top.
- Identify the north side for each plant. (Mark north somewhere in the plot and where the base point was when marking north so each observer on the team may know for certain which way to face when recording observations.
- If the leaves are opposite each other, designate the leaves to the left of the middle of the plant as “B” and the right side “A” as you are facing North. Using a permanent marker, put a small dot on the stem of the leaf close to where it joins the plant. This will ensure

that you do not assign “A” to a leaf one week and “B” to the same leaf the next week. The plant may twist as it grows so this is an important step.

- If the leaves alternate on the stem, number the leaves in sequence beginning with leaf #1-the first leaf at the bottom, just above the florette of basal leaves. If any leaf has fallen off (feel for a leaf scar remain on the main stem), continue to include it in your count.

### Preparing to Observe

Use a 10X magnifying glass to check leaves and record your observations. Ozone injury may become visible as early as late May in areas with higher ozone levels, while other areas never experience ozone symptoms (and this is also important data). The first symptom of stippling on the upper leaf surface provides the baseline date of occurrence and initial data to begin assessing foliar injury. Data may be collected every two weeks, but it is fine to collect it weekly. Stop collecting data once the plants have been through their first frost event of the late summer or fall season. Each leaf is measured for the amount of “C” – chlorosis (yellowing), “P” – purpling (ozone damage) and “N” – necrosis (death of leaf tissue). The percentage of the leaf that is missing should be recorded in the notes area of the data sheet for each leaf.

On the data sheet, each leaf will be rated based on the following codes.

- 1 - 0%
- 2 - 1-6%
- 3 - 7-25%
- 4 - 26-50%
- 5 - 51-75%
- 6 - 76 - 100%

As the exposure to ozone increase and foliar injury increases, a leaf may break off. Make sure to assign it the appropriate leaf loss code using the following criteria:

- 7 - leaf gone with no prior symptoms
- 8 - leaf gone with prior chlorosis (yellowing) only
- 9 - leaf gone with prior purpling (the brownish purplish dots called stippling) only
- 10 - leaf gone with prior purpling and chlorosis

When a leaf falls off, carry its last data entry over into all of the following data collection entries. This is to show leaf loss on a graph or other visualizations as opposed to leaf improvement. Never make assumptions about what you are seeing, just rate what you see at that particular point in time. Insects will also eat part of some leaves during the season. Rate the leaves on what is visible, and note in the comments area for that leaf the rating for the percentage of the total leaf is missing i.e. 2 would mean 1%-6% of the leaf is gone.



## Training to Assess Foliar Injury

An “Ozone Bioindicator Garden” provides the opportunity to measure the amount of ozone induced injury to plants and to observe the impact of ozone air pollution on ozone-sensitive plant species over time. For this study, we are using a rating scale developed by the National Park Service to assess the percentage of foliar or leaf injury caused by ozone. Anyone observing and recording foliar damage and submitting data to Hands on the Land website must participate in a formal training to assess foliar injury. Accuracy in assessing foliar injury requires practice and an observer can practice and refine his or her skills at: <http://www.nature.nps.gov/air/edu/O3Training/index.cfm>

- Select the number of leaf images for training. You may want to change the number of specimens to 10 rather than 20.
- Click select if you made changes.
- Click on the image of the leaf you would like to practice with, we suggest Common Milkweed.
- Select the proper percent range and click “okay”
- An observer must score at least 80% correct with errors being no more than + or – one injury class before proceeding to the field.
- Practice estimating folia injury on species for which you will be collecting data.
- You may want to practice estimating foliar injury on the website using your identification chart.
- Have each student do this exercise at least three times or until they score 80%.

## Registering Your Site

If you are an educator and are already involved in a partnership with a public land management agency that meets HOL membership criteria, we encourage you to work with your agency contact(s) to fill out and submit the Membership Application. NOTE: Applications must be received from the agency provider.

Agencies involved in the Hands on the Land network include the National Park Service, National Fish and Wildlife Service, US Forest Service, Bureau of Land Management, NOAA, EPA, and NRCS. Within each of those agencies, there are member sites. You can contact [Susan\\_Sachs@nps.gov](mailto:Susan_Sachs@nps.gov) for your application sponsorship.

## Entering Data into the Hands on the Land Website

(Note: You will need to be a registered member to enter data on the Hands on the Land website)

Go to the data entry website:

<http://www.handsontheland.org/environmental-monitoring/ozone-bio-monitoring.html>

- 1) Under Datasheet
  - a. Select your site
  - b. Click on POST
- 2) Log in to access your data page-at the password prompt, enter the following:  
User Name: your email address  
Password: selected by you when you initially registered your site.
- 3) You should now have a blank data sheet for your garden, check the garden name and location. If you have the wrong datasheet, go back to the beginning and make sure you have the proper garden name selected from the dropdown menu
- 4) Write in the name of the collectors, and for groups of students team names work well.
- 5) Change the date to the day data were collected, not the date they are entered.
- 6) Select the species initials from the drop down list  
BB = Blackberry  
CB = Crownbeard  
CF=Cut-leaf coneflower  
CFD = Cut-leaf Coneflower (var. Digitalis)  
EB = Elderberry  
MW=Common milkweed  
WS = Wingstem
- 7) Enter the plant height in centimeters
- 8) Enter the total leaf sets (include missing leaves)
- 9) Enter data for each leaf (or leaf set). Leaf 1 is the lowest leaf on the plant. Only enter data for the lowest 8 leaves. For alternate leaf plants, only enter data codes in the “A” column.
- 10) When all data have been checked, click the “Insert” button. You will be able to edit and make corrections using the link at the bottom of the page.

If you have any problems with this section, contact Susan Sachs at (828) 926-6251 or email at [susan\\_sachs@nps.gov](mailto:susan_sachs@nps.gov)

## Retrieving Data

Data can be viewed in several different ways. You can view just the data, or you can develop graphs, animate change to one plant over time or compare data sets from one or up to four gardens. One way to compare gardens is by elevation. To determine which sites you want to compare, click on the Garden Map link under the Reports, Graphs and Maps section. This will allow you to lump gardens based on topography; thus you can compare mountaintop tops to valleys or valleys to other valleys. Once you have the site locations, you can do the following searches.

1) Reviewing the data for just one garden: To do this, click on “Advanced Search” off of the main ozone bio-monitoring page. Select the garden and the plant species using the drop down list. From here, specify the date range you want to isolate. Are you wanting to see all of the data or just one year or season? Hit search once you are done.

If you want to view data from a particular elevation, you can enter a range in the middle portion of the search page. From here, enter the date range and hit search.

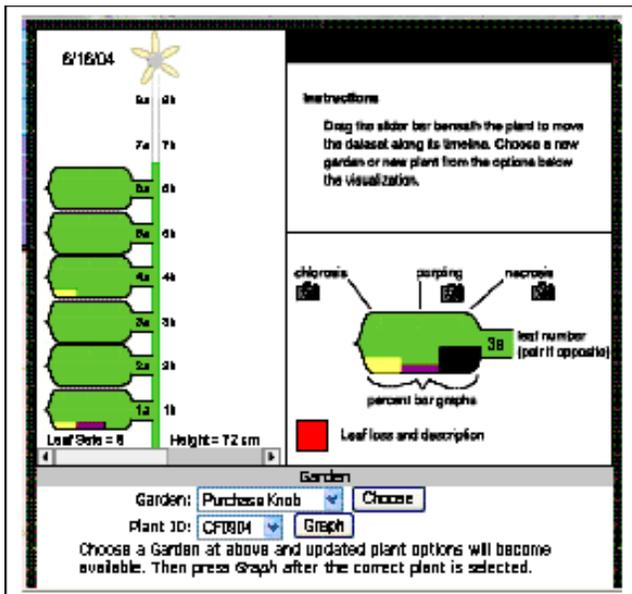
2) Comparing Data for Multiple Gardens: If you are interested in comparing your garden with up to four other gardens, click “graph” in the “compare up to four datasets from any garden” in the Reports, Graphs and Maps section.

- First you must select the gardens to compare using the drop down list that appears after you hit the “graph” button. You may select one site or up to four.
- After you select garden locations, hit the “choose garden” button.
- Next, choose a plant to compare. In our example, we randomly chose leaf #4 from a Cut-leaf Coneflower plant from Purchase Knob, Cradle of Forestry and Tuscola gardens in 2004. We selected purpling as the characteristic to compare and then hit “graph”.
- The graph showed that only the plant at Purchase Knob showed symptoms of purpling in 2004.

3) Animation of a Plants Foliar Injury Over Time: If you are interested in seeing an illustration of the plants progression over the growing season, choose a garden and hit “graph” in the animation section of Reports, Graphs and Maps.

- If you haven’t already, choose a garden.
- Choose a plant to animate through the growing season.
- We randomly chose CF0904 from the Purchase Knob garden for the example.





To see the change, slowly drag the slider bar under the graphic of the plant. You should see the dates change and might see the leaf colors change to reflect the data collected.

The animation examples show that the plant showed dramatic changes through the growing season. On June 16, 2004, the first data collection date, on Leaf 1 the plant showed a rating of 2 (1%-6%) for both chlorosis and purpling. Leaf 4 shows a rating of 2 for chlorosis. By dragging the slider bar to the third data collection date of June 29, we notice an increase for Leaf 1 to a rating of 4 for purpling (26%-50%) and a rating of 2 for both chlorosis and necrosis. Both Leaf 2 and 3 are now showing purpling symptoms and necrosis is apparent on the plant Leaf 5.

