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HANDBOOK FOR ASSESSMENT OF FOLIAR OZONE INJURY ON VEGETATION IN THE NATIONAL PARKS

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Copies are available from:

Air Resources Division
National Park Service
P.O. Box 25287
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Or, the report can be downloaded from:
http://www2.nature.nps.gov/air/permits/aris/networks/docs/Handbook_Ozone_Injury_Assessment.pdf

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INTRODUCTION

Ozone is the most widely distributed phytotoxic air pollutant in the United States. It is not released directly into the atmosphere by any source, but produced by photochemical reactions in the atmosphere powered by ultraviolet light from the sun and involving oxides of nitrogen and volatile organic compounds. Many of the gaseous precursors are emitted in the urban environment by automobiles, power plants, and industries using fossil fuels and organic chemicals, and some volatile organic compounds are also released by vegetation. Since intense sunlight powers the photochemical reactions, ozone is a concern primarily in the summer. The production of ozone takes place as air parcels are carried on the prevailing wind, and the pollutant transported long distances into rural forests and wildlands. This long-distance transport of ozone, as well as its presence in urban centers, makes it a concern for the US National Park Service. Foliar injury on plants from ozone has been documented in studies in a number of national parks (Bartholomay et al. 1997, Benoit et al. 1982, Chappelka et al. 2003, Chappelka et al. 1997, Chappelka et al. 1999a, Chappelka et al. 1999b, Duchelle and Skelly 1981, Duchelle et al. 1983, Eckert et al. 1999, Hildebrand et al. 1996, Neufeld et al. 1992, Peterson et al. 1987, Williams et al. 1977). The extent to which vegetation in other parks is being impacted by ozone is not known, but such impacts are of interest and potential concern to park managers.

This handbook is intended to provide both the broad background and the specific information necessary to design and implement a field program to assess the presence and extent of foliar ozone injury on plants. It provides guidance to managers and biologists who are interested in determining whether ozone is injuring plants in their park and assessing the extent of ozone injury in a given year and over time. It describes in detail a number of assessment programs that may be implemented to address specific management objectives.

The handbook begins by providing an understanding of how plants, ozone exposure and the exposure environment interact to produce foliar injury. It describes and illustrates various kinds of foliar injury produced by ozone and identifies ozone-sensitive species that are most useful in field surveys.

The focus of the assessments is on visible foliar injury since it constitutes a readily identifiable signal that ambient levels of ozone are producing effects on plants. However, it is important to recognize that the production of visible foliar injury is the result of a series of biochemical, physiological and physical responses as the plant is exposed to ozone. Many responses have taken place in the plant before foliar injury becomes manifest. Conversely, it is likely that many responses to ozone have taken place in other plants that have not developed visible foliar injury. The presence of foliar ozone injury does not necessarily indicate there are effects on plant growth, development or reproduction. Depending on the plant species, nature and extent of injury, time of the growing season in which the injury develops, recovery time, and impacts on carbon fixation, the plant may or may not experience long-term consequences from the injury. This handbook, and field surveys in general, do not attempt to assess the incidence or consequences of the biochemical and physiological responses preceding the development of foliar injury, or assess the consequences of foliar injury on the growth, development or reproduction of the plant.

Since field surveys are conducted to satisfy various management and biological objectives, three assessment approaches are presented that require different commitments of resources and produce different levels of information. The handbook describes the objectives of each approach, how field sites are located and evaluated, how field assessment plots are established, and how the assessments of foliar injury are conducted. Guidance is also provided on compiling foliar injury data, implementing a quality assurance program, and conducting fieldwork safely and efficiently.

This handbook can be used in conjunction with the assessments of risk of foliar ozone injury to plants conducted for the 270 national parks in the 32 networks in the Vital Signs Monitoring Program. The assessments used site-monitored or kriged ozone values for the years 1995 through 1999 to assess risk. If the exposure regimes at a site change significantly in subsequent years, it will be appropriate to reassess the level of risk for the site. The Ozone Injury Risk Assessment reports for the sites in the Vital Signs Program are available on the National Park Service Air Resources Division web site at http://www2.nature.nps.gov/air/Permits/ARIS/networks/index.cfm.

The risk assessment for each park was conducted by obtaining information on the ozone-sensitive plant species found there, the levels of ozone exposure that occurred over a five-year period, and, since soil moisture is a critical variable controlling gas exchange, the levels of soil moisture that existed during the periods of ozone exposure. The information was evaluated to determine the degree to which the levels of ozone and soil moisture integrate to create an exposure environment that can lead to the production of foliar injury on sensitive species at the site.

The risk of foliar ozone injury at each site was classified as high, medium or low. Sites receiving a risk rating of high have a probability of experiencing foliar injury in most years. Those rated low are not likely to experience injury in any year, but the risk is not zero for most of them. A rating of moderate was assigned to sites where analyses indicated injury was likely to occur at some point in the five-year period. That is, foliar injury will probably occur at sites rated moderate, but will not likely occur regularly or frequently. Sites rated moderate are likely to experience a wide temporal variation in the occurrence of injury, and may experience injury for one or more years, but also experience several years without injury.

Understanding the risk of ozone injury can help resource managers decide whether an effort should be made to determine whether foliar injury is occurring in a park or to systematically assess the incidence of injury. This handbook provides managers with the guidance necessary to decide which type of assessment is appropriate for their park's level of risk and their management objectives, and presents detailed information on how to design and conduct an assessment.

THE TRIAD CONCEPT OF INJURY

The production of foliar ozone injury is the result of interactions among the plant species, level of ozone exposure, and exposure environment. Injury is produced on plants when certain properties of this triad of variables are satisfied, and the interactions of the variables foster the effects of the exposure. First, the plant species must be genetically predisposed to be sensitive to ozone. Differences in genetic disposition to ozone are most evident at the species level, but significant differences in sensitivity are also manifest among clonal lines and individuals within a species. Classifications of the sensitivities of various plant species to ozone are reflections of fundamental differences in responses that have been observed in laboratory research and field studies. These generalizations, such as the statement that quaking aspen is more sensitive to ozone than is black oak, are the consequence of differences grounded in the genetic properties of the species. The biochemical and physiological bases for these genetically-induced differences are not clearly understood.

Second, the plant must be exposed to ambient levels of ozone that exceed the threshold required for injury. There are two basic types of exposures that can produce injury on plants: acute and chronic. Acute exposures are characterized by the presence of a high concentration of ozone for a relatively short period of time, while chronic exposures involve lower concentrations that persist or recur over an extended period of time. In reality, ambient ozone regimes provide both acute and chronic exposures with variations in exposure occurring throughout the growing season and from year to year. Ambient ozone monitoring stations in the local vicinity provide the best indication of the level of exposure plants receive. However, it is important to recognize that ozone exposure concentrations at a specific site can vary significantly from those at a nearby monitoring station depending upon elevation, aspect, and openness of the site.

Lastly, the plant must experience environmental conditions that foster the uptake of ozone from the atmosphere. Environmental conditions that favor photosynthesis also promote gas exchange and the uptake of ozone along with carbon dioxide. Conditions of optimum temperature, humidity, illumination, and soil moisture will facilitate photosynthesis and the associated uptake of ozone. Low levels of soil moisture and high temperatures can lead to stomatal closure in plants, and thereby significantly reduce the uptake of ozone. These soil moisture and temperature conditions often exist when ozone concentrations are high. This combination of conditions creates a situation in which ambient levels of ozone are above the threshold for plant response, but reduced rates of gas exchange effectively exclude ozone from the interior of the plant leaf. Under these circumstances, the effective exposure dose is considerably less than that in the ambient atmosphere. It is also important to remember that within a generally adverse environment at a field site, a more favorable environment for pollutant uptake, particularly as determined by soil moisture, can exist on the microsite level. When this condition exists, plants on favorable microsites may express foliar ozone injury while adjacent plants do not.

Production of foliar injury is greatest when all variables of the response triad are optimized. Maximum foliar injury occurs when a particularly sensitive plant species or genotype is exposed to concentrations of ozone above the injury threshold under environmental conditions that foster pollutant uptake. Movement of any of the three variables away from the optimum will reduce the level of injury produced. Significant movement of a variable away from the optimum can preclude the production of injury, no matter how favorable the other variables may be. The most common conditions that suppress or preclude the production of foliar ozone injury by reducing its uptake by plants are high temperatures and low precipitation. Under these conditions foliar ozone injury may be entirely absent, or found only on favorable microsites, even though ambient levels of ozone may significantly exceed threshold injury levels.

OZONE INJURY

Types of Foliar Injury

Foliar ozone injury on broadleaf plants is generally categorized in increasing levels of severity as stipple, chlorosis, fleck, and bifacial necrosis. Stipple is characterized by the appearance of interveinal, dot-like areas of tan, red, brown, purple, or black pigmentation on the upper surface of the leaf. Stipple can be either uniformly distributed over the surface of the leaf or concentrated in certain areas. It may range from widely scattered dots to dots whose density nearly covers the surface of the leaf. Coloration, density, and distribution are functions of plant species and the duration and nature of exposure. The production of stipple requires exposure of the leaf surface to direct sunlight. A closely overlapping leaf can protect the lower leaf by covering it and producing a "shadow" in the injury on the leaf surface.

Figure 1. Widely-scattered stipple on spreading dogbane (*Apocynum androsaemifolium*).

Chlorosis is a loss of chlorophyll in the leaf and can appear as a generalized effect over the leaf, as relatively discrete patches known as mottle, or as highly localized spots. Chlorosis may appear in conjunction with other symptoms of foliar ozone injury. Extensive chlorosis, especially when accompanied by other ozone-induced injury, can lead to premature senescence of foliage and its abscission from the plant.

Figure 2. Spreading dogbane (*Apocynum androsaemifolium*) with extensive chlorosis and associated stipple on leaflets.

Fleck is characterized by small, discrete areas of dead tissue that are visible only on the upper surface of the leaf. Fleck is produced by the death of cells in the palisade mesophyll of the leaf. The lesions may be irregular in shape and range in color from tan to black. Bifacial necrosis is a more severe form of injury resulting from cell death in both the palisade and spongy mesophyll and epidermal tissues of the leaf. Injury appears on both sides of the leaf and dead tissue may take on a papery texture. Coloration in bifacial necrosis can range from light tan to black.

Fig 3. Bifacial necrosis on aspen (*Populus tremuloides*).

Ozone injury on needles of conifers generally appears as either tipburn or chlorotic mottle. Tipburn is considered to be primarily induced by acute exposures and mottle by chronic exposures, although variations exist in these responses. Tipburn consists of tissue that has been killed either through dieback of the needle's tip or has died following the development of a band of dead tissue along the needle. The dead tissue may become red to brown. Mottle consists of discrete patches of yellow tissue and can be further classified as hard- or soft-edged. Ozone-injured needles may be shed prematurely, leaving the tree with less than its normal complement of needles.

Foliar injury from acute exposure to ozone is usually evident within hours or days of exposure. Common symptoms produced by acute exposure are stipple, fleck, and bifacial necrosis. Injury from chronic exposure becomes evident over a period of weeks or months and is often characterized by chlorosis, stipple, premature senescence, and necrosis. Symptoms intergrade between the two general types of injury and can vary depending upon species, physiological state of the plant, environmental conditions, and the history of exposure.

Figure 4. Spreading dogbane (*Apocynum androsaemifolium*) leaflet showing co-occurrence of chlorosis, stipple and developing fleck.

Foliar markings can serve as important tools in diagnosing ozone injury on broadleaf species of plants. Stipple is a classic symptom associated with injury by ozone and its presence and coloration are diagnostic for the pollutant. The pattern of expression of injury on individual leaves and on a series of leaves provides strong complementary information for diagnosis. The presence of stipple, fleck and bifacial necrosis and their patterns of expression are strong diagnostic tools. Chlorosis is the least valuable aid to diagnosis due to its generalized appearance and ability to be induced by a range of stresses.

Figure 5. Bifacial necrosis on aspen (*Populus tremuloides*) with the greatest injury on older leaves that were exposed to ozone for the longest period of time.

The use of foliar markings as diagnostic tools is more demanding with conifers than with other species. Tipburn and mottle on needles can be induced by a variety of stresses and their value in diagnosis is highly dependent on the ability of the observer to make subtle discriminations and judgments. In general, assessment of foliar ozone injury on conifers requires more training and experience than does assessing injury on herbaceous and broadleaf tree species. While conifers have been used in many field surveys, the probability of misdiagnosing markings on their needles is likely greater than for other species. In the West and areas where information on the ozone-sensitivity of resident species is limited, the use of conifers as the primary bioindicator in an assessment may be a necessity. However, in the East and in areas where other bioindicators are found, conifers should be considered secondary choices as bioindicator species.

Illustration and descriptions of foliar ozone injury, depictions of mimicking symptoms, and information on foliar markings from other pollutants and biotic and abiotic stresses is found in several comprehensive publications (Flagler 1998, Innes et al. 2001, Pennsylvania State University 1987).

A collection of images illustrating ozone injury on bioindicator and other ozone-sensitive species is found at the National Park Service AirWeb site at http://www2.nature.nps.gov/air/Pubs/bioindicators/index.cfm.

Mimicking Symptoms

It is essential that full consideration be given to biotic and abiotic stress agents when foliar markings are being examined in the field. Markings produced by a variety of insects, pathogens, nutritional imbalances, and environmental factors can mimic foliar ozone injury. Attention must be paid to assure that mite or leafhopper injury is not interpreted as stipple; close examination will usually detect signs of the insects themselves. Drought, sunscald, viruses, and herbicides can produce foliar injury similar to that from ozone. Field diagnosis requires consideration and exclusion of other agents of stress before ozone is identified as the causal agent of the injury observed. Thus, a working knowledge of insects and pathogens, as well as an understanding of other abiotic stresses, is important in conducting field assessments of ozone injury.

Approach to Diagnosis

Diagnosis of foliar injury is to a great extent an art that reflects the scientific, analytical, and deductive abilities of the individual conducting the assessment, as well as their inherent philosophy toward decision making. The approach each individual adopts in making a diagnosis reflects their willingness or reluctance to identify markings as being caused by ozone. These differences among individuals speak to the importance of having consistency in the evaluator(s) for a field program. The ideal is to have the same person(s) perform the evaluations over time. Regardless of whether one or more individuals conducts the assessments, a training program should be used to both familiarize them with the appearance of ozone injury and mimicking injury, and to condition their eye in estimating the level of injury.

The evaluator needs to understand basic principles associated with the pattern and appearance of ozone injury as well as know the specific characteristics of ozone injury on the plant species being evaluated. Some useful concepts to employ when diagnosing foliar ozone injury include the following:

- On plants with indeterminate growth and foliage of different ages, symptoms will be more pronounced on older and mid-aged foliage since these leaves have had the longest period of exposure. The younger leaves may have little or no injury.

- If injury is the result of an acute exposure, it may be restricted to only those leaves whose physiological age (recently matured) at the time of exposure made them most susceptible to the exposure.

- Symptoms are frequently confined to the upper leaf surface and are evident as purple-red to black stipple. The stipples are generally discrete and uniform in size, but may coalesce and cover much or all the leaf surface as exposure continues.

- Symptoms are interveinal and do not occur on the veins or veinlets.

- Where leaves overlap and marking appears on the lower leaf or on both leaves, the lower leaf will often display a "shadow effect" apparent as a zone of unaffected tissue beneath the overlap area.

- With extended or high exposure, stipple can mix with chlorosis or fleck on foliage and make markings less distinct and more difficult to diagnose.

- If exposure has been significant throughout the growing season, premature senescence may have resulted in the casting of heavily injured leaves. Cast leaves can significantly reduce the level of injury determined from assessing the leaves that remain on the plant. Check the ground for marked, fallen leaves and make note of them since they represent an effect of ozone on the plant that cannot be readily quantified.

Conclusions drawn with regard to the etiology of markings on plant foliage are always accompanied by varying degrees of certainty. Markings associated with insects and diseases are generally diagnosed with a high degree of certainty since they are often associated with signs of the pathogen or the presence of the insect. Markings of abiotic origin are often more difficult to associate with a specific stress due to the more generalized response (e.g., chlorosis, necrosis, and pigmentation) they elicit from the plant.

While knowing the characteristics of the typical symptoms associated with ozone provides a starting point for diagnosis of injury, being able and willing to assign a level of certainty to the diagnosis completes the process. Under circumstances in which there is uncertainty as to the origin of markings that look like those caused by ozone, the evaluator should feel at liberty to categorize them as "ozone-like". Uncertainty may arise from minor deviations from the expected in the color, distribution, or size of the markings under conditions in which other features of the symptoms agree with expectations. Voucher specimens and digital images of foliage with injury whose nature is uncertain should be provided to experts for examination and diagnosis. The evaluator also needs to recall the nature of the markings observed from year to year since deviation from those typically expected may be a result of the overall environment at the site or be a characteristic response of a particular genotype.

Timing of Symptom Evaluation

Ideally, the evaluation of foliar ozone injury should be conducted when the seasonal exposure to ozone has been maximized and the probability of injury expression is highest, and before environmental conditions at the end of the growing season foster the development of foliar senescence that can mask ozone injury. In the eastern United States this window of opportunity generally extends from mid-July through August, and varies largely with latitude. In the western United States the optimum period for injury assessment is significantly influenced by local geographic and environmental conditions that affect the growing season and the period of maximum ozone exposure. In these instances, decisions on when to conduct the annual assessment must be based on site-specific conditions with the goal of assuring that plants have had the opportunity to receive the maximum seasonal ozone exposure, but that deteriorating growing conditions will not produce foliar senescence that compromise assessment of foliar ozone injury.

Developing Expertise in Identifying Ozone Injury

Developing a familiarity with and understanding of the symptoms ozone can produce on plant foliage requires study and experience. Symptoms vary among species, and their appearance can be influenced by the nature of the exposure regime and environmental conditions during exposure. The ability to accurately and confidently identify foliar ozone injury is a skill gained through studying images of foliar injury, inspecting plants injured in controlled laboratory exposures, and examining ozone-injured plants in the field. Images of foliar ozone injury are found in several books (Flagler 1998, Innes et al. 2001, Pennsylvania State University 1987) and on the NPS web site http://www2.nature.nps.gov/air/Pubs/bioindicators/index.cfm. Inspecting foliage of plants exposed to ozone in the laboratory provides an opportunity to see typical injury first-hand, but access to such material is limited. Ultimately, developing skill and confidence in identifying ozone injury is most successfully achieved by spending time examining plants in the field with a person experienced in field diagnosis. Again, these opportunities are limited since the pool of experienced people skilled in field diagnosis is small. While it may be difficult to spend time in the field with these persons, doing so will greatly improve the diagnostic skill and confidence of the assessor and the accuracy and quality of the assessment. To build confidence in diagnosing ozone injury, the evaluator should send samples of foliage identified as injured by ozone and accompanying photographs from the field to experts for confirmation.

OZONE-SENSITIVE SPECIES AND BIOINDICATORS

Not all species of plants are sensitive to ozone and range from those that are highly resistant to those that are very sensitive and readily injured. Highly sensitive species of plants are injured when exposure levels increase only slightly above background. Most species that respond to ozone are intermediate in sensitivity, and injured by extended periods of exposure to moderately elevated levels of ozone. However, most species of plants are resistant to ozone and able to withstand extended periods of exposure to elevated levels without the development of foliar injury.

Plants injured at levels of ozone slightly above background are termed bioindicators; they serve to indicate that the levels of exposure are above background, are affecting species of plants highly sensitive to ozone, and may be reaching levels harmful to plants of intermediate sensitivity. Although these species are most sensitive to ozone, genetic differences among individual plants result in variations in sensitivity that may be expressed as differences in the levels in foliar injury on plants in a population. One should not expect uniform levels of injury among bioindicator plants of the same species on an assessment plot.

Ozone exposure research conducted in the laboratory and field provides information on the relative sensitivity of plant species. However, some studies employed ozone exposure regimes that do not mimic those occurring in nature and thus their findings regarding the ozone-sensitivity of plant species and the nature of the symptoms produced are subject to question.

A workshop sponsored by the National Park Service was held in June 2003 with the objectives of assessing existing information on ozone-sensitive species of plants, deriving a consensus on the classification of the species in the studies, and developing lists of ozone-sensitive species and those that are highly sensitive and used as bioindicators for ozone. The workshop produced a report that classifies plants into three categories: ozone-sensitive species, bioindicator species, and suspect species (National Park Service 2003). Ozone-sensitive species typically exhibit foliar injury to exposures slightly or moderately above ambient levels of ozone.   Bioindicator species are a subset of the sensitive species and exhibit foliar injury that is readily recognized and diagnostic for ozone exposure at ambient concentrations of ozone slightly above background. Suspect species are those for which there is some indication of sensitivity to ozone, but because of the high levels of ozone used in controlled exposures or limited observation and verification of foliar markings in the field, there is uncertainty about the sensitivity of the species. The workshop concluded that suspect species need additional research to determine their sensitivities to ozone, and should not be used in field assessments at this time.

Plant bioindicators for ozone produce consistent and diagnostic types of foliar injury when exposed to slightly elevated ambient levels of the pollutant. The foliar symptoms produced by the exposures are unique in appearance and distinguishable from injury produced by other stresses. The nature of the markings and the general levels of exposure at which they occur have been confirmed by controlled exposures. To be most useful, bioindicator species should be widely distributed and readily identifiable in the field.

Plant species determined at the workshop to be sensitive to ozone are presented in Table 1, and species that are bioindicators are presented in Table 2. The report from the workshop, Ozone Sensitive Plant Species on National Park Service and U.S. Fish and Wildlife Service Lands (US National Park Service 2003), is found at http://www2.nature.nps.gov/air/Pubs/index.cfm.