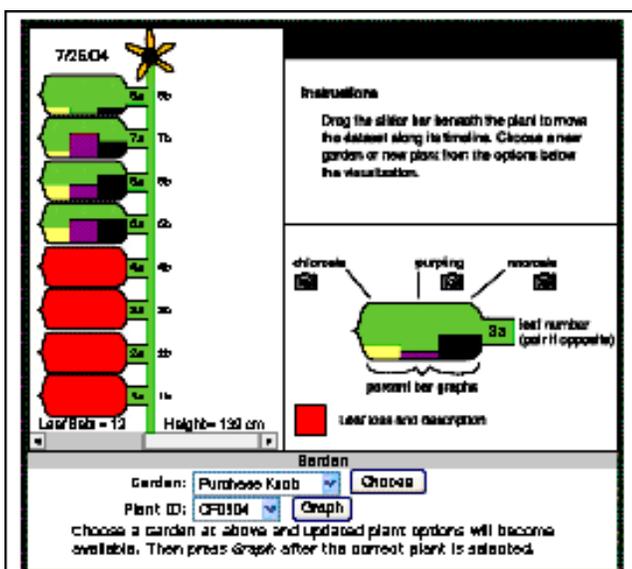
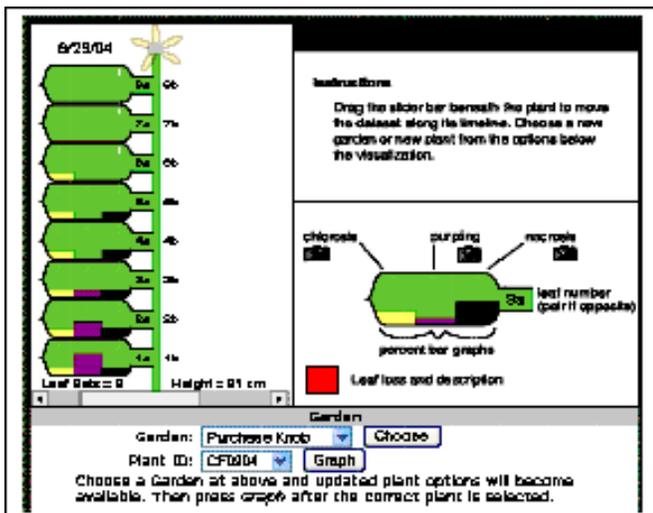
By the last data entry on July 26, 2004, the plant has lost the lowest 4 leaves and the purpling is visible on the plant up to Leaf 7 with a rating of 4 (26%-50%).

## Looking at Data

### Are the data reasonable?

Average plant injury from ozone air pollution can range from 0% to 100% depending upon the time of year and the cumulative ozone exposures that have occurred throughout the spring and summer seasons. Research has shown that different amounts of ozone-induced foliar injury occur amongst plants of the same species because of the differences in soil, amount of water, ozone exposures and available sunlight on the leaf surfaces. However, there are some correlations that usually apply:

1. Sunlight drives the amount of air taken in for photosynthesis. The more active the plant on days with elevated ozone air pollution, the more likely the ozone-sensitive plants will show ozone-induced injury.
2. Increased ozone concentrations over time produces increased plant injury. Studies in different regions have shown that different elevations and levels of sunlight influence the severity of plant injury.
3. Shaded plants or even leaves will show less injury than those plants and leaves located in full sunlight with the lower older leaves showing most of the injury due to season-long exposures.



Students taking bi-weekly measurements of the percentage of plant injury should observe a general increase in the amount of damage over time. They should take particular note of the weather conditions (temperature, days with intense sunlight, cloud cover and type, wind direction, and humidity). These factors influence the concentrations of surface ozone present. Are there several consecutive days when the ozone level is high? Were there any dramatic changes recorded in the amounts of injury to the plants? On a longer time scale, how did the ozone induced plant injury vary each month? What can be learned about the effects of ozone air pollution from the observations made of the incidence and severity of foliar injury to plants?

Additional information about surface ozone and ozone data may be found at: [www.epa.gov](http://www.epa.gov). There will be a daily air quality map. Click on "Ozone" in

the heading of the home page and it will provide background information and a site to click on for ozone levels where you live. This will take you to AIRNOW. This site provides today's forecast and archives to research different months and years. It does not provide raw data. You will need to go to your state Environmental Protection Office or Air Quality office to get raw data. Some states post it, but others do not.

### What do people look for in the data?

One method of collecting data is to estimate foliar injury once a week on the same day of each week. However, bi-weekly is also acceptable. A sample of one season of data for one pair of leaves is recorded and presented in Table I. (Data is posted on <http://www.handsontheland.org/environmental-monitoring/ozone-bio-monitoring.html>)

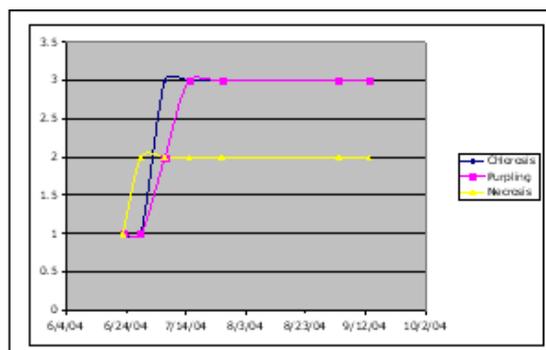
Table 1: Garden Site: Purchase Knob

Plant ID: CB0104

Definitions: Chlorosis=yellowing, Purpling=stippling, and necrosis = death of leaf tissue

Foliar Area Injury: 1=0%, 2=1%-6%, 3=7%-25%, 4= 26%-50%, 5= 51%-75%, 6=76%-100%

	LEAF 1 A			L
	C	P	N	
6/23/04	1	1	1	
6/29/04	1	1	2	
7/7/04	3	2	2	
7/15/04	3	3	2	
7/26/04	3	3	2	10
9/3/04	3	3	2	10
9/13/04	3	3	2	10



### Interpreting Data for Leaf 1A

This data is showing us that Leaf 1A had no imperfections of any sort the first week. During the second week, there was a rating of 2 for necrosis which means 1%-6% of the leaf had dead tissue but there was no other damage noted. In the third week, 7%-25% of the leaf showed yellowing (chlorosis) and 1% - 6% of the leaf showed both purple stippling and necrosis. In the next three weeks, the data stays the same which indicates the leaf fell off of the plant with prior purpling and chlorosis giving it a loss code of 10. Once a leaf falls off, carry over the previous data so graphing does not show improvement.

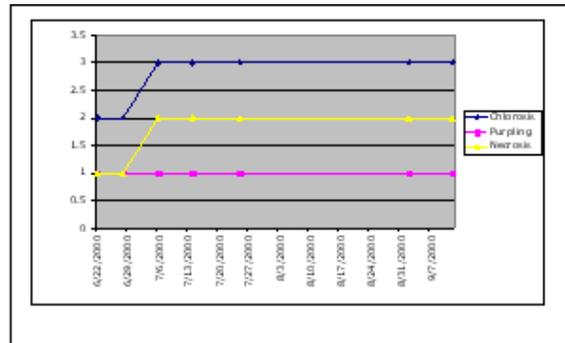
Table 1: Garden Site: Purchase Knob

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Foliar Area Injury: 1=0%, 2=1%-6%, 3=7%-25%, 4= 26%-50%, 5= 51%-75%, 6=76%-100%

	LEAF 1 B			
	C	P	N	L
6/23/04	2	1	1	
6/29/04	2	1	2	
7/7/04	3	1	2	
7/15/04	3	1	2	8
7/26/04	3	1	2	8
9/3/04	3	1	2	8



**Interpreting Data for Leaf 1B**

This data shows a similar progression as 1A except the leaf fell off with only prior chlorosis, therefore it gets a leaf loss code of 8.

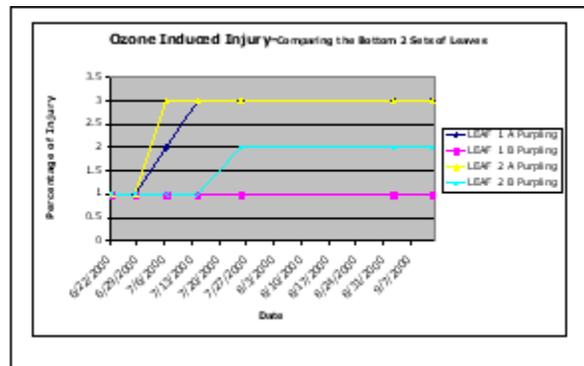
Table 2: Stippling on Leaves 1A,!b and 2A,2B

Plant ID: CB0104

Definition: Stippling is the color change that occurs in the leaf due to ozone injury.

Foliar Area Injury: 1=0%, 2=1%-6%, 3=7%-25%, 4= 26%-50%, 5= 51%-75%, 6=76%-100%

	LEAF 1 A	LEAF 1 B	LEAF 2 A	LEAF 2 B
	Purple Stippling	Purple Stippling	Purple Stippling	Purple Stippling
	6/23/04	1	1	1
6/29/04	1	1	1	1
7/7/04	2	1	3	1
7/15/04	3	1	3	1
7/26/04	3	1	3	2
9/3/04	3	1	3	2
9/13/04	3	1	3	2



Patterns observed: The bottom two pairs of leaves showed no ozone injury during June. Beginning damage of 1%-6% began to show in July but only on the two leaves on the East side of the plant. Towards the end July the ozone induced injury increased to between 7%-25%. The second leaf on the West side of the plant began to show injury but the leaf on the East side of the stem of the plant continue to show the most ozone injury. The leaf loss codes are not shown in this table but they appear as data carried over in the table and show up as a straight line in the graph.

Additional ideas for data analysis:

- Do high ozone episodes result in an increases in symptoms?
- Comparisons between MW and CF at each site.
- Comparisons between elevations of the sites.
- Comparisons between rural and urban garden sites.
- Tracking flower and seed production through the years
- Monarch butterfly feeding on MW leaves.

## An Example of a Student Research Investigation

### Forming a Hypothesis

A student enrolled within a Milan school in Italy has decided to focus on the interconnection between the exposures of ozone air pollution on the amount of ozone-induced injury to the Cut-leaf coneflower. She has decided to begin her research process by looking at the visualizations of her measurements of the ozone exposures and the pattern of ozone-induced plant injury during the months of July through September. Her hypothesis is, the severity of ozone-induced plant injury is directly related to specific events of high ozone levels.

### Collecting and Analyzing Data

Monitoring the level of ozone induced plant injury at Milan School is a new protocol, but Hands on the Land posts several months of data gathered at various garden locations during the past year. She decides to randomly pick a plant from Purchase Knob, NC in Great Smoky Mountains National Park and correlate the rate of damage to the 1-hour ozone average in ppb. While doing an internet search, she discovers that the state of North Carolina posts ozone levels measured by professional equipment throughout the state at <http://daq.state.nc.us/monitor/data/>.

She begins by organizing a spreadsheet of ozone measurements from the week prior to each data collection event and the amounts of ozone-induced plant injury for Purchase Knob. She generates a plot of the ozone measurements using the current U. S. Environmental Protection Agency Index Values to determine healthy and unhealthy exposures to ozone air pollution.