In this handbook, plants used in ozone injury assessments are divided into two categories: ground layer and trees. "Ground layer" is a term commonly used to identify the collection of mostly vascular plants generally less than 1.0 meter in height found on the forest floor and in open, non-forested habitats are ground layer and herbaceous layer (Gilliam and Roberts 2003). The term "tree" refers to ozone-sensitive and bioindicator tree species generally over 10 cm in diameter found singly or in stands. While a tree species may be sensitive to ozone, seedling and small sapling trees growing under the tree canopy are generally not included in the tree category since they are often not responsive to ozone due to the reduced levels of ozone and light occurring in their environment. If trees in these size-classes are found in open, well-illuminated sites they may be considered for use in an assessment.

TABLE 1. PLANT SPECIES SENSITIVE* TO OZONE

*Species considered "sensitive" are those that typically exhibit foliar injury at or near ambient ozone concentrations in fumigation chambers and/or are species for which ozone foliar injury symptoms in the field have been documented by more than one observer.

Scientific Name	Common Name
Aesculus octandra	Yellow buckeye
Ailanthus altissima	Tree-of-heaven
Alnus rubra	Red alder
Alnus rugosa	Speckled alder
Amelanchier alnifolia	Saskatoon serviceberry
Apios americana	Groundnut
Apocynum androsaemifolium	Spreading dogbane
Apocynum cannibinum	Dogbane, Indian hemp
Artemisia douglasiana	Mugwort
Artemisia ludoviciana	Silver wormwood
Asclepias exaltata	Tall milkweed
Asclepias incarnata	Swamp milkweed
Asclepias syriaca	Common milkweed
Aster acuminatus	Whorled aster
Aster macrophyllus	Big-leaf aster
Cercis canadensis	Redbud
Clematis virginiana	Virgin's bower
Corylus americana	American hazelnut
Eupatorium rugosum	White snakeroot
Fraxinus americana	White ash
Fraxinus pennsylvanica	Green ash
Gaylussacia baccata	Black huckleberry
Krigia montana	Mountain dandelion
Liquidambar styraciflua	Sweetgum
Liriodendron tulipifera	Yellow-poplar
Lyonia ligustrina	Maleberry
Oenothera elata	Evening primrose
Parthenocissus quinquefolia	Virginia creeper
Philadelphus coronarius	Sweet mock orange
Physocarpus capitatus	Ninebark

Physocarpus malvaceum	Pacific ninebark
Pinus banksiana	Jack pine
*Pinus jeffreyi***	Jeffrey pine
*Pinus ponderosa****	Ponderosa pine
Pinus pungens	Table-mountain pine
Pinus radiata	Monterey pine
Pinus rigida	Pitch pine
Pinus taeda	Loblolly pine
Pinus virginiana	Virginia pine
Platanus occidentalis	American sycamore
Populus tremuloides	Quaking aspen
Prunus pensylvanica	Pin cherry
Prunus serotina	Black cherry
Quercus kelloggii	California black oak
Robinia pseudoacacia	Black locust
Rhus copallina	Winged sumac
Rhus trilobata	Skunkbush
Rubus allegheniensis	Allegheny blackberry
Rubus canadensis	Thornless blackberry
Rubus cuneifolius	Sand blackberry
Rubus parviflorus	Thimbleberry
Rudbeckia laciniata	Cutleaf coneflower
Salix goodingii	Gooding's willow
Salix scouleriana	Scouler's willow
Sambucus canadensis	American elder
Sambucus mexicana	Blue elderberry
Sambucus racemosa	Red elderberry
Sapium sebiferum	Chinese tallowtree
Sassafras albidum	Sassafras
Solidago altissima	Goldenrod
Spartina alterniflora	Smooth cordgrass
Symphoricarpos albus	Common snowberry
Vaccinium membranaceum	Huckleberry
Verbesina occidentalis	Crownbeard
Vitis labrusca	Northern fox grape

Vitis vinifera	European wine grape

** *P. jeffreyi* and *P. ponderosa* may hybridize, making identification difficult.
*** *P. ponderosa* var. *ponderosa* is the more sensitive variety; *P. ponderosa* var. *scopulorum* is not as sensitive.

US National Park Service 2003

TABLE 2. PLANT SPECIES CONSIDERED BIOINDICATORS* FOR OZONE

*Bioindicator species for ozone injury meet all or most of the following criteria:
- species exhibit foliar symptoms in the field at ambient ozone concentrations that are easily recognized as ozone injury by subject matter experts
- species' ozone sensitivity has been confirmed at realistic ozone concentrations in exposure chambers
- species are widely distributed regionally
- species are easily identified in the field

Scientific Name	Common Name
Ailanthus altissima	Tree-of-heaven
Alnus rubra	Red alder
Alnus rugosa	Speckled alder
Apios americana	Groundnut
Apocynum androsaemifolium	Spreading dogbane
Artemisia douglasiana	Mugwort
Artemisia ludoviciana	Silver wormwood
Asclepias exaltata	Tall milkweed
Asclepias syriaca	Common milkweed
Aster acuminatus	Whorled aster
Aster macrophyllus	Big-leaf aster
Cercis canadensis	Redbud
Corylus americana	American hazelnut
Eupatorium rugosum	White snakeroot
Fraxinus americana	White ash
Gaylussacia baccata	Black huckleberry
Liriodendron tulipifera	Yellow-poplar
Lyonia ligustrina	Maleberry
Oenothera elata	Evening primrose
Physocarpus capitatus	Ninebark
Physocarpus malvaceum	Pacific ninebark
*Pinus jeffreyi***	Jeffrey pine
*Pinus ponderosa****	Ponderosa pine
Platanus occidentalis	American sycamore
Populus tremuloides	Quaking aspen
Prunus serotina	Black cherry
Rhus trilobata	Skunkbush

Rubus allegheniensis	Allegheny blackberry
Rubus canadensis	Thornless blackberry
Rudbeckia laciniata	Cutleaf coneflower
Salix scouleriana	Scouler's willow
Sambucus canadensis	American elder
Sambucus mexicana	Blue elderberry
Sambucus racemosa	Red elderberry
Sapium sebiferum	Chinese tallowtree
Symphoricarpos albus	Common snowberry
*Vaccinium membranaceum*****	Huckleberry
Verbesina occidentalis	Crownbeard
Vitis labrusca	Northern fox grape
Vitis vinifera	European wine grape

** *Pinus jeffreyi* and *P. ponderosa* may hybridize, making identification difficult.
*** *P. ponderosa* var. *ponderosa* is the more sensitive variety; *P. ponderosa* var. *scopulorum* is not as sensitive.
****Sensitivity of *Vaccinium membranaceum* has been demonstrated in chambers, but not in the field, possibly because of low ozone levels throughout its range.

US National Park Service 2003

ASSESSMENT APPROACHES

Three general approaches are used to assess the effects of ozone on plants: scouting, surveying, and monitoring. Each approach addresses different management objectives, provides different information, and requires different investments of effort, manpower and time. Selecting the type of assessment to be conducted is primarily a function of the information park management seeks to obtain, but the decision is also influenced by the size of the park and the commitment of financial and personnel resources that can be made to the effort.

Scouting

A scouting assessment is used to determine whether foliar ozone injury is occurring on plants in a park, and to document its presence over time. It is intended to both produce a yes/no answer with regard to the presence of foliar injury and provide largely qualitative information on its continued occurrence. It requires the smallest investment of time, personnel and funds, however it provides limited information regarding the incidence, severity, spatial distribution, and long-term occurrence of ozone injury.

A scouting assessment may be the best initial effort for a park that is uncertain about the presence of ozone impacts within its boundaries. It can serve to document injury and thus establish a rationale for considering a more quantitative evaluation through a survey assessment. Annual scouting assessments can provide information on the continued presence of ozone injury that has long-term value with respect to air quality concerns. If a scouting survey is the only assessment a park can conduct, it should do so since the confirmation of foliar ozone injury has management and regulatory implications.

Surveying

Surveying and monitoring assessments are quantitative and comprehensive in nature and differ from each other primarily with respect to the intensity and duration of effort. Each approach entails the commitment of different levels of time, manpower and funding, and a selection between the two is made on the specific needs and resources of the park. A survey can be employed to provide a one-time assessment of injury, performed only when exposure and environmental conditions warrant, or conducted annually to assess the incidence of foliar injury and its trend over time. A survey uses field sites that are located using both random and non-random means, considers known locations of plant communities containing bioindicator species, and insures sites are distributed to provide spatial coverage throughout the park. A survey assessment extensively evaluates several ground layer bioindicator species, with trees having secondary importance. A survey yields quantitative information on the incidence and severity of ozone injury and its spatial distribution. Annual assessments are conducted on permanent plots, but not necessarily on the same plants. A survey assessment is likely to be appropriate for most parks since it has the greatest structural and operational flexibility and requires a moderate commitment of resources and effort.

Monitoring

Monitoring is the most intensive and longest duration assessment, and provides the most detailed information on species affected, the nature and extent of foliar injury, and the spatial distribution of impacts within the park. Monitoring generally focuses on two or more bioindicator tree species, but allows ground layer species to be included with generally secondary emphasis. Sites employed in the monitoring assessment are located using random and non-random methods. The randomly established sites are located by overlaying a grid on a map of the park to determine the locations of potential assessment sites. Plant community maps and other local information are used to establish non-random sites. Plots are permanently marked in the field, and trees are permanently tagged and mapped so they can be assessed annually. Foliar injury assessments provide a complete set of data for annual and trend analysis. Because of its objectives, a monitoring program is long-term in nature and requires an appropriate commitment of manpower, financial resources and time. Monitoring is most appropriate for parks where some or all of the following conditions prevail: injury has been previously documented, the level of risk is consistently high over time, management requires a scientifically rigorous documentation of the severity and spatial extent of injury, and appropriate resources can be dedicated to a long-term effort.

Selecting an Approach

When considering which approach to implement, it is important to clearly specify the management or biological objectives to be pursued. In brief, scouting determines whether foliar ozone injury is present in a park, surveying provides evaluation of injury in a specific year or in a few, possibly intermittent, years, and monitoring provides long-term evaluation of injury with annual observations on the same trees. Distinctions among the approaches are clear, but somewhat arbitrary, and the protocols employed in each are not exclusive, but gradate from one to another. Protocol features such as quantitative assessment of injury, permanently tagging plants for repeated evaluation, and mapping the locations of plants can be added to an assessment strategy to meet the needs of management.

The protocols for locating and evaluating candidate field sites, establishing tree and ground layer assessment plots, and assessing foliar ozone injury are drawn largely from US Forest Service protocols for assessing ozone impacts on trees in the southern Sierra Nevada mountains (Miller et al. 1996), the US Forest Service FHA/FIA protocols for assessing ozone injury (U.S. Department of Agriculture 2003), and the protocols employed in a National Park Service study assessing ozone injury on plants at Acadia National Park (Kohut et al. 2000).