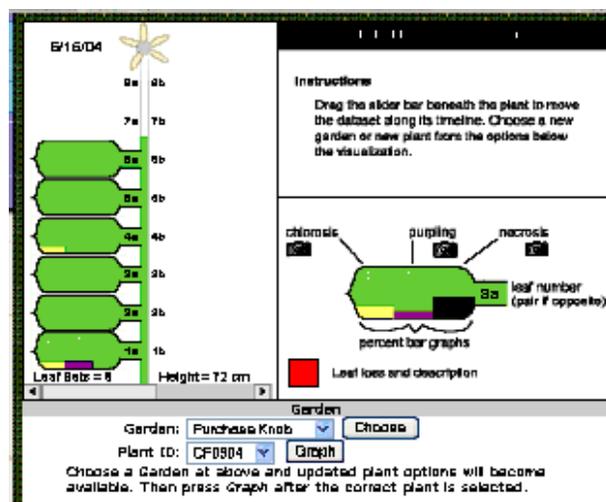
She determines that 65 ppb and higher are relatively high levels of ozone. Below is the data that the student examined for her analysis.

### WEEK 1

1-hour ozone average for the week before June 16

June 09	46 ppb
June 10	68 ppb
June 11	60 ppb
June 12	51 ppb
June 13	47 ppb
June 14	51 ppb
June 15	32 ppb

Injury animation for June 16



Week 1 shows ozone levels between 32 ppb and 68 ppb with 1%-6% damage on only the bottom leaf.

### Air Quality Index for Ozone

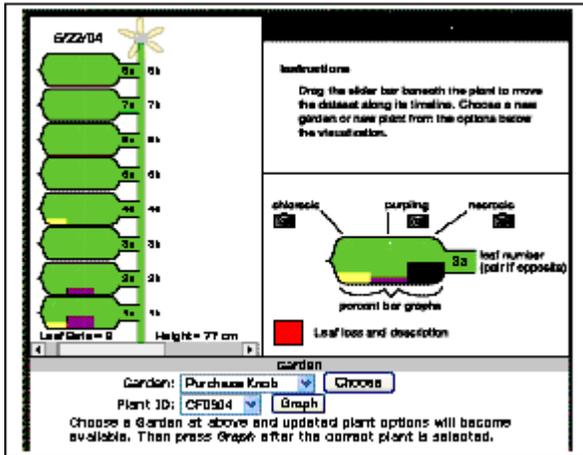
Index Values	Category	Cautionary	ppb (8-hr)	(1-hr)
0-50	Good	None	0-64	
51-100	Moderate	Unusually sensitive people should consider reducing prolonged or heavy exertion.	65-84	
101-150	Unhealthy for Sensitive Groups	Sensitive groups should reduce prolonged or heavy exertion.	85-104	125-164
151-200	Unhealthy	Sensitive groups should avoid prolonged or heavy exertion; everyone else should reduce prolonged or heavy exertion.	105-124	165-204
201-300	Very Unhealthy	Sensitive groups should avoid all physical activity outdoors; everyone else should avoid prolonged or heavy exertion.	> 125	> 205

## WEEK 2

1- hour ozone average for the week before June 22

June 16	31 ppb
June 17	34 ppb
June 18	56 ppb
June 19	52 ppb
June 20	59 ppb
June 21	47 ppb
June 22	49 ppb

Injury animation for June 22



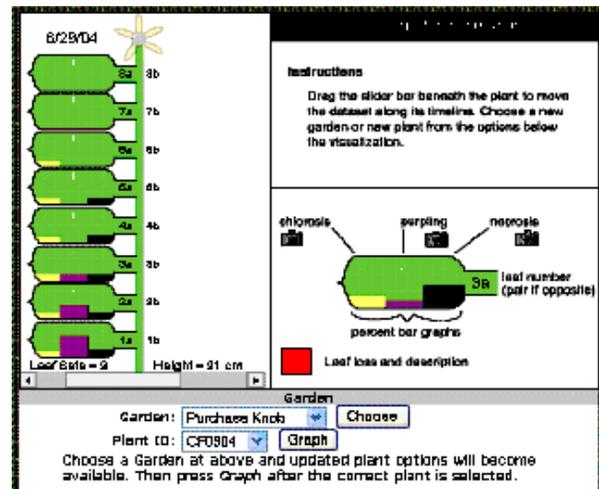
Week 2 shows ozone levels between 31 ppb and 59 ppb with Leaf 1 progressing to a purpling rating of 3 (7% - 25%) and Leaf 2 with a purpling rating of 2 (1%-6%). The ozone levels were fairly low and the progression moved slowly.

## WEEK 3

1- hour ozone average for the week before June 29

June 23	41 ppb
June 24	40 ppb
June 25	35 ppb
June 26	52 ppb
June 27	44 ppb
June 28	49 ppb
June 29	52 ppb

Injury animation for June 29



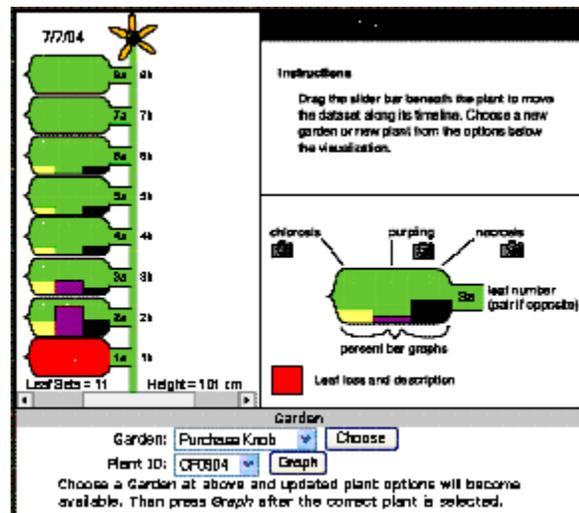
Week 3 shows ozone levels between 35 ppb and 52 ppb with Leaf 1 progressing to a purpling rating of 4 (26%-50%) and Leaf 2 with a purpling rating of 3 and Leaf 3 developing a purpling rating of 2. The ozone levels were fairly low and the progression continues to move slowly. The student is curious about the progression since the levels are well below 65 ppb.

## WEEK 4

1- hour ozone average for the week before July 7

June 30	55 ppb
July 1	37 ppb
July 2	40 ppb
July 3	47 ppb
July 4	49 ppb
July 5	53 ppb
July 6	53 ppb

Injury animation for July 7



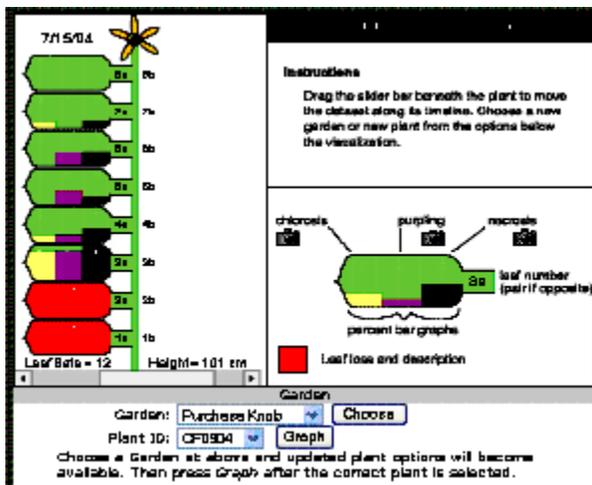
Week 4 shows ozone levels between 37 ppb and 55 ppb with Leaf 1 falling off the plant. Leaf 2 has progressed to a purpling rating of 5 (51%-75%) and Leaf 3 to a purpling rating of 3.

### WEEK 5

1- hour ozone average for the week before July 15

July 8	57 ppb
July 9	57 ppb
July 10	60 ppb
July 11	57 ppb
July 12	51 ppb
July 13	58 ppb
July 14	59 ppb

Injury animation for July 15



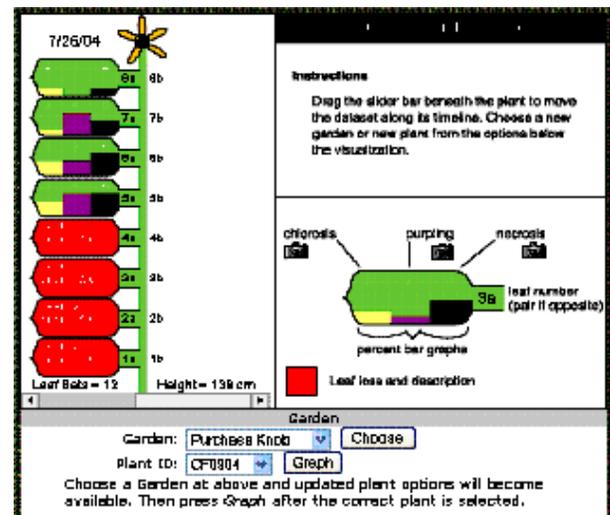
Week 5 shows ozone levels between 51 ppb and 60 ppb. Compared to the last few weeks, these levels are more constant at the higher end of the healthy ratings according to EPA. Both Leaf 1 and Leaf 2 have fallen off and Leaf 3 has progressed from a purpling rating of 3 to 5. Leaf 4 is now showing purpling at a rating of 2, Leaf 5 and 6 have developed a purpling rating of 3.

### WEEK 6

1- hour ozone average for the week before July 26

July 19	61 ppb
July 20	73 ppb
July 21	67 ppb
July 22	71 ppb
July 23	66 ppb
July 24	60 ppb
July 25	52 ppb

Injury animation for July 26



Week 6 shows ozone levels between 52 ppb and 73 ppb. While reviewing her data from each week, she notices that none of the levels are particularly high, only 5 days exceed 65 ppb and no days are considered unhealthy. At the same time, the rate of progression of symptoms is fairly dramatic.

The results have raised more questions for the student than answers. She is now wondering about the total number of hours the plants received certain ozone exposures. The data she received only gave the maximum 8-hour average for the day. The student decides she wants to do more research on the internet to determine if there is a ppb standard for plant health since the plants appear to react to ozone in weeks when ozone levels are as low as 50 ppb at a maximum. Her conclusion is that sustained exposure at low levels of ozone exposure seems to be just as likely to produce symptoms as a short-lived high exposure.