The numbers of plots and plants sampled on the plots, mix of ground layer and tree species, and other details in the protocols are intended to assure that the findings of the assessment are based on adequate field data. The numbers do not establish rigid requirements that preclude conducting a field assessment if they cannot be satisfied. The protocols are somewhat flexible, and a specific program should be designed with this in mind. However, significant departure from the suggested protocol should be undertaken with caution and for legitimate reasons that do not compromise the science of the assessment process. Inadequate time, funding and manpower are not appropriate reasons for compromising the requirements of an assessment, and it is imperative that sound judgment be used to assure the scientific credibility of the effort. In general, if the requirements for a monitoring assessment cannot be satisfied, a survey assessment may be appropriate. Likewise, if the requirements for a survey assessment cannot be satisfied, a scouting assessment should be considered.

PROTOCOL FOR SCOUTING

Objectives

The objectives of a scouting assessment are to determine whether ozone is producing foliar injury on plants in a park, and to document its presence over time. This is accomplished by examining ozone bioindicator species on a number of non-randomly selected plots. Scouting can be a one-time effort to determine whether ozone injury is occurring within a park, or conducted annually to document its continued occurrence. Scouting requires the lowest commitment of time, money and manpower and yields relatively limited quantitative data. Scouting does, however, enable a park to establish a foundation of information about the occurrence of ozone injury that can be used to determine whether a more detailed evaluation of impacts is required.

Selecting Bioindicator Species

Several of the most common bioindicator species in the park are selected for examination. The more species examined, the greater the value of the assessment. The bioindicators selected should generally be broadleaf ground layer species that are abundant, easily examined, and on which ozone injury is readily and accurately diagnosed. However, hardwood bioindicator tree species may also be selected if they are of concern and accessible.

Identifying Candidate Field Sites

The locations of candidate field sites for assessing foliar ozone injury in a scouting assessment are determined using a non-random approach, and are ones on which the bioindicator species are known to be abundant. Information on the distribution of plant communities and species within the park is used in making the selections, or sites are selected by walking through the park looking for the bioindicator species. The sites selected are not intended to provide a random sample of the plant population, but an attempt is made to obtain spatial coverage of the park. The site locations should be broadly representative of the habitats of the species to allow potential influences of environment on response to be taken into account. While the sites are usually readily accessible, care should be taken to assure the site is not influenced by its proximity to a road or trail. If more than one species is selected, the selection of sites should assure that all species are examined equally to provide optimum opportunity to detect foliar injury.

Evaluating Candidate Field Sites

The presence, abundance, and distribution of bioindicator species at the site are critical considerations when evaluating a candidate site, but the overall site environment is also important. Sites are evaluated for the bioindicator species of interest, the presence of other bioindicator species, overall site conditions that may influence ozone exposure and pollutant uptake, prior disturbance, and nearby activities that may affect the integrity of the plot. Accessibility is also a concern, and since scouting is a relatively low-level effort, sites that are remote and time-consuming to access should not be used in favor of using additional sites that are more readily accessible.

Establishing Assessment Plots

Assessment plots are selected with the objective of having access to many individual plants of the bioindicator species. While the locations of the plots in a scouting assessment are not permanently marked in the field, their locations should be accurately identified on a map and established using a global positioning system (GPS). The plot has no fixed dimensions and as many plants as feasible should be examined for ozone injury. However, care must be exercised to assure that some minimum number of plants, probably 50 per species, is examined at each plot.

Care must be exercised when assessing a ground layer species that is clonal in nature. It is important to make sure that a number of clones are examined at a site. To do this, it may be necessary to expand the size of the plot or to employ multiple smaller plots to assure that several clonal lines are assessed. As a guide in the field, clonal lines may be separated by a physical barrier such as a stream, roadway, or rock outcropping that prevents clonal spread, or may occur as groups of plants separated by habitat in which the species does not occur.

PROTOCOL FOR SURVEYING

Objectives

A survey provides a systematic, quantitative assessment of foliar ozone injury, primarily on selected ground layer bioindicator species. The survey assessment can be used to provide a one-time quantitative evaluation of injury, performed only when exposure and environmental conditions warrant, or conducted annually to assess the incidence and severity of foliar injury and their trends over time. Incidence is a count of the number of plants or leaves affected, while severity is a measure of the percent of the leaves or the leaf surface area affected. A survey assessment should be implemented for a period of years compatible with the objectives of the program. When the time period is completed, all data should be evaluated and a decision made on whether to continue. The survey approach to assessment is flexible and can address diverse management and biological objectives.

While permanent assessment plots are established in a survey, repeated observations on the same plant are not part of the protocol. Plot center points and centerlines are permanently marked and documented for future use, but individual plants are not tagged nor are their positions mapped. This protocol can, of course, be modified at the discretion of the park to include tagging and mapping if desired.

Selecting Bioindicator Species

Several of the most common ground layer bioindicator species in the park are evaluated in the assessment. Emphasis is on ground layer species since they are easily examined, display ozone injury that is readily identified, and are found in large numbers on moderate sized field plots. If bioindicator trees species are of concern, they can be included in the assessment. However, it can take extra effort to find appropriate numbers of trees to sample and additional time to properly examine each tree. If it is desirable to have trees serve as a major component of the program, it may be more appropriate to adopt a monitoring approach to the assessment.

Identifying Candidate Field Sites

The locations of candidate field sites for assessing foliar ozone injury in a surveying program are identified using both random and non-random approaches. The spatial distribution of populations of a species should first be determined by using the best available plant community or habitat maps; however, such maps are usually not available at the individual species level. If necessary, an initial reconnaissance of the area is conducted to verify the distribution of the species of interest.

It is generally useful to stratify the geographic extent of the species of interest based on elevation, soil type, or other physical or biological criteria that appropriately delineate environmental variables that influence the plant community. The target population is further defined by progressively eliminating strata that meet rejection criteria such as slope angle, canopy closure level, associated species, and other environmental variables. The overlay of these criteria and the area they jointly define constitute the area to be eliminated from sampling, and the area remaining constitutes the target population. This can be quickly accomplished with a geographic information system (GIS), if it contains data on the criteria of interest.

After the target population is defined, it may be further stratified into smaller sampling units by watershed or elevation as a means of increasing sampling efficiency. Smaller sampling units will often contain less within–unit variability than larger units. Other factors that may be considered for defining strata are associated species, site class, landform type, and soil moisture-holding capacity (which is modeled from an estimate of soil depth, slope position, slope angle, and/or aspect).

Random sampling is used to locate potential sites in each stratum. Random sampling is conducted by placing an appropriately scaled Cartesian coordinate system on a 1:24,000 scale (USGS 7.5 minute) topographic map of the study area: larger scale maps may be required for smaller parks. Random x and y coordinates to locate potential sites are created using a scientific calculator as a random number generator or an appropriate GIS program that will locate random sites. Randomly-located sites are rejected if they occur on private land, in a developed area, in a body of water, near a trail or roadway, are too densely forested, or are so remote that sampling the point is not logistically feasible.

Additional candidate sites are selected using a non-random approach. These sites are located using historical records, information provided by park or professional botanists, or by searches of likely habitats. Non-random sites are used to increase the number of species and populations surveyed, provide needed geographical coverage, and to assure, when appropriate, that herbaceous species are sampled in both open and forested habitats.

Evaluating the Acceptability of Candidate Field Sites

Candidate field sites are inspected on the ground to evaluate their suitability for use in the surveying program. The presence, abundance, and distribution of bioindicator species at the site are critical considerations, but the overall site environment is also important.

Sites are examined for the bioindicator species of interest, the presence of other bioindicator species, overall site conditions that may influence ozone exposure and pollutant uptake, prior disturbance, and nearby activities that may affect the integrity of the plot. Accessibility is also a concern, but is secondary to making sure an adequate number of suitable plots are established within a stratum.

After all candidate sites are visited, information on the presence, abundance and spatial distribution of species of interest at each site is assessed to select the sites at which plots will be established. Sites that have more than one bioindicator species, that are reasonably accessible, and are unaffected by adjacent land-use or environmental variables are preferred. If a stratum contains more suitable sites than deemed necessary or that can be assessed with the resources available, the sites to be employed are selected at random from the suite of candidates.

Site rejection criteria and a procedure for selecting an alternate site if the sample point fails (such as another random site in the stratum, a random offset from the original point, or the site nearest the failed site) should be established before venturing into the field. In general, candidate sites should be rejected if the plants show obvious stresses from natural or human–caused factors that would mask or confound any observable ozone injury.

The total number of assessment sites established is a function of the size of the park, number and size of strata, and the number and distribution of bioindicator species. There must be a sufficient number of sites with appropriate spatial coverage to produce an accurate estimate of foliar effects on a given species in each stratum. It is possible that all suitable sites in a stratum may have to be used to obtain an adequate number of observations. The number of sites employed is also a function of the time and manpower that can be committed to the assessment effort. While these constraints are often significant, care must be exercised to assure they do not impact the effort to the degree that the assessment is compromised. If resource limitations prevent the implementation of a sound monitoring program, park administrators need to consider whether they can more effectively implement a survey-level effort, or whether assessing the potential impacts from ozone deserves a higher priority in the park's management plans and a greater commitment of park resources.

Evaluating Ground Layer Bioindicator Sites

In assessing the suitability of a candidate site for use with ground layer bioindicator species, the area within a 100-meter radius of the point is searched for the selected species. One way to conduct the search at each site is to walk a transect 100 m long in each of the four cardinal compass directions. At the end of each transect, the survey member turns right, walks about halfway to the next transect azimuth, and returns to the starting point. The locations of populations of bioindicator species encountered along these systematic walks are recorded.

Exposure to ambient air and sunlight are important for the development of foliar injury from ozone. Thus, to assure that the responses of ground layer plants on the site reflect the response in the larger environment, it is important that the site not be beneath a dense tree canopy that can take up and reduce the concentration of ozone reaching ground level and also reduce the level of sunlight incident on ground layer plants. Sites in the open, on or near the edge of a forest stand, or under an open tree canopy are preferred.

Evaluating Tree Bioindicator Sites

A population of trees suitable for use is one that has crowns that are readily accessed for injury assessment, is not located in an unusual microhabitat, is not impacted by foliar insects and diseases that would confound the evaluation of ozone injury, and is readily relocated. Accessibility is also a concern, but is secondary to making sure an adequate number of suitable plots are established within a stratum.

The density, spatial distribution, and exposure of trees to ambient air and light are important in determining whether an adequate sample of trees can be obtained at a site. To assure that the responses measured on the sampled trees are representative of trees in the larger environment, it is important that the canopies of the trees assessed are exposed to ozone in the ambient environment and receiving high levels of sunlight. If tree foliage can be sampled in-hand without pruning, it facilitates the assessment process and reduces possible long-term impacts from removing foliage. There is significant flexibility in the plot configuration that can be used to obtain a sample randomly selected trees, and options need to be considered when the site is being evaluated.

Establishing Assessment Plots

Plots for Ground Layer Bioindicator Species

Ground layer bioindicator species are evaluated using circular plots established as close to the sampling site point as feasible. If the populations of plants at a site are spatially distinct and of various sizes, a population close to the site point and of suitable size is used. The approximate center of the population is found and the distance to the closest edge of the population estimated. In large or continuous populations, the center of the sampling plot is established as a point within the population that is close to the site point and surrounded by bioindicator plants for at least 20 m in all directions. If more than one ground layer bioindicator is being assessed at a site, it may be necessary to establish multiple plots.

The center of the plot is permanently marked, a description of the location of the plot is written, and the azimuth and distance of the plot center relative to a reference point such as a witness tree or rock monument is recorded so the plot can be readily located in the future. The plot's location should also be established using a GPS.

Care must be exercised when assessing a ground layer species that is clonal in nature. It is important to make sure that a number of clones are examined at a site. To do this, it may be necessary to expand the size of the plot or to employ multiple smaller plots to assure that several clonal lines are assessed. As a guide in the field, clonal lines may be separated by a physical barrier such as a stream, roadway, or rock outcropping that prevents clonal spread, or may occur as groups of plants separated by habitat in which the species does not occur.

In a population of a ground layer bioindicator species, 60 plants are selected for evaluation. To select specific plants, 30 polar coordinates consisting of 30 random compass bearings and 30 random distances from the population center are used. The maximum distance from the center of the plot is set at 20 m, although the plot radius may vary due to the shape of the population of plants. Sets of random coordinates can be created using spreadsheet software on a personal computer before going to the field. Several sets are created for each maximum radial distance of 2, 5, 10, and 20 m from the population center. These are printed on weatherproof paper and stored in a binder for field use. Random bearings and distances for use in selecting plants are provided in Appendix A. At each assessment plot, one of the coordinate sets for the appropriate maximum radial distance is chosen and then used to select 30 points within the population at which plants are examined. A compass and laser rangefinder are used to locate the points. The two bioindicator plants closest to each of the 30 are selected for assessment of foliar injury thus providing a sample population of 60 plants at each plot. Each plant to be assessed is marked with a flagged wand stuck into the ground. If it is necessary to use a population in which there are fewer than 60 plants, all are examined, however the number of plants should not be fewer than 40.

For most ground layer species, a 20 m radius assessment plot will be adequate, but the radius can be increased where the density of plants is low. In cases where the margin of the population is irregular, it is sometimes necessary to use longer or shorter radial distance values for azimuths where the population ranges for greater or lesser distances, respectively, from the plot center.

Plots for Tree Bioindicator Species

If trees are selected for use as bioindicator species, 20 to 30 individuals are assessed at a site. Finding a suitable number of trees can be problematic depending on the spatial distribution and size of the trees at a site. The protocol for a survey assessment does not require trees to be tagged or their locations mapped since repeated observation of the same tree is not a part of the approach. However, if management objectives require repeated observations, trees can be permanently tagged and their positions mapped relative to the plot reference point and to each other. GPS may be used to map the locations of the selected trees if sufficient accuracy can be attained. Information on tagging and mapping is found in the Protocol for Monitoring Assessment.

Trees selected for assessment should be >10 cm diameter-at-breast-height (DBH) and in the dominant or co-dominant crown classes. Trees in the intermediate and overtopped classes should not be included in the sample since they are probably not exposed to ambient concentrations of ozone or to higher levels of sunlight. Trees are generally unsuitable for assessment if they have severe mechanical wounds, an unpruneable crown (lowest branches > 10 m above ground), evidence of root disease, excessive lean, or significant foliar insect or disease injury.

There are three types of plots that can be used and on which trees to be assessed are randomly selected: circular plots, strip plots, and stand-based plots.

Circular Plots. A 40 m radius circular plot may provide an adequate number of trees for assessment if the trees are moderate in size and density. Trees to be assessed are identified using pairs of polar coordinates consisting of random compass bearings and random distances from the plot center. The tree meeting the diameter, crown class, and other suitability criteria that is closest to the point designated by each pair of azimuth and distance values is selected for assessment. If trees are widely scattered, it may be difficult to locate the desired number, and increasing the distance from the center point within which selections are made can facilitate the process. In such cases, it may be necessary to sample all of the acceptable trees in the plot, and expand the plot size until the desired number is reached.

Strip Plots. A strip plot 80 m wide and up to 250 m long (2 hectare) may be used to identify candidate trees for evaluation. To establish the plot, a compass direction is selected that is likely to include a suitable number of candidate trees, is parallel to the contour of the slope, and remains within the same landform type. On flat areas, it is possible to select a random compass direction. The bearing establishes the long axis of the plot and also represents the plot's centerline. A tape or laser rangefinder is used to measure distances and if the slope in either the long or the short dimensions of the plot exceeds 10 percent, distance is added or subtracted as appropriate.

All bioindicator trees suitable for monitoring and within 40 m each side of the centerline are mapped, assigned a number, classified by crown position as dominant or co-dominant, and measured for DBH. Mapping is done with compass and rangefinder, or by using GPS if suitable accuracy can be attained. Ideally, at least 50 dominant and co-dominant trees >10 cm DBH will be found within the assessment plot and comprise the population from which the trees to be evaluated for foliar ozone injury are randomly selected.

Stand-Based Plots. If a stand of trees at the site has a well–defined boundary, is less than about 2 hectares in size, and contains at least 50 acceptable candidate trees, it may be effective to map and enumerate the suitable trees in the stand and make a random selection of those to be assessed from that population.

PROTOCOL FOR MONITORING

Objectives

In a monitoring program, evaluations of trees are conducted annually to provide repeated, quantitative, long-term evaluations of the incidence of foliar ozone injury. Trees are tagged and their positions mapped to facilitate repeated observations on the same individual. Ground layer species may also be used in a monitoring program, but while herbaceous plants generally cannot be permanently marked, it is possible to obtain repeated observations from clusters of plants over time. Monitoring provides the most detailed record of the responses of plants to long-term ozone exposure.

Repeated, annual assessments are central to a monitoring program since they provide detailed information on the extent and frequency of injury, and how the interaction of exposure and the environment control the occurrence of foliar injury. Spontaneously skipping years or conducting assessments only in high exposure years will compromise the continuity and completeness of the data and reduce their value in understanding when injury occurs and how exposure and environment influence injury at the site. If it is not possible to make a commitment to conduct annual assessments, the monitoring program can be modified as follows. Prior to initiating the program, a decision is made to monitor for several sequential years, to then omit several years, and to then repeat the monitoring period. This cycle is repeated as desired. This approach provides a less complete and discontinuous set of data than obtained with annual assessments, but is better than making spontaneous annual decisions about assessments. The program should be conducted for a pre-determined number of years at which time all data are analyzed and a decision made on whether to continue. If financial, logistical and personnel constraints limit the ability to implement a monitoring program that will produce meaningful results, consideration should be given to adopting a survey program since it is more flexible and likely to be compatible with the resources available.

Selecting Bioindicator Species

Bioindicator species used in a monitoring program are generally tree species since an objective is to obtain long-term assessments of injury on the same plant. Assessing injury on trees, particularly conifers, can be a labor- and time-intensive process, and these considerations generally constrain the number of assessment plots and species employed. Ground layer bioindicator species may be included as secondary indicators, however it is generally not possible to permanently identify individual plants or obtain repeated observations on the same plant. In either case, assessment is best served by selecting species that are widely distributed and abundant within the park. It is conceivable, however, that ozone effects on a bioindicator species with limited distribution and prevalence may be a concern in some parks.

Identifying Candidate Field Sites

The protocols for identifying candidate field sites for monitoring are the same as those for a survey assessment, and the previous section on survey protocols should be consulted for information.

Evaluating the Acceptability of Candidate Field Sites

The general protocols for evaluating candidate field sites for monitoring are the same as those for a survey assessment, and the previous section on survey protocols should be consulted for information. Specific protocols for evaluating sites for suitability as ground layer and tree bioindicator plots are presented below.

Evaluating Tree Bioindicator Sites

A population of trees suitable for use is one that will permit the long-term collection of data, has crowns that are readily accessed for injury assessment, is not located in an unusual microhabitat, is not impacted by foliar insects and diseases that would confound the evaluation of ozone injury, and is readily relocated so it can be revisited on a regular basis. Accessibility is also a concern, but is secondary to making sure an adequate number of suitable plots are established within a stratum

The density, spatial distribution, and exposure of trees to ambient air and light are important to determining whether at least 50 candidate trees can be obtained at a site. To assure that the responses measured on the sampled trees are representative of trees in the larger environment, it is important that the canopies of the trees assessed are directly exposed to ozone in the ambient environment and receiving high levels of sunlight. If tree foliage can be sampled in-hand without pruning, it facilitates the assessment process and reduces possible long-term impacts from removing foliage. To allow an adequate number of sample trees to be obtained at a site, it will probably be necessary to establish a plot in a strip or belt configuration approximately 250 m long and 80 m wide that runs along the contour line. The circular and stand-based plots described in the Protocol for Surveying section may also be considered. It is important to keep the plot requirements and options in mind when evaluating the suitability of the site.

Evaluating Ground Layer Bioindicator Sites

In assessing the suitability of a candidate site for use with ground layer bioindicator species, the area within 100 meters of the point is searched for the presence of the selected species. One way to conduct the search at each site is to walk a transect 100 m long in each of the four cardinal compass directions. At the end of each transect, the survey member turns right, walks about halfway to the next transect azimuth, and returns to the starting point. The locations of populations of bioindicator species encountered along these systematic walks are recorded.

Exposure to ambient air and sunlight are important for the development of foliar injury from ozone. Thus, to assure that the responses of ground layer plants on the site reflect the response in the larger environment, it is important that the site not be beneath a dense tree canopy that can serve to take up and reduce the concentration of ozone reaching ground level and also serve to reduce the level of sunlight incident on ground layer plants. Sites in the open, on or near the edge of a forest stand, or under an open tree canopy are preferred.

Establishing Assessment Plots

Plots for Tree Bioindicator Species

The emphasis in a monitoring program on repeated evaluations over time to assess changes and trends dictates that the number of trees assessed on each plot is large enough to provide an accurate estimate of the incidence of foliar ozone injury. The procedures for establishing plots use a sample size of 30 dominant and co-dominant trees on each plot. A sample of this size is recommended to assure that an accurate estimate of injury is obtained for the plot. However, the recommendation to use a 30-tree sample can be modified by the program manager after appropriate consideration of the potential consequences of reducing the number. The number of trees assessed on each plot is established by balancing two considerations: science dictates the sample must be large enough to accurately estimate the level of foliar injury on the plot, and logistics dictates the program must be manageable with respect to the time and manpower resources available. It is the responsibility of the program manager to assure that an appropriate balance is maintained between science and logistics, and that constraints on them neither overwhelm nor undermine the successful execution of the program. If the monitoring program is not able to employ a 30-tree sample, a smaller sample may be acceptable as long as the rationale for the change is sound and documented, and there is reasonable assurance that the smaller sample will not compromise the validity of the entire monitoring program.

Trees selected for assessment should be >10 cm DBH and in the dominant or co-dominant crown classes. Trees in the intermediate and overtopped classes should not be included in the sample since they are probably not exposed to ambient concentrations of ozone or to higher levels of sunlight. Trees are generally unsuitable for assessment if they have severe mechanical wounds, an unpruneable crown (lowest branches > 10 m above ground), evidence of root disease, excessive lean, or significant foliar insect or disease injury.