### Further Analysis

A next step for the student from Milan School might be to compare the Purchase Knob garden with one or two gardens at different elevations. Examination of research shows that elevation may have an impact on the rate of symptoms. She could try to find gardens with similar ozone levels for each week and see if there is a correlation between elevation, ozone levels and the rate of symptoms. Another analysis could be to look at the correlation between the rates of purpling and necrosis.

# Activities

## Before Collecting Data

**The Leaf Game** - This activity allows students to practice the estimation skills they will need to collect data.

**Virtual Ozone Injury Data Collection** - Set up on the Hands on the Land website are photographs from one field season at the Purchase Knob garden in Great Smoky Mountains National Park. Visit <http://www.handsontheland.org/educator-resources/hol-games/hol-sponsored-games/ozone-inquiry.html> for the activity that goes along with the worksheets on pages 32 - 33 of this guide.

## Collecting Data

**Rating Ozone-Induced Foliar Injury on Ozone-sensitive Plants** - This guides you in collecting data. Choose the version of the data sheet and Ozone Garden Data Collection field guide that will work best for your class. Also, note that there are two data sheets for Version I and II, these differ for the various plant growth forms (alternate vs. opposite leaves).

**Version I** - This is the simplest data collection method. Use this for upper elementary or remedial middle/high school groups.

**Version II** - This is the intermediate data collection method. Use this for advanced upper elementary, middle school or remedial high school groups.

**Version III** - This is the tracking data sheet and it can be used with advanced high school or college groups.



# The Leaf Game

Navigate to the Foliar Injury Assessment Module website:

<http://www.nature.nps.gov/air/edu/O3Training/index.cfm>

- Change the number of leaf images for training to 10
- Click “select”
- Start training on Common Milkweed

## Record your results here

Trial 1 = 10 leaves

1. Which species did you use?

2. Errors are to be expected, but it is important to record and track the categories in which you have wrong answers. Please write the total wrong answers in each category in the spaces below.

\_\_ 0%    \_\_ 1%-4%    \_\_ 5%- 12%    \_\_ 13%-25%    \_\_ 26% - 50%    \_\_ 51% - 75%    \_\_ 76% - 100%

---

Trial 2 = 10 leaves

1. Which species did you use?

2. Errors are to be expected, but it is important to record and track the categories in which you have wrong answers. Please write the total wrong answers in each category in the spaces below.

\_\_ 0%    \_\_ 1%-4%    \_\_ 5%- 12%    \_\_ 13%-25%    \_\_ 26% - 50%    \_\_ 51% - 75%    \_\_ 76% - 100%

---

Trial 3 = 10 leaves

1. Which species did you use?

2. Errors are to be expected, but it is important to record and track the categories in which you have wrong answers. Please write the total wrong answers in each category in the spaces below.

\_\_ 0%    \_\_ 1%-4%    \_\_ 5%- 12%    \_\_ 13%-25%    \_\_ 26% - 50%    \_\_ 51% - 75%    \_\_ 76% - 100%

---

Trial 4 = 10 leaves

1. Which species did you use?

2. Errors are to be expected, but it is important to record and track the categories in which you have wrong answers. Please write the total wrong answers in each category in the spaces below.

\_\_ 0%    \_\_ 1%-4%    \_\_ 5%- 12%    \_\_ 13%-25%    \_\_ 26% - 50%    \_\_ 51% - 75%    \_\_ 76% - 100%

---

Did you get any better with each trial? In order to collect data in the ozone garden, you must get at least 80% correct on one of the 4 trials and be no more than one category off on incorrect answers.



Name \_\_\_\_\_

## O<sub>3</sub> Skills Center Student Worksheet

Go to the O<sub>3</sub> Inquiry website: <http://www.handsontheland.org/ozone-inquiry>

Answer the following questions in a few phrases or in a sentence.

1. What does chlorosis look like?
2. What does chlorosis mean for the plant?
3. What does necrosis look like?
4. What does necrosis mean for the plant?
5. What does purpling/stippling look like?
6. What does purpling/stippling mean for the plant?
7. What is happening inside of the leaf?
8. How do we know that the symptoms are from ozone?
9. Choose one Plant in the drop down menu. Circle the one chosen: Plant 1, Plant 2, Plant 3, Plant 4
10. Choose one of the following Dates in the drop down menu.  
Circle which one you chose: June 29, July 6, July 13, July 20, July 27, August 4

11. Complete the following table using the following foliar area injury codes: 0 = leaf missing    1 = 0%  
2 = 1%-6%    3 = 7%-25%    4 = 26%-50%    5 = 51%-75%    6 = 76%-100%

	Chlorosis		Purpling		Necrosis	
	Leaf A	Leaf B	Leaf A	Leaf B	Leaf A	Leaf B
Set 8						
Set 7						
Set 6						
Set 5						
Set 4						
Set 3						
Set 2						
Set 1						

12. Insert the above data into the O<sub>3</sub> Skills Center website and click the “Check” button. What is your score? \_\_\_\_\_

13. When was the first observed date the selected plant flowered (click at the top of the plant for each week to determine if the plant has flowered)?

Circle which date the selected plant flowered: July 27, August 4, August 11, August 31, September 7, September 14

14. Choose another Date (use a date listed below) in the drop down menu using the same selected Plant as before. Circle which one you chose: August 11, August 31, September 7, September 14, September 20, September 28

15. Complete the following table using the following foliar area injury codes: 0 = leaf missing 1 = 0%  
2 = 1%-6% 3 = 7%-25% 4 = 26%-50% 5 = 51%-75% 6 = 76%-100%

	Chlorosis		Purpling		Necrosis	
	Leaf A	Leaf B	Leaf A	Leaf B	Leaf A	Leaf B
Set 8						
Set 7						
Set 6						
Set 5						
Set 4						
Set 3						
Set 2						
Set 1						

16. Insert the above data into the O<sub>3</sub> Skills Center website and click the “Check” button. What is your score? \_\_\_\_\_

17. Describe three differences you observed between the two dates selected.

1.

2.

3.

# Lesson Plan: Rating Ozone-Induced Foliar Injury on Ozone-sensitive Plants

## Purpose

To measure the percentage of ozone-induced injury to plants.

## Overview

- Determine an average percent area of the ozone-induced injury on each plant leaf.
- Use the National Park Service's rating scale to quantify the area of leaf affected. The rating classes of 1-6 represent a percentage range of foliar injury (1= 0%, 2=1-6%, 3= 7-25%, 4= 26-50%, 5= 51-75% and 6= 76-100%) and will be identified using a field guide for the assessment.

## Educational Outcomes

Participants will learn to identify the percent of ozone-induced injury and changes occurring to the plant over time as ozone pollution exposures change.

## Science, Geography, Mathematics Concepts

### Inquiry

- Ask a question about plant injury and events in the environment
- Plan and conduct an investigation
- Use data to construct reasonable explanations
- Maintain a journal

### Person and Social Perspectives

- Changes in the environment can be natural or influenced by humans
- Material from human societies affect both physical and chemical cycles of earth

### Earth and Space

- The atmosphere is made up of different gases
- Materials from human societies affect the chemical cycles of the Earth

### Life Science

- Cells carry on many functions needed to sustain life.
- Demise is a breakdown in the structure and functions of an organism

### Geography

- Human activity can modify the physical environment.

### Mathematics

- Understand and apply ratios, proportions, and percents in a wide variety of situations
- Formulate and solve problems that involve collecting and analyzing data
- Construct, read, and interpret displays of data
- Communicate results

## Observation Support

Ozone induced foliar injury is commonly measured using the National Park Service's rating scale. The graphic chart identifies the varying percentage of ozone injury to a plant using the ratings of: 0%, 1-6%, 7-25%, 26-50%, 51-75% and 76-100%. The greater the amount of ozone air pollution present, the more foliar injury that will likely be observed on ozone-sensitive plants. The plant is observed daily using a 10X magnifying glass until the beginning of stippling is seen on a leaf. The plant data are then recorded. The first data collecting event should occur no later than June 15 each year, subsequent observations should be taken one day each week or every two weeks, trying to maintain a regular schedule. Remember, no injury is relevant data.

The amount of foliar (leaf) injury is likely to increase shortly after days with high levels of ozone air pollution. This would be an opportune time for a diurnal study of surface ozone and correlating that data with the level of injury observed on the leaf of the plant. If taking hourly ozone measurements is not possible, the hourly measurements may be gotten from the local Environmental Protection Agency or local air quality-monitoring device. An internet search will reveal if your state posts air quality data.

## Measurement Logistics

The need to assess the plant daily for ozone injury until the first symptoms, may pose a logistics challenge. One approach might be to use the magnifying glass to check the lowest leaves of the plant during a recess or lunch break, or immediately after school is out. Identifying the day each of the plants show symptoms of foliar injury and rating the amount of injury provides a baseline of data for monitoring the pattern of foliar injury over time.

After establishing a base line of data, the plant is assessed once a week preferably, or every two weeks on the same day. Establish a schedule so everyone involved knows what is expected and when to do it.

## Supporting Measurements

The percentage of injury to a plant depends upon the level or amount of ozone present and the amount of sunlight. Strong sunlight supports photosynthesis, producing food the plant needs. This requires more intake of air by the plant, and increased intake of air containing higher levels of ozone damage the plant. Hot sunny days, high temperatures, wind direction, humidity, and cloud cover all affect the concentration of ozone produced. Levels of ozone can be measured with a hand-held optical scanner using the Surface Ozone Protocol found in the GLOBE (Global Learning and Observation to Benefit the Environment) Program ([www.globe.gov](http://www.globe.gov)). Participating in the training for the ozone protocol enables you to submit your data to the GLOBE Student Data Server. The data can be retrieved in raw form or through visualizations making it a natural extension to the study of plant injury.

## Participant Preparation

Anyone assessing foliar (plant) injury and submitting data Hands on the Land website must be trained. It is important to the accuracy of the measurements that participants are able to:

1. Work in cooperative groups of 2-4 to setup the site and gather, analyze, and discuss results.
2. Organize all materials needed to assess plant injury.
3. Follow a schedule for taking measurements.
4. Identify and record, date, time, garden location, plant ID number, number of leaves on the entire plant and other observations required on the data sheet.
5. Estimate the percentage of foliar injury using the graphically designed chart summarizing the National Park Service's rating scale.
6. Practice estimating foliar injury using the website: <http://www.nature.nps.gov/air/edu/O3Training/index.cfm>
7. Record data accurately and completely for submitting to the Hands on the Land website .

## Helpful Hints

- » Designate an area to keep the clipboard, data sheet and all materials for gathering observations.
- » Check the written records from time to time for completeness and accuracy.

## Questions for Further Investigation

How is the amount of foliar injury related to other atmospheric phenomena?

Which one?

How?

What variability of foliar injury did you observe over a period of time?

Daily? Weekly? Seasonally?

How could you use your data on plant injury to characterize any changes in the ozone exposures...

weekly?, seasonally?, between years?

## Site Definition

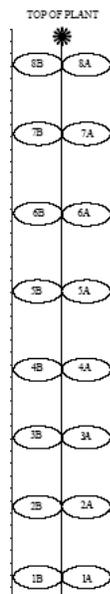
Choosing the location for your “Ozone Bioindicator Garden” and arranging the ozone sensitive plants are important to your successful implementation of observing plants for ozone injury. Assessment of plant injury begins with using a 10X magnifying glass to observe each plant at the beginning of the ozone season (May or June) for determining the initial symptoms of injury. Observers need to be able to get close to each plant without walking on or damaging another plant.

The ideal garden may be circular or rectangular with 4 plants each of the same species planted 18 inches apart. Each plant is tagged with a number using CF for coneflower and MW for milkweed. Place a metal rod with a red flag with the individual plant’s number on the flag, at the base of each plant. This will also be a marker to identify the new plant for the next year.

Once each plant has been given an ID tag, identify North. Using North as the focal point, each leaf on each plant will need to be identified. Start at the bottom of the plant to number the leaves. If a leaf has fallen off of the plant, it should still be counted and labeled. The last data for that leaf will carry over from week to week throughout the data collection period.

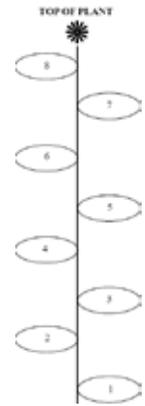
### Plant with parallel/opposite leaves

- Leaves are directly across from each other, the leaves to the right of the stem (facing North) will be labeled 1A, 2A, 3A, 4A, 5A, 6A, 7A, and 8A.
- The leaves on the same plant to the left of the stem (you facing North) will be labeled 1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B.



### Plant with alternating leaves

- Leaves alternate on the stem. Only the 8 bottom leaves are numbered.
- If a leaf has fallen off near the bottom of the plant, it is counted as one of the 8 bottom leaves.
- Starting from the bottom, feel along the stem to make sure you are counting leaf scars as well. The first leaf above any basal leaves is 1. Continue numbering leaves up the main stem until 8.



An open area of at least 15-20 meters on a side square is necessary to allow good air movement and normal precipitation without “rain shadows”, and of greatest importance, the plant must receive direct sunlight. Use the natural soil in the area for planting each species in their own garden. The garden’s location must be away from areas where pesticides and fertilizers are used. It is important that no pesticides or fertilizers are used in the “Ozone Bioindicator Garden”. In selecting your site, some compromise may be necessary between the ideal site for scientific observations and the logistical constraints of the area and its surroundings of the site available to use. To ensure the value of your data document the nature of your bioindicator site and its surroundings.

The spreading of the plants is controlled by digging up roots at the end of the season, and trimming back any excess growth. You may want to let a second plant grow next to each of the original plants to double each species for data gathering. If not, then trim back each original plant. Be sure to place a small flag in the location of the growth of each plant and have the plant’s ID number on the flag. Also, designate and be consistent year to year with which side of the plant is “A” or “B”. Make a map of the layout of your site and mark N, S, E, and W and the location of each labeled plant.

# Site Definition Sheet

## Ozone Biomonitoring Garden

School Name: \_\_\_\_\_ Class or Group Name \_\_\_\_\_

Name(s) of student(s) filling in Site Definition Sheet: \_\_\_\_\_

Date: \_\_\_\_\_ Check one: Coneflower Site Milkweed Site

Site Name: (Give your site a unique name) \_\_\_\_\_

Location: Latitude: \_\_\_\_\_ N or S Longitude \_\_\_\_\_ E or W

Elevation: \_\_\_\_\_ meters

Source of Location (Check one) GPS Other (please specify) \_\_\_\_\_

Describe site: (Include trees, buildings, etc. that are near your site).

---

Send a picture of your site and orientation of garden layout to Hands on the Land.

Draw a model of your garden layout, label North, South, East and West, and place markers in garden to identify N, S, E. and W. Number each plant and place a marker at the base of each plant. You will use the same number for the plant that grows in the same area the following year.

# Field Instruction

## Rating Ozone-Induced Foliar Injury

### Materials

- Meter stick
- Tags with string, and black permanent markers
- Site location flag for each plant with the plant number on the flag
- Metric measuring tape
- % ozone leaf injury identification chart
- Clipboard and Pencil
- Small hand mirror to place under the leaf to see if damage goes through leaf
- Small 10X hand lens

NOTE: The first evaluations should occur at least by June 15. The final evaluation should be before the typical first hard frost for your area.

### Procedures for Plot Evaluation

1. Begin collecting data on plants once they tall enough to have at least three sets of leaves growing from the main stem. This date will vary according to locale.
2. Measure the individual heights of the plants from base to apex. using a meter stick and record height in centimeters, a thin piece of wood should be carried to the base of each plant to prevent the meter stick from depressing into the ground.
3. When measuring the height of the plant, measure up to the bottom of the highest, most open leaf. Once the plant is in bud or flower, hold the plant straight, follow the main stem (the thickest branch) and measure to the top of the tallest bloom on the main stem.
4. When collecting data on the individual leaves, start at the base of the plant.
5. Count the total number of leaves on the plant that come directly off of the main stem. Do not count apical leaves that are not fully expanded. (In general, evaluate only leaves that are at least 65% expanded). Do not count leaves that are on sub-branches to the main stem. Do count leaf scars where leaves have fallen off (run your hand up the stem to feel for leaf scars).

6. Estimate the percentage of total leaf area of each leaf that has ozone-induced injury. Rating classes are 1=0%, 2=1%-6%, 3=7%-25%, 4=26%-50%, 5=51%-75%, 6=76-100%. The plants are rated on C= chlorosis (yellowing), P= purpling (stippling), N = necrosis (death). Necrosis is visible on both sides of a leaf unlike purpling which only appears on the upper leaf surface.

7. As ozone injury progresses, purpling will turn into necrosis. So it doesn't appear that the plant has improved, it is important to carry over the previous week's data for purpling (e.g. if a leaf's necrosis rating goes up to a 5, the purpling may no longer be apparent. It hasn't gotten better, it has just progressed to a point where it has changed from injury to dead tissue. Carry over the last data entry for purpling).

8. Ideally, data should be collected once a week. If that is not possible, try for at least once per month.

9. As the summer season comes to a close, record the number of flowers and seedpods per plant.

10. For plants with opposite leaves, each facing pair of leaves is one set. For alternate leaf plants, such as the coneflower, count only the leaves coming off of the main stem.

11. Use caution when checking the back of the leaf, especially if you notice any symptoms. These leaves will become fragile and will break off easily. Get down close and gently turn the leaf over to make your observation or use a hand mirror to reflect the underside.

12. If a leaf breaks off, make sure to assign it the appropriate leaf loss code.

- 7 - leaf gone with no prior symptoms
- 8 - leaf gone with prior chlorosis (yellowing) only
- 9 - leaf gone with prior purpling only (ozone)
- 10 - leaf gone with prior purpling stippling and chlorosis

13. When a leaf falls off, carry its last data entry over into all of the following data collection entries. This is to show leaf loss on a graph as opposed to leaf improvement.

14. Never make assumptions about what you are seeing, just rate what you see at that point in time. (What of insect feeding or some other symptom that is uncertain, contact Susan Sachs at: [Susan\\_Sachs@nps.gov](mailto:Susan_Sachs@nps.gov) for assistance? Include digital photos if possible?

15. During the season, insects will eat part of some leaves. Rate the leaf surface area that is visible, but then also note in the comments area for that leaf the percentage of the total leaf area missing.

16. Students need to be aware of mimicking symptoms and other pest problems that look like ozone air pollution injury to sensitive plants. Some strategies are:

a. Look at older leaves as they should have the most injury. If younger leaves have the most injury, then it is not ozone damage that you are observing.

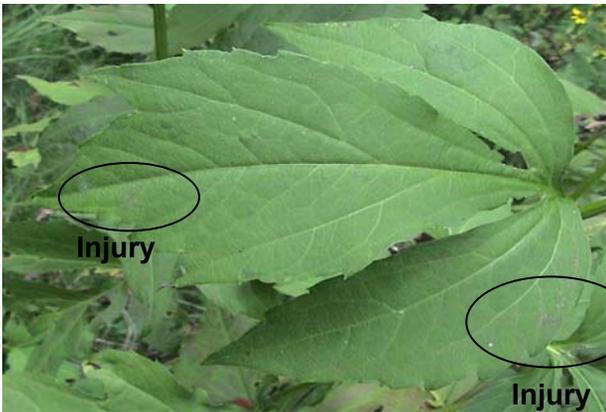
b. The purple stippling should be on the surface of the leaf only and not on the under side of the leaf.

c. The purple stippling (or small purple dots) do not enter any of the veins.

d. Be aware of mimicking symptoms, and other pest problems that look like ozone injury.