A strip plot 80 m wide and 250 m long (2 hectare) is recommended for use in assessments for most stands of trees. To establish the plot, a compass direction is selected that includes a suitable number of candidate trees, is parallel to the contour of the slope, and remains within the same landform type. On flat areas it is possible to select a random compass direction. This becomes the long axis of the plot and also represents the plot’s centerline. A tape or laser rangefinder is used to measure distances and if the slope in either the long or the short dimensions of the plot exceeds 10 percent, distance is added or subtracted as appropriate.

The locations of all suitable candidate bioindicator species trees within the 250 m by 80 m (40 m each side of the centerline) plot are mapped, assigned a number, classified by crown position as dominant or co-dominant, and have their DBH measured.   Mapping is conducted using a compass and laser rangefinder, and GPS may be used if sufficient accuracy can be attained. This group of trees constitutes the population from which those to be assessed for foliar ozone injury are randomly selected.

Mapping and tallying continues along the plot centerline from the origin point until one of the following conditions is met: a population of candidate trees large enough, at least 50, to allow 30 dominant and co-dominant trees to be randomly selected for long-term monitoring in obtained; a different landform type is encountered; the transect extends out of the species population; or the transect is extended to 250 m from the origin point. If 50 dominant and co-dominant trees of the species of interest are not found, the transect may be extended beyond 250 m if the landform type does not change. If this is not possible, widening the transect or displacing it upslope or downslope may be necessary.

Ideally, 30 dominant and co-dominant trees > 10 cm DBH are randomly selected from the population within the plot and comprise the sample to be assessed for foliar ozone injury. The selections are made using a random number generator or table of random numbers.

Trees selected for injury assessment should be permanently identified with a numbered tag attached by an aluminum nail at breast height on the uphill side of the tree. If the plot is in an area that is readily accessed by the public, tags may be placed near ground level on the uphill side of the tree to be more discrete. Tree locations should be mapped as accurately as possible relative to each other and the plot centerline. A GPS or hand–held compass and a tape or laser rangefinder should be used.

If a discrete stand of trees is found on the site that is irregular in shape with well–defined boundaries, is less than about 2 hectares in size, and contains more than 50 suitable trees, it may be more efficient to sample the stand by enumerating the entire stand and mapping the stand boundaries rather than trying to fit a rectangle to nature. If such a stand is used, trees to be assessed should be selected from the population at random with the same attention to crown class as in the belt plot. A center point should be selected for the stand and the locations of the sampled trees mapped relative to the point and to each other.

Mortality of selected trees may occur in long–term monitoring. Selection of replacement trees is not recommended, unless the number of surviving trees falls below 15 or 20. At that level, the useful life of the plot may be over, and a new plot of 30 trees should be established at the site, if necessary. Data from the old plot trees should not be directly compared to data from the newly established trees because the natural tree–to–tree variability in sensitivity to air pollution is high.

Plots for Ground Layer Bioindicator Species

Ground layer bioindicator species are evaluated using circular plots established as close to the sampling site point as feasible. If the populations of plants at a site are spatially distinct and of various sizes, a population close to the site point and of suitable size is used. The approximate center of the population is found and the distance to the closest edge of the population estimated. In large or continuous populations of plants, the center of the sampling plot is established as a point within the population that is close to the site point and surrounded by plants for at least 20 m in all directions. If more than one bioindicator species is being assessed at a site, it may be necessary to establish multiple plots.

The center of the plot is permanently marked, a description of the location of the plot is written, and the azimuth and distance of the plot center relative to a reference point such as a witness tree or rock monument is recorded so the plot can be readily located in the future. The plot's location should also be established using a GPS.

Care must be exercised when assessing a ground layer species that is clonal in nature. It is important to make sure that a number of clones are examined at a site. To do this, it may be necessary to expand the size of the plot or to employ multiple smaller plots to assure that several clonal lines are assessed. As a guide in the field, clonal lines may be separated by a physical barrier such as a stream, roadway, or rock outcropping that prevents clonal spread, or may occur as groups of plants separated by habitat in which the species does not occur.

In a population of a ground layer bioindicator species, 60 plants are selected for evaluation. To select specific plants, 30 polar coordinates consisting of 30 random compass bearings and 30 random distances from the population center are used. The maximum distance from the center of the plot is set at 20 m, although the plot radius may sometimes be less than this due to the shape of the population of plants. Sets of random coordinates can be created using spreadsheet software on a personal computer before going to the field. Several sets are created for each maximum radial distance of 2, 5, 10, and 20 m from the population center. These are then printed on weatherproof paper and stored in a binder for field use. Random bearings and distances for use in selecting plants are provided in Appendix A. At each assessment plot, one of the coordinate sets for the appropriate maximum radial distance is chosen and then used to select 30 points within the population at which plants are examined. A compass and laser rangefinder are used to locate the points. The two bioindicator plants closest to each of the 30 points are selected for assessment of foliar injury thus providing a sample population of 60 plants at each plot. Each plant to be assessed is marked with a flagged wand stuck into the ground. If it is necessary to use a population in which there are fewer than 60 plants, all are examined, however the number of plants should not be fewer than 40.

For most ground layer species, a 20 m radius assessment plot will be adequate, but the radius can be increased where the density of plants is low. In cases where the margin of the population is irregular, it is sometimes necessary to use longer or shorter radial distance values for azimuths where the population ranges for greater or lesser distances, respectively, from the plot center.

Since repeated observations on the same plant are a fundamental aspect of a monitoring program, individual plants should be tagged to facilitate identification in subsequent years. Most ground layer plants cannot be permanently tagged, however species such as huckleberry, elderberry, and grape have woody, persistent parts that may be tagged to allow future identification. Some ground layer plants have crowns that persist and these may be carefully marked for identification. In some cases, sets of azimuth and distance values can be permanently adopted, or if accurate enough, GPS coordinates obtained and used annually to allow repeated measurements on plants near specific points in the assessment plot.

DOCUMENTING THE LOCATIONS OF ASSESSMENT PLOTS

It is essential that the locations of assessment plots be well documented so they can be readily and accurately relocated. Whenever possible, a plot's location is documented using a witness tree or a rock monument as a reference, and GPS coordinates obtained.

A witness tree is a large, conspicuous, or uniquely–shaped tree within or near the plot that is readily identified. The witness tree is photographed from a known point (roadside turnout, mile marker, prominent ridge top located on topographic map, etc.) to document its appearance and facilitate its relocation. The distance and compass direction from the witness tree to the plot marker is measured and recorded, and GPS coordinates obtained. The DBH of the witness tree is measured and a small aluminum plate with the plot identification and date etched on it is nailed discretely at the base on the uphill side of the tree.

If bedrock outcrops are in the vicinity of the plot, a stainless steel or brass tag can be imbedded in the rock with a concrete nail and epoxy, and the position of the rock monument referenced using GPS.

When the center point or origin of the centerline of an assessment plot is mapped relative to the witness tree or rock monument reference point, the location of the plot and its selected trees can be reconstructed for many years regardless of changes in the appearance of the vegetation. The location of a plot should also be established using GPS. A written description of how to find the witness tree or rock monument should be compiled.

EVALUATING FOLIAR OZONE INJURY

Scouting Assessment

The evaluation of foliar ozone injury in a scouting assessment is intended to simply determine whether or not ozone injury is present in the field. To achieve this objective, as many plants as feasible are examined for foliar injury, and records maintained of the number of plants examined and the number found injured.

When ozone injury is found, the species and type of injury are recorded. Data on the number of leaves injured and the surface area affected on each leaf are generally not recorded in a survey assessment. Data on the number of plants examined and the number injured allow the percent of plants injured to be calculated.

Surveying and Monitoring Assessments

Ground Layer Bioindicator Species

Assessment of foliar ozone injury involves evaluating its incidence and severity. The incidence of foliar injury is determined either by counting the number of plants observed and affected, counting the number of leaves observed and affected, or estimating the overall percent of foliage affected on a plant. Which approach is employed is a function of the objectives of the assessment, the size of the plant, the number of leaves on a plant, and the size of the leaves. If the plant has relatively few leaves (less than 50, as a rough guide) of moderate to large size, the total number of leaves and the number of injured leaves should be determined by counting. If the plant is large, has many leaves, or has small leaves that make counting impractical, incidence is assessed by estimating the percent of leaves injured using the Horsfall-Barratt Scale (Horsfall and Barratt 1945) in Table 3.

Table 3. Application of the Horsfall and Barratt Scale to estimate the percent of a plant's total leaves affected or the area of individual leaves injured by ozone.

Index	Percent Affected
1	0
2	0 to 3
3	3 to 6
4	6 to 12
5	12 to 25
6	25 to 50
7	50 to 75
8	75 to 87
9	87 to 94
10	94 to 97
11	97 to 100
12	100

(Horsfall and Barratt 1945)

It is essential that the incidence and severity of foliar ozone injury be estimated accurately and consistently. Use of the Horsfall-Barratt Scale for assessing severity of injury on individual leaves somewhat alleviates the problem since the scale is designed to work with the capabilities of natural visual acuity. It is important that the scale is used consistently and that the observer's ability to do so be developed. Formalized methods for learning to make estimates and testing accuracy and consistency are limited. It is recommended that the assessor train using the Foliar Injury Assessment Module at http://mona.psu.edu/scripts/FhWeb2.dll/intro (Nash et al. 1992, Pennsylvania State University 1992).

For each bioindicator species, either counting or estimating is selected and used consistently. Once a decision is made, the same procedure is used for that species throughout the course of the entire surveying or monitoring assessment so the units remain consistent over the duration of the program.

The severity of foliar ozone injury is determined either for individual leaves or for the overall plant, depending on the size of the leaves. If leaves are large enough so they can be readily examined individually, severity should be determined on an individual leaf basis. If leaves are too small to effectively examine, an overall estimate of the severity of injury is made after considering the entire group of affected leaves. Severity is estimated using the Horsfall-Barratt Scale in Table 3.

Leaves on each of the selected ground layer plants at a site are examined, and data collected on the nature, incidence and severity of ozone injury, and the presence of other foliar markings. For plants on which it is feasible to assess individual leaves, the total number of leaves on the plant is recorded whether ozone injury is present or not, and the number of injured leaves counted. A random sample of 20 injured leaves on each affected plant is examined to assess the nature and severity of injury. The specific 20 leaves assessed are selected randomly out of those injured using random numbers generated on a pocket calculator or a random number table. If fewer than 20 leaves are injured on a plant, each leaf is assessed. For each injured leaf examined, the type of injury is identified as chlorosis, stipple, fleck, or necrosis, and its color noted. The severity of injury on each leaf is estimated using the scale presented in Table 3.

If the number, size, or clustering of foliage make it impractical to assess ozone injury on individual leaves, overall assessments of incidence and severity are made on the plants using the Horsfall-Barratt Scale. If possible, an estimate of severity should be made for each symptom type on the foliage.

After assessing all plants, the evaluator should systematically walk through the plot looking for ozone injury symptoms on plants not included in the random sample. Observations of foliar injury made in the walk-through should be noted in the record for the plot and considered supplemental to the quantitative data obtained from the randomly selected plants.