**Injury level 1  
0%**



**Lower end of Injury  
level 2 (1-6%)**



**Upper end of Injury  
level 2 (1-6%)**



**Lower end of injury  
level 3 (7-25%)**



**Upper end of injury  
level 3 (7-25%)**



**Lower end of injury  
level 4 (26-50%)**



**Upper end of injury  
level 4 (26-50%)**



**Lower end of injury  
level 5 (51-75%)**



**Lower end of injury  
level 5 (51-75%)**

Must notice the small green  
Areas that make this leaf a  
high  
Level 5 as opposed to a 6



Complete foliar injury

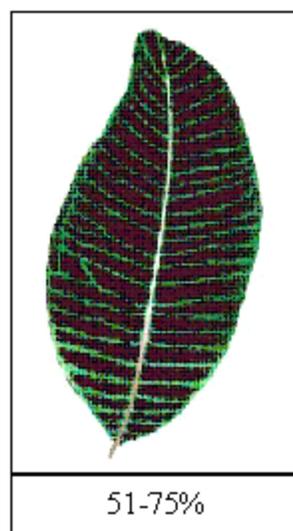
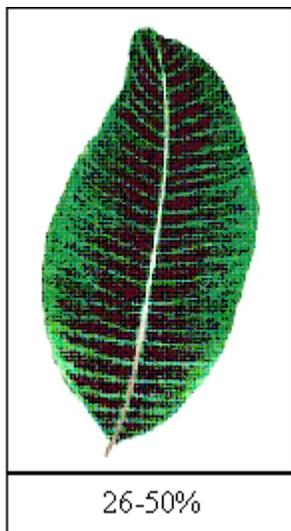
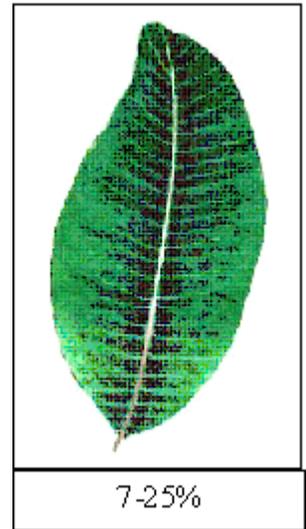
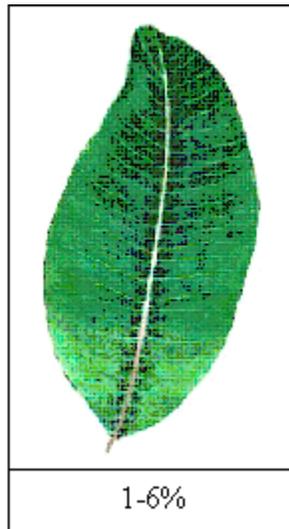
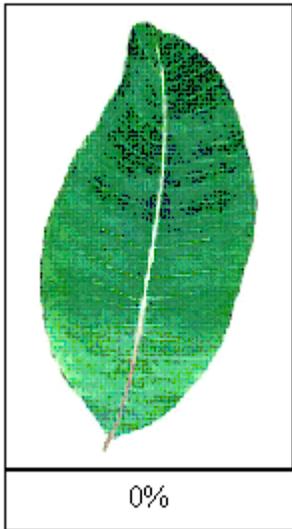
**Injury level 6  
76-100%**

# Leaf Field Guide

## Estimating Foliar Injury-Common Milkweed

Use the following chart to guide the estimation of foliar damage to each leaf. Remember, the stippling (also called purpling) is only on the top side of the leaf and the discoloration does not occur on any of the leaf veins.

National Park Service Categorization Method



## Ozone Bio-monitoring Garden Data Sheet - Alternate Leaves

Group Name: \_\_\_\_\_ Date: \_\_\_\_\_

Garden Location: \_\_\_\_\_ Plant ID #: \_\_\_\_\_

Plant species: \_\_\_\_\_ Plant height (cm): \_\_\_\_\_

Total # of leaves on the entire plant: \_\_\_\_\_

Observations (in flower, in bud...) \_\_\_\_\_

**TOP OF PLANT**



leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

8

7

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

6

5

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

4

3

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

2

1

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>			
0%	1-6%	7-25%	26-50%	51-75%	76-100
P					
Observations					

**BOTTOM OF PLANT**

Rate the % of ozone symptoms covering the entire leaf  
 P= purpling (reddish-purple injury spots caused by ozone)

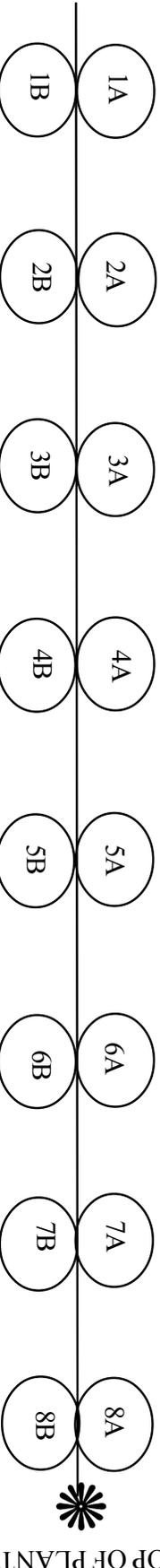
# Ozone Bio-monitoring Garden Data Sheet - Opposite Leaves

School: \_\_\_\_\_ Date: \_\_\_\_\_ Plant species: \_\_\_\_\_ Plant ID #: \_\_\_\_\_

Plant height (cm): \_\_\_\_\_ Total # of leaf sets on the entire plant: \_\_\_\_\_ Observations (in flower, in bud...): \_\_\_\_\_

Current 1 hour ozone: \_\_\_\_\_

leaf gone	P																		
1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%
2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%
3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%
4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%
5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%
6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100
notes		notes		notes		notes		notes		notes		notes		notes		notes		notes	



leaf gone	P																		
1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%	1	0%
2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%	2	1-6%
3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%	3	7-25%
4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%	4	26-50%
5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%	5	51-75%
6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100	6	75-100
notes		notes		notes		notes		notes		notes		notes		notes		notes		notes	

### Ozone Bio-monitoring Garden Data Sheet - Alternate Leaves

Group Name: \_\_\_\_\_ Date: \_\_\_\_\_

Garden Location: \_\_\_\_\_ Plant ID #: \_\_\_\_\_

Plant species: \_\_\_\_\_ Plant height (cm): \_\_\_\_\_

Total # of leaves on the entire plant: \_\_\_\_\_

Observations (in flower, in bud...) \_\_\_\_\_

TOP OF PLANT

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>							
0%		1-6%	7-25%	26-50%	51-75%	76-100			
C									
P									
N									
Observations									

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>							
0%		1-6%	7-25%	26-50%	51-75%	76-100			
C									
P									
N									
Observations									

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>							
0%		1-6%	7-25%	26-50%	51-75%	76-100			
C									
P									
N									
Observations									

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>							
0%		1-6%	7-25%	26-50%	51-75%	76-100			
C									
P									
N									
Observations									

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>							
0%		1-6%	7-25%	26-50%	51-75%	76-100			
C									
P									
N									
Observations									

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>							
0%		1-6%	7-25%	26-50%	51-75%	76-100			
C									
P									
N									
Observations									

leaf present <input type="checkbox"/>		missing <input type="checkbox"/>							
0%		1-6%	7-25%	26-50%	51-75%	76-100			
C									
P									
N									
Observations									

BOTTOM OF PLANT

Rate the % of ozone symptoms covering the entire leaf  
 P= purpling (injury dots), C= chlorosis (yellowing), N=necropsy (death)

# Ozone Biomonitoring Data Sheet - Opposite Leaves

Collector Names: \_\_\_\_\_ Date: \_\_\_\_\_

Garden Location: \_\_\_\_\_

Plant Species: \_\_\_\_\_ Plant ID#: \_\_\_\_\_

Plant Height (cm): \_\_\_\_\_ Total # of leaf pairs: \_\_\_\_\_

Observations (in flower, in bud...) \_\_\_\_\_

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

8A

8B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

7A

7B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

6A

6B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

5A

5B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

4A

4B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

3A

3B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

2A

2B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						

1A

1B

	Leaf present			Leaf missing		
	0%	1-6%	7-25%	26-50%	51-75%	76-100%
P						
C						
N						



# Ozone Garden Data Collection

## Version I

A. Two most important pieces of data in data collecting:

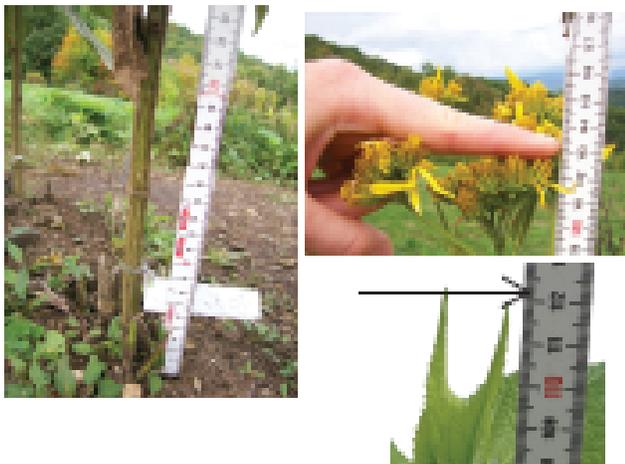
1. Collector name or group name
2. Date of data collection

B. Find the plant ID# with the metal hang tag

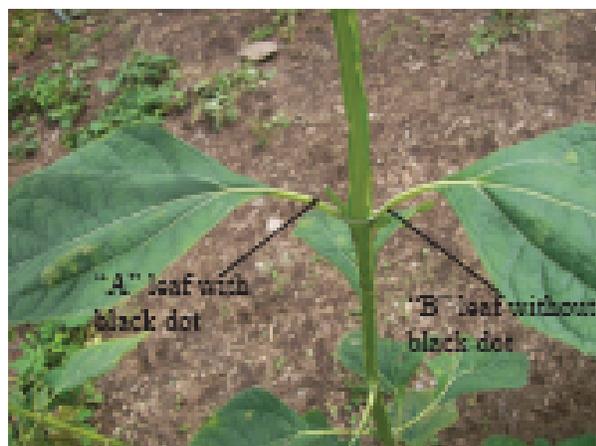


C. Measure the HEIGHT of the plant

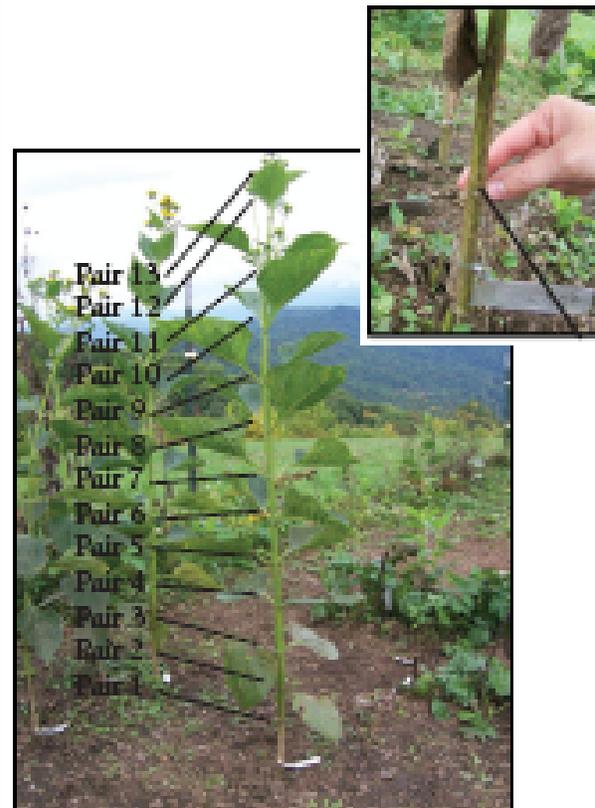
1. Use the metric side of the ruler (the side with the small numbers)
2. Measure from the base of the plant (beginning at zero) to the tallest leaf pair or bloom (if flowering) of the plant (apex) on the main stem



D. For plants such as Crownbeard, opposite leaves are identified as "A" leaf or "B" leaf



E. Count the # of leaf pairs that come off of the main stem, start at the base of the plant (make sure to count the pairs that may be missing at the base of the plant, leaf scars)



F. Observations

**NO BUD OR FLOWER**

**IN FLOWER**



**IN BUD**



# Ozone Garden Data Collection

## Version I (page 2)

G. Estimate the percentage of total leaf area of each leaf that has foliar injury area using the NPS foliar injury scale

P = Purpling or Stippling

Example of Purpling in two  
on top (epigynous) of leaf, this on the  
underside of leaf



If the leaf is not present check box "leaf gone"

✓	Leaf gone	P
1	0%	
2	1-6%	
3	7-25%	
4	26-50%	
5	51-75%	
6	75-100%	
notes		

If leaf is present estimate the percentage of foliar area injury in the purpling category. An example of the rating is shown regarding the leaf 7A on the right side of the page

	Leaf gone	P
1	0%	
2	1-6%	✓
3	7-25%	
4	26-50%	
5	51-75%	
6	75-100%	
notes		

7A

H. Hints:

1. Use caution when checking the back of the leaf, especially if you notice any symptoms. These leaves will become fragile and will break off easily.
2. For plants with opposite leaves, each facing pair of leaves is one set. For alternate leaf plants, such as the coneflower, count only the leaves coming off of the main stem.



Leaf 7A



# Ozone Garden Data Collection

## Version II

A. Two most important pieces of data in data collecting:

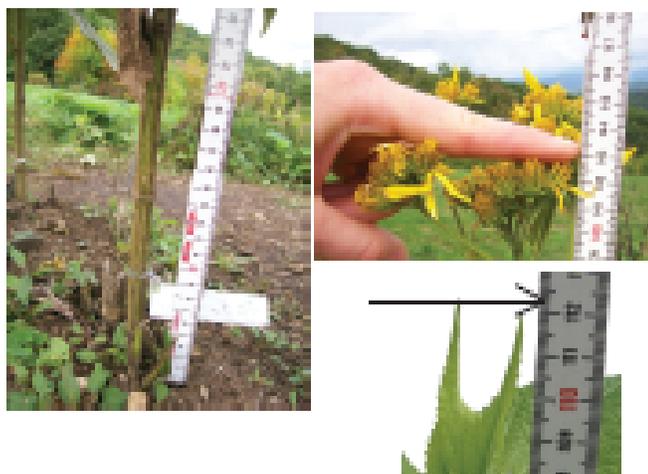
1. Collector name or group name
2. Date of data collection

B. Find the plant ID# with the metal hang tag



C. Measure the HEIGHT of the plant

1. Use the metric side of the ruler (the side with the small numbers)
2. Measure from the base of the plant (beginning at zero) to the tallest leaf pair or bloom (if flowering) of the plant (apex) on the main stem



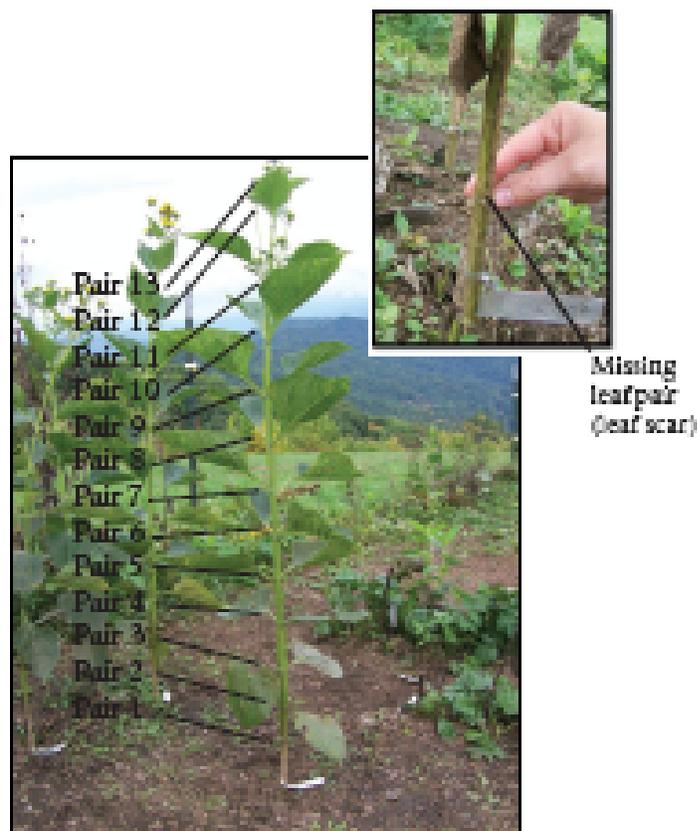
D. For plants such as Crownbeard, opposite leaves are identified as "A" leaf or "B" leaf



"A" leaf with black dot

"B" leaf without black dot

E. Count the # of leaf pairs that come off of the main stem, start at the base of the plant (make sure to count the pairs that may be missing at the base of the plant, leaf scars)



F. Observations

**NO BUD OR FLOWER**

**IN FLOWER**



**IN BUD**



# Ozone Garden Data Collection

## Version II (page 2)

G. Estimate the percentage of total leaf area of each leaf that has foliar injury area using the NPS foliar injury scale

P = Purpling or Stippling

C = Chlorosis (yellowing)

N = Necrosis (death)

Example of Purpling is seen on top (epidermal) of leaf (P) on the underside of leaf

Example of Chlorosis (yellowing)

Example of Necrosis, visible on both sides of a leaf



If the leaf is not present check box "leaf missing"

	leaf present			leaf missing		
	0%	1-25%	26-50%	51-75%	76-100%	
P						
C						
N						

If leaf is present check box "leaf present" and estimate the percentage of foliar area injury in the three categories. An example of the ratings is shown regarding the leaf 7A on the upper right side of the page

✓	leaf present			leaf missing		
	0%	1-25%	26-50%	51-75%	76-100%	
P		✓				
C		✓				
N		✓				

Leaf 7A

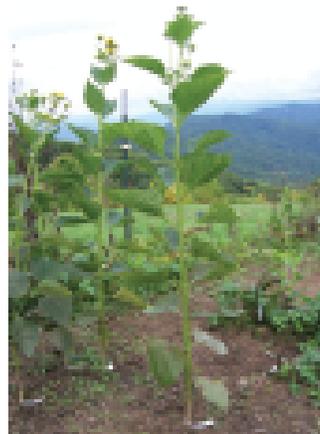


H. Hints:

1. As ozone injury progresses, purpling will turn into necrosis. If a leaf's necrosis rating goes up to a 51-75%, the purpling may no longer be apparent. The purpling has not gotten better, it has just progressed to a point where it has changed from injury to dead tissue.

2. Use caution when checking the back of the leaf, especially if you notice any symptoms. These leaves will become fragile and will break off easily.

3. For plants with opposite leaves, each facing pair of leaves is one set. For alternate leaf plants, such as the coneflower, count only the leaves coming off of the main stem.



# Ozone Garden Data Collection

## Version III

A. Two most important pieces of data in data collecting:

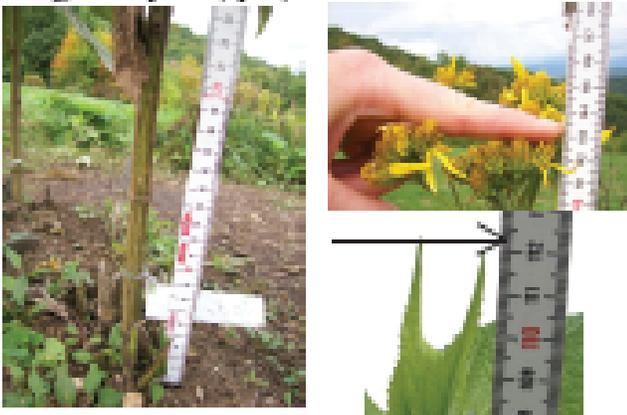
1. Collector name or group name
2. Date of data collection

B. Find the plant ID# with the metal hang tag at the base of the plant



C. Measure the HEIGHT of the plant

1. Use the metric side of the ruler (the side with the small numbers)
2. Measure from the base of the plant (beginning at zero) to the tallest leaf pair or bloom (if flowering) of the plant (apex) on the main stem



D. For plants such as Crownbeard, opposite leaves are identified as "A" leaf or "B" leaf



A leaf with black dot

B leaf without black dot

E. Flower

(Y= if flowered, N= if in bud or not flowering)