Foliar injury or leaf markings and coloration due to biotic and other abiotic stress agents are also recorded for each of the plants, but no quantitative data are collected on these variables.

Tree Bioindicator Species

When a tree species is used as a bioindicator, sampling can be labor and time consuming, particularly if the trees are large or widely distributed. The basic approach to sampling a tree is to randomly select branches from the four cardinal aspects of the crown and to assess leaves or needles for ozone injury either on branches that remain on the tree or branches that are clipped since they cannot be reached from the ground. When possible, foliage should be assessed in-hand on branches that remain on the tree and are temporarily flagged for identification. This approach reduces the possibility of long-term effects from branch pruning, and facilitates reassessment of foliage for quality assurance purposes. If branches are to be clipped, it may be necessary to use a pole pruner to reach them. Large trees can also be sampled by climbing; however manpower, time, equipment requirements and costs for this method may be prohibitive.

Branches selected for sampling should be well exposed to light and the open atmosphere. It is important that a minimal amount of foliage be removed from the tree since repeated sampling over time may have a cumulative effect. For conifers, it is important to obtain both the current and several previous years of growth in the branch sample so injury on and retention of previous year’s needles can be assessed. For hardwoods that have indeterminate growth, it is essential that the sample include the oldest leaves of the year since they have been exposed to ambient ozone for the longest period of time and have the highest probability of developing injury.

Conifers

Assessing foliar ozone injury on conifers involves sampling open-exposed branches from the middle of the lower crown at the four cardinal aspects to obtain foliage from current and previous years of growth. Lateral branches with at least two and ideally three or more years of growth and accompanying whorls of needles should be selected from near the crown edge. The leader of major branches should not be pruned. The lowest branches on mature trees should be avoided because they may soon self-prune with age and their overall vigor may be less than others in the lower crown.

Branches with dead terminal buds, severe fungal infections, insect infestations, or injury from insects or animals should be rejected. Branches with cones or those that branch again within the selected length may be accepted, although their evaluation may be more time–consuming. Specific branches with markings that may confound the evaluation of ozone injury should be avoided if the markings are not present on the majority of branches in the lower crown.

Assessment of foliar ozone injury on conifers focuses on evaluating the number and retention of whorls of needles and the nature, incidence and severity of injury on needles on current and previous years of growth.

Years of needles —The number of annual whorls of needles retained on each branch sample is counted. This is a measure of the age of the foliage retained on the branch in years. If a whorl of needles for a particular year is missing, it should be noted on the data sheet. Whorls with short needles are included in the count.

Fascicle retention —This is a measure of the portion of the full complement of needle fascicles that remain for each year of growth. Retention is assessed using the following classes:

1 = 1–33 percent of fascicles retained
2 = 34–66 percent of fascicles retained
3 = 67–100 percent of fascicles retained

To estimate fascicle retention, abscission scars (small pits in the scaly branch surface) should be noted between the whorl nodes. On the current year of growth there will generally be no or only a few abscission scars and the retention class will be 3 (100 percent). It is not necessary to count the abscission scars and retained fascicles, and a visual estimate is sufficient.

Foliar injury —The incidence of ozone injury across all fascicles and the severity of ozone injury on the affected fascicles are assessed for each year of growth. The incidence of injury in an age-class is the number of fascicles with ozone injury compared to the total number of fascicles.

The severity of foliar injury is assessed on a random sample of 20 injured fascicles in each age-class of growth. A separate assessment of severity is made for each type of foliar injury. The most common types of foliar injury on conifers are chlorotic mottle and tip necrosis, although necrotic banding, flecking and needle chlorosis may also occur. A visual estimate is made of the needle area injured in each fascicle using the Horsfall-Barratt Scale in Table 3.

The presence of foliar injury or leaf markings and coloration due to biotic and other abiotic stress agents is also recorded for each tree. No quantitative data are collected on these variables, but photographs may be taken for documentation.

Hardwoods

Assessing foliar ozone injury on hardwoods involves sampling open-exposed branches from the middle of the lower crown at the four cardinal aspects to obtain foliage for evaluation. It is important to obtain representative branches, but care must be exercised to cut no more foliage than necessary and small trees should not be over–pruned. Lateral branches that are well foliated should be cut from near the crown edge without pruning the leader of major branches. The lowest branches on mature trees should be avoided because they may soon self-prune with age and their overall vigor may be less than others in the lower crown.

Branches with dead terminal buds, severe fungal infections, insect infestations, or injury from insects or animals should not be evaluated. Branches that are themselves branched within the selected length may be used, although their evaluation may be more time–consuming. Specific branches with markings that may confound the evaluation of ozone injury should be avoided if the markings are not present on the majority of branches in the lower crown.

All leaves on each branch are examined and data collected on the nature, incidence and severity of ozone injury and the presence of other foliar markings. At least 60 leaves should be examined on branches from each aspect of the tree. Leaves on most hardwood bioindicator species are large enough to allow collection of incidence and severity data on an individual leaf basis.

The total number of leaves examined on each aspect of the tree is recorded whether ozone injury is present or not, and the number of injured leaves counted. A random sample of 20 injured leaves on each affected aspect is examined to assess the nature and severity of injury. The specific 20 leaves are selected randomly out of those injured using random numbers generated on a pocket calculator or a random number table. If fewer than 20 leaves are injured, each leaf is assessed. For each leaf examined, the type of injury is classified as chlorosis, stipple, fleck, or necrosis, and characterized by color when appropriate. The severity of injury on each leaf is estimated using the Horsfall-Barratt Scale in Table 3.

The presence of foliar injury or leaf markings and coloration due to biotic and other abiotic stress agents is also recorded for each tree. No quantitative data are collected on these variables, but photographs may be taken for documentation.

DATA COMPILATION AND SUMMARIZATION

Scouting Assessment

Scouting provides documentation of the presence of foliar ozone injury, or the lack thereof. It provides information on the spatial distribution of injury, its incidence within the population, and its continuing occurrence over time.

A record is compiled of the presence or absence of foliar ozone injury at each plot. The type of injury found and its incidence, and the number of plants injured compared to the total number of plants examined are recorded for each plot. The plot-level data are used to provide data at higher levels of resolution such as by species, spatial strata, or the entire park.

Surveying and Monitoring Assessments

Data on foliar ozone injury are summarized for each site and for each species evaluated to reflect the nature, incidence and severity of the injury observed. The objectives of the summarizations are to characterize the annual levels of injury for each bioindicator species in each stratum, the injury for a species across strata, the average level of injury for a species within the park, the differences in injury among bioindicator species, and the changes in injury for a species over years.

The following variables may be used to provide a concise overview of the nature and level of injury for each species on a plot. The plot-level values are used to calculate values for each stratum and for the entire park.

Incidence of Injury - The variables described below are based on counts of plants assessed and plants injured, and of leaves examined and leaves injured. If whole-plant incidence is estimated using the Horsfall-Barratt index, only the number and percent of plants injured, average index for all plants, and average index for the injured plants can be calculated.

- Plot Incidence - The number and percent of plants assessed injured on a plot.

  Number of plants injured on a plot
  Percent of plants injured on a plot
  (Number of plants injured ÷ Number of plants assessed) X 100%

- Individual Affected Plant Incidence - The number and percent of leaves injured on an affected plant.

  Number of leaves injured on an affected plant.
  Percent of leaves injured on an affected plant.
  (Number of leaves injured on a plant ÷ Number of leaves assessed) X 100%

- Average Affected Plant Incidence – The average number and percent of leaves injured on all affected plants.

  Average number of leaves injured on all affected plants.
  (Sum of number of leaves injured on all affected plants ÷Number of affected plants)

  Average percent of leaves injured on all affected plants.
  (Sum of the Individual Affected Plant Incidence values in percent ÷ Number of plants injured)

- Average Plant Incidence - The average percent of leaves injured for all plants examined on a plot.

  (Total number of leaves injured on all plants ÷ Total number of leaves assessed on all plants) X 100%

Severity of Injury - The variables below are calculated by using the Horsfall-Barratt index to assess injury on individual leaves and are determined only for plants with foliar ozone injury. If the index was used to assess whole-plant injury, the individual plant values can be averaged to calculate a mean index for all affected plants.

- Individual Plant Severity - The mean index for each affected plant.

  (Sum of the indices for individual leaves ÷ Number of leaves assessed)

- Average Plant Severity - The mean index for all affected plants.

  (Sum of the Individual Plant Severity indices ÷ Number of plants injured)

- Average Plot Severity - The mean index for all plants on a plot.

  (Sum of the Average Plant Severity for all plants examined on a plot ÷ Total number of plants examined on a plot)

Summary variables may be modified depending on whether conifer, hardwood or ground layer bioindicator species are assessed. Other response variables may be calculated to address the specific interests or needs of the park.

The nature and presence of foliar injury not attributable to ozone are summarized for each species examined.

At each plot where foliar ozone injury is found, a sample of affected leaves is collected, pressed, and dried for future reference, and digital images obtained of representative affected foliage. These reference samples should be appropriately and fully documented so the location and date of harvest are readily identified. The samples should be maintained in long-term storage as documentation for the assessment, and sent to experts in the identification of ozone injury in the field for examination and validation. The protocol for archiving plant foliage is presented in the section, Training Personnel and Quality Assurance.

PROCEDURAL, EQUIPMENT AND SAFETY CONSIDERATIONS

Procedures

When possible, assessments should be performed using field crews of adequate size to allow the work to be conducted efficiently. Tasks include using a compass and laser rangefinder to locate trees or ground layer plants, obtaining GPS coordinates, marking trees, pruning branches, assessing foliar injury, recording data, and mapping plots and trees for future reference. Depending on species and plot particulars, a three- or four-person crew is required to efficiently conduct the assessments. A two-person crew is generally not effective, except for a scouting assessment.

The process of selecting ground layer plants and examining their foliage can create considerable disruption and injury in the plant community on some assessment plots. Care must be exercised to minimize these impacts and attention must be paid to their potential long-term effects when plots are repeatedly evaluated.

Branches to be assessed without pruning should be appropriately flagged and tagged so they are readily identified and can be relocated if they are to be reassessed for quality assurance proposes.

Pruned branches should be retained in shade at the base of each tree. If branches cannot be evaluated in less than 1 hour, they should be placed in labeled plastic bags and maintained in a cooler or ice chest.

A ball point pen or permanent fine–tipped marker should be used to write the number of each branch on the cut surface or, if resin is too heavy, a 1 to 2 cm strip of bark should be sliced off with a pocket knife and the branch number written on the surface of the wood. This is important for quality assurance checks and for re–checking foliage before leaving the field.

For conifers it is usually faster to observe all whorl-level variables in a sequence (e.g. number, retention, injury, etc), however beginners find it easier to concentrate on one variable and complete the estimates or measurements on all whorls first. Because this requires repeated separation or isolation of whorls, it is not efficient in terms of hand motions required. As soon as the observer is confident about the estimation or measurement of individual variables, assessment becomes most time-efficient if all variables are measured one whorl at a time.