**NO BUD OR FLOWER**

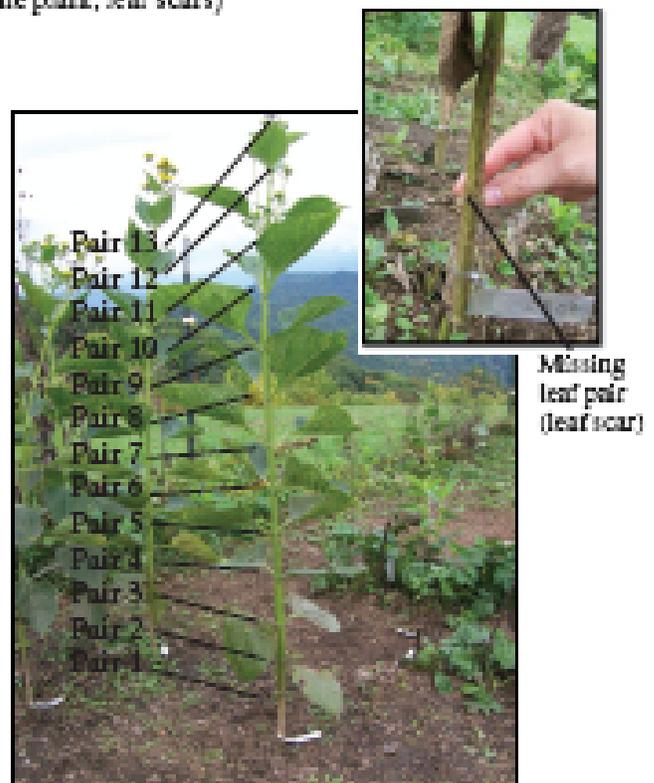
**IN FLOWER**



**IN BUD**



F. Count the # of leaf set or leaf pairs that come off of the main stem, start at the base of the plant (make sure to count the pairs that may be missing at the base of the plant, leaf scars)

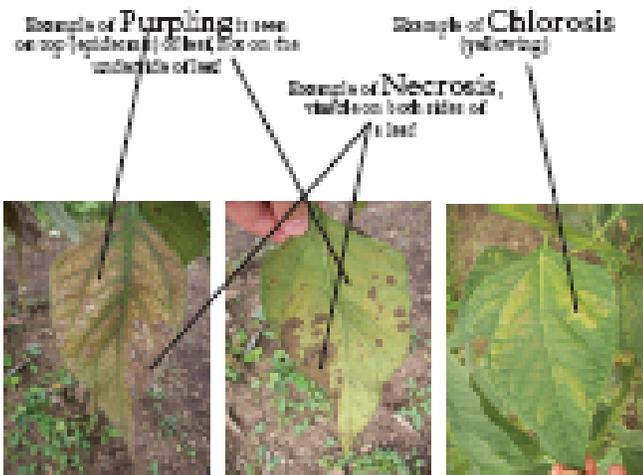


# Ozone Garden Data Collection

## Version III (page 2)

G. Estimate the percentage of total leaf area of each leaf that has foliar injury area using the NPS foliar injury scale

- P = Purpling or Stippling
- C = Chlorosis (yellowing)
- N = Necrosis (death)



If a leaf breaks off or has broken off, make sure to assign it the appropriate leaf loss code:

- 7 - leaf gone with no prior symptoms
- 8 - leaf gone with prior chlorosis (yellowing) only
- 9 - leaf gone with prior purpling only (ozone)
- 10 - leaf gone with prior purpling (stippling) and chlorosis

When a leaf falls off, carry its last data entry over into all of the following data collection entries. See example below

Date: 8/20/09

Leaf 7				
	C	P	N	L
A	2	1	1	8
B	2	2	1	-

Between 8/20/09 and 8/30/09 Leaf 7B fell off

Date 8/30/09

Leaf 7				
	C	P	N	L
A	2	1	1	8
B	2	2	1	10

An example is shown below of the ratings regarding the leaf on the upper left side of the page.

Leaf 7				
	C	P	N	L
A	2	2	2	-
B				

Leaf 7A



H. Hints:

1. As ozone injury progresses, purpling will turn into necrosis. If a leaf's necrosis rating goes up to a 5, the purpling may no longer be apparent. The purpling has not gotten better, it has just progressed to a point where it has changed from injury to dead tissue. Carry over the last data entry for purpling.
2. Use caution when checking the back of the leaf, especially if you notice any symptoms. These leaves will become fragile and will break off easily.
3. For plants with opposite leaves, each facing pair of leaves is one set. For alternate leaf plants, such as the coneflower, count only the leaves coming off of the main stem.

# Some Useful Webpages

Provided by Dr. Art Chappelka

These Web pages should prove helpful, and will provide many other links.

1. [www.epa.gov](http://www.epa.gov)

US Environmental Protection Agency homepage: Lots of information regarding air quality (standards, emissions, effects, etc.), climate change, ecosystem effects.

2. [www.noaa.gov](http://www.noaa.gov)

National Oceanic & Atmospheric Administration homepage: Good information regarding air quality (mainly atmospheric chemistry & meteorology), climate change.

3. [www.ncar.ucar.edu](http://www.ncar.ucar.edu)

National Center for Atmospheric Research homepage: Lots of good information related to global climate change, carbon budgets, & atmospheric chemistry. Description of a large-scale global climate change model.

4. [www.ncl.ac.uk/gane](http://www.ncl.ac.uk/gane)

Webpage related to a major program dealing with nitrogen deposition & effects. Follow the links for detailed information.

5. [www.ncl.ac.uk/airweb](http://www.ncl.ac.uk/airweb)

Webpage related to a description of biological effects for ozone, fluoride, and sulfur dioxide. Follow the links for detailed information.

6. [www.ipcc.ch](http://www.ipcc.ch)

Intergovernmental Panel on Climate Change homepage. Some good information related to global climate change.

7. [www2.nature.nps.gov/air/](http://www2.nature.nps.gov/air/)

National Park Service's website on air quality in parks. Includes issues, data and webcams.

8. [nadp.sws.uiuc.edu](http://nadp.sws.uiuc.edu).

A description of the National Atmospheric Deposition Program/ National Trends Network (NADP/NTN) national network for monitoring acidic deposition.

9. [www.asl-associates.com](http://www.asl-associates.com).

Webpage dedicated to information about air pollution, primarily ozone, particulates and sulfur dioxide.

10. [www.epa.gov/airnow](http://www.epa.gov/airnow).

Realtime image demonstration of ozone buildup over time and categories of severity.

11. [www.ace.mmu.ac.uk](http://www.ace.mmu.ac.uk).

Go to the Resources Index. There are many different fact sheets and teaching resources that will be of help to you. I suggest you look at this site.

12. [www.ozone.wsl.ch](http://www.ozone.wsl.ch)

Webpage showing visible ozone injury of several sensitive plants and how to use this information in assessment.

13. [www.fia.fs.fed.us](http://www.fia.fs.fed.us) & [www.fiaozone.net/index.html](http://www.fiaozone.net/index.html)

Interesting information concerning the USDA Forest Service's Forest Health Monitoring Program.

14. <http://reports.eea.eu.int>

European Environment Agency Website.

15. [www.heinzcenter.org/ecosystems/index.htm](http://www.heinzcenter.org/ecosystems/index.htm)

A report on the state of the Nation's ecosystems. Good information regarding indicators of ecosystem change.

16. [mona.psu.edu](http://mona.psu.edu)

A link to a foliar injury assessment module. A training tool used for individuals in the assessment of air pollu-

tion injury to vegetation.

17. [www2.nature.nps.gov/air/pubs/index.htm](http://www2.nature.nps.gov/air/pubs/index.htm).

A link to various publications related to air quality by the NPS.

18. [airlichen.nacse.org/](http://airlichen.nacse.org/).

This is a good website for information on lichens as bioindicators of air pollution.

19. [toxmap.nlm.nih.gov/toxmap/main/index.jsp](http://toxmap.nlm.nih.gov/toxmap/main/index.jsp).

This website provides some information and maps regarding environmental health and toxicology.

20. [reports.eea.eu.int/EMEPCORINAIR4/en](http://reports.eea.eu.int/EMEPCORINAIR4/en).

Links to various inventory models, etc. in Europe.

## REFERENCES

- Horsefall, J. G., Barreatt, R. W. 1945. An Improved Grading system for Measuring Plant Disease. *Phytopathology* 35, 655.
- Duchelle, S. F., Skelly, J. M. and Kress, L. W. 1980. The impact of photochemical oxidant air pollution on biomass development of native vegetation and symptom expression of *Asclepias* spp. *Phytopathol.* 70:689.
- Duchelle, S. F. and Skelly, J. M. 1981. The response of *Asclepias syriaca* to oxidant air pollution in the Shenandoah National Park of Virginia. *Plant Disease* 65:661-663.
- Skelly, J. M., Davis, D. D., Merrill, W., Cameron, E. A., Brown, H. D., Drummond, D. B., and Dochinger, L. S. (eds.) 1987. *Diagnosing Injury to Eastern Forest Trees*. USDA-For. Ser., Veg. Survey Res. Coop. and Penn State Univ., University Park, PA. 122 p.
- Hildebrand, E.S., Skelly, J. M. and Fredericksen, T.S. 1996. Foliar response of ozone sensitive hardwood tree species from 1991-1993 in the Shenandoah National Park, VA. *Can. J. For. Res.* 26:658-669.
- Neufeld, H.S., Renfro, H.S., Hacker, W.D., and Silsbee, D. 1992. Ozone in Great Smoky Mountains National Park: Dynamics and Effects on Plants. In "Tropospheric Ozone and the Environment II", edited by R. L. Berglund, Air & Waste Management Assoc., pp. 594-617
- Skelly, J.M. 1998. A brief assessment of forest health in northeastern United States and southeastern Canada. *Northeastern Naturalist* 5 (2) 137-142.
- Skelly, J. M. 2000. Tropospheric ozone and its importance to forests and natural plant communities of the northeastern United States. *Northeastern Naturalist* (3) 221-236.
- Innes, J. L., Skelly, J.M. and Schaub, M. 2001. A guide to the identification of ozone-induced foliar injury on broadleaved tree, shrub and herb species. Paul Haupt Publishing, Bern, Switzerland. ISBN No. 3-258-06384-2. 136pp.
- Chappelka, A.H., Neufeld, H.S., Davison, A.W., Somers, G.L., and Renfro, J. R. 2002. Ozone injury on cutleaf coneflower (*Rudbeckia laciniata*) and crown-beard (*Verbesina occidentalis*) in Great Smokey Mountains National Park. *Environmental Pollution* 125 (2003) pp. 53-59.
- Orendovici, T., Skelly, J. M., Ferdinand, J. A., Savage, J. E., Sanz, M-J. and Smith, G. C. 2003. Response of native plants of northeastern United States and southern Spain to ozone exposures: Determining exposure/response relationships. *Environmental Pollution* 125: 31-40
- 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.
- Skelly, J.M. 2003. Native plants as bioindicators of air pollutants: contributed papers to a symposium held in conjunction with the 34th air pollution workshop. *Environmental Pollution* 125: 1-2.