Visual estimates do not require prolonged scrutiny, and experience has shown that the first impression is usually the most accurate. However, training is essential and use of the FIAM training module at http://mona.psu.edu/scripts/FhWeb2.dll/intro is highly recommended (Nash et al. 1992, Pennsylvania State University 1992). If working in a small crew, the observer can use an audio tape recorder to read data into and keep both hands on the branch and ruler. Data can be entered into the computer later.

Equipment

A map, compass, GPS unit, and flagging should be carried to facilitate navigation in the field. Carrying a back-up rangefinder or replacement batteries is often helpful.

Field crews should carry photographs or pressed samples of ozone-injured foliage of the bioindicator species being assessed to serve as references in the field. The crew should also have one or more large-diameter 10X hand lenses to facilitate examination of injured foliage.

A plant press should be carried to allow voucher specimens of injured foliage to be collected and preserved. The field crew should be familiar with how to properly press and conserve foliage samples; directions are provided in the Quality Assurance section of the handbook. The press must be carried to the assessment plot to assure that samples are fresh when being archived. Selected symptomatic leaves should also be photographed using a digital camera.

Data can be recorded on hand-held data loggers and/or entered by hand on paper data sheets. If data are to be stored electronically in the field, it is prudent to carry paper data sheets as backup in case of a problem with the electronic system.

Safety

The primary safety concerns are those typically associated with working outdoors and include hazards related to weather, topography, poisonous flora and fauna, and route-finding in remote areas. Tick-borne diseases, such as Lyme disease, are a significant concern and appropriate precautions must be exercised. Biting and stinging insects can also be problems, particularly for individuals with allergic reactions to them. A first aid kit should always be carried in the field, and it is advantageous to have one crew member qualified in administering first aid.

Use of a 30–ft telescoping pole–pruner can be hazardous under adverse conditions of slope, inclement weather (lightning), and fatigue. Power lines must be avoided when using a pole pruner. The pruner should be stable if it is leaning against a tree in an extended position. Back injury can result from hoisting the pole pruner from ground to vertical when fully extended. When extended or while being collapsed in a vertical position, pole segments in some designs can slide rapidly downward causing injury to fingers and hands if they are between the locking ferrules. Wearing leather gloves improves grip and may help prevent injury.

A hard hat should be worn when working with trees since dead branches, cones or the pruned branches themselves fall from the trees. Safety glasses are also recommended to prevent direct injury from a heavy falling object and also to protect eyes from the accumulation of fine debris that is dislodged from bark, leaves, and needles.

Steep slopes are more hazardous because the pruner’s attention is less focused on immediate surroundings while trying to get positioned to prune a branch. The field crew should be alert to poison ivy and oak and poisonous snakes. They should avoid working alone and always carry a hand–held radio or cellular phone so assistance can be requested if needed.

A cell phone or radio is a useful aid for safety depending on the location and geography of the park and plots. However, it is not appropriate to depend on a cell phone to resolve emergencies in the field since their utility is significantly affected by local topography. Field crews should be prepared to address most field emergencies without depending on outside assistance.

PERSONNEL TRAINING AND QUALITY ASSURANCE

It is important that field personnel be trained and competent. Identifying ozone injury in the field is both a science and an art that is mastered through experience. It is essential that a quality assurance plan be adopted and followed so the data collected are valid and can withstand scrutiny and challenge. The main elements of the plan focus on the training and use of personnel and verifying the quality of data collected in the field.

Personnel Training

The crew conducting the assessment of ozone injury will usually consist of three or four people. To the extent feasible, only one person should be assigned the responsibility for identifying and quantifying ozone injury. In addition to being able to readily identify the bioindicator species used in the assessment, this person must be familiar with the symptoms ozone produces, able to recognize mimicking symptoms, and trained to consistently estimate the incidence and severity of foliar markings.

Accurately diagnosing foliar ozone injury is a skill that is best acquired through training and experience in the field. Working with photographs, observing fresh pressed plant foliage with injury, and reviewing field diagnostic handbooks can provide an understanding of the appearance of foliar ozone injury on various species of plants and a sense of the variation in its appearance. Similar resources can also provide insight to the nature and appearance of mimicking symptoms produced by insects, pathogens, and other environmental stresses. Ultimately, it is most beneficial for the trainee to spend time in the field with a person experienced in diagnosing ozone injury on plants under field conditions. In the field, an experienced person is able to describe and demonstrate the diagnostic process used in determining whether markings are ozone-induced, point out the variation that can occur in symptom expression, and indicate the role of the micro-environment in conditioning symptom expression. In addition, there is often a degree of uncertainty associated with making a diagnosis. It is useful to have the instructor discuss his or her approach to addressing uncertainty, and instill in the trainee the understanding that making decisions about markings is frequently associated with some degree of uncertainty.

It is essential that the incidence and severity of foliar ozone injury be estimated accurately and consistently. Use of the Horsfall-Barratt Scale for assessing severity of injury on individual leaves somewhat alleviates the problem since the scale is designed to work with the capabilities of natural visual acuity. However, it is still important that the scale is used consistently and that the observer's ability to do so be developed. Formalized methods for learning to make estimates and testing accuracy and consistency are limited. It id highly recommended the assessor train using the Foliar Injury Assessment Module at http://mona.psu.edu/scripts/FhWeb2.dll/intro (Nash et al. 1992, Pennsylvania State University 1992).

All members of the crew must understand the importance of following the assessment protocols and the significance of attention to detail. Each person should be familiar with the assessment strategy and recognize how each part of the effort contributes to the success of the whole. One member of the crew should be designated its leader and have responsibility for assuring protocols are followed and for verifying that all relevant data are recorded at each assessment plot. When it is not possible to explicitly follow a protocol in the field, it is this person's responsibility to consider alternative procedures and make a sound and scientifically valid decision regarding what will be done.

Quality Assurance

Voucher specimens. Foliage voucher specimens are collected for several purposes: to document the occurrence and appearance of foliar ozone injury on a plot for future reference, and to provide foliage samples with markings identified as ozone injury or suspected of being ozone injury for examination by an expert. The procedure for obtaining and pressing foliage must be carefully followed to assure leaves are properly preserved and labeled for future identification.

At each plot where foliar ozone injury or markings suspected to be ozone injury is found, three leaves should be collected from each of several injured plants. Collections should be made for each species injured. The leaves selected should be ones that show obvious and typical injury for the plant, not ones that reflect the range of markings that is present. Once the leaves are cut from the plant, they should be immediately labeled for identification and placed into a plant press. Labels should be attached to the leaf petiole, with doubled-over tape or strings with paper tags, and be large enough to allow writing on them. Each leaf should have its own space on the blotter paper and should not overlap other leaves. Labels should identify the park, plot, plant species, plant number, and date of assessment. Leaves must be pressed immediately otherwise they will wrinkle, become brittle, and lose their value as voucher specimens. Leaves should be photographed immediately with a digital camera to capture the color and nature of the foliar injury.

Pressed leaves may be removed from the plant press after 36 to 48 hours. They should be maintained between sheets of protective paper and stored in 10”x12” envelopes that are labeled to fully identify the contents. Envelopes should be stored so they are protected and not subject to pressure or weight that may bend or shatter the pressed leaves.

Pressed foliage and digital images should be sent to an expert in the identification of foliar ozone injury for examination and validation. This is particularly important when an assessment program is first initiated and whenever there is uncertainty about the causal agent of foliar injury.

Reassess Plots. Plots are reassessed for two purposes: to evaluate the accuracy, the representation of the true level of injury on the plot, and precision, the reproducibility or consistency, of the assessment.

To assess the accuracy of the injury evaluations, 5% of the field plots are re-evaluated after the assessments of all plots are completed. The plots to be re-evaluated are selected at random, and a full assessment of the bioindicator species on each plot is performed using different ground layer plants and new branch samples from the tagged trees. The resulting data provides insight to the variation in the level of injury on a plot.

The reassessment to evaluate the precision of the injury evaluation process is conducted immediately after the initial assessment of a plot and uses the same individual ground layer plants and branch samples as the initial assessment. Under high temperatures, leaves on branches that have been cut may deteriorate significantly before they can be reassessed. Under these circumstances, foliage should remain on the tree and be evaluated in-hand whenever possible, and the reassessment of cut foliage conducted as soon as possible to assure its integrity is maintained. Five percent of the plots are reassessed in this manner. Differences in the data obtained in the two assessments are measures of the precision of the evaluation process.

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APPENDIX A

SETS OF RANDOM AZIMUTHS AND DISTANCES FOR LOCATING POINTS ON ASSESSMENT PLOTS

SET 1

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
014	1.5	3.6	7.3	10.3	15.1
019	1.9	4.3	8.3	13.8	17.8
035	1.1	2.0	5.8	8.1	11.4
043	1.0	2.2	5.5	6.6	9.7
088	1.7	5.0	8.3	13.2	16.7
089	1.4	3.4	6.8	10.1	12.4
095	1.1	2.5	4.8	06.7	10.4
097	0.5	1.3	4.0	4.3	4.9
107	2.0	4.2	9.0	13.0	17.5
116	0.5	1.6	2.2	3.4	5.2
117	1.7	4.9	8.9	14.6	17.0
118	0.4	1.3	2.2	6.0	5.6
118	1.9	4.4	9.8	13.9	16.2
159	1.3	3.9	6.3	10.4	14.0
173	1.7	4.1	9.9	13.3	16.8
183	0.9	2.9	4.4	7.1	10.2
186	1.8	4.6	8.3	12.5	19.0
191	0.1	0.7	0.9	3.0	0.6
202	1.3	3.8	6.7	10.5	12.7
212	1.5	3.5	7.2	9.3	13.2
238	1.4	3.5	6.1	10.5	13.2
242	1.8	4.4	9.6	12.2	16.3
256	1.8	4.4	9.9	14.4	18.6
264	1.2	2.7	5.5	6.3	11.7
296	0.5	1.7	2.8	3.9	4.4
301	1.5	3.1	6.2	9.2	15.6
308	1.9	4.8	9.6	14.5	16.5
322	1.2	2.5	5.2	6.9	9.4
326	1.7	4.1	9.8	13.2	18.2
345	1.4	3.3	6.1	11.7	15.8

SET 2

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
039	1.2	3.4	7.3	10.4	15.9
042	0.0	0.1	1.5	2.0	2.8
048	0.7	1.0	3.0	3.3	4.7
052	0.7	1.8	2.6	3.4	6.4
065	1.1	2.9	4.9	7.4	9.1
068	1.6	4.3	8.6	12.8	16.1
076	1.5	3.7	7.5	9.9	15.8
104	1.6	3.9	6.4	10.7	14.5
112	1.8	4.3	9.0	12.1	17.9
130	1.7	4.5	8.9	12.1	18.1
131	1.8	4.5	8.7	14.0	16.2
149	0.8	2.3	5.3	7.2	10.2
149	1.4	3.5	7.5	10.5	12.5
171	1.9	4.2	8.1	13.1	17.1
185	0.8	2.8	4.7	8.1	11.9
186	1.7	4.3	8.9	12.7	19.0
260	1.8	5.0	9.0	14.7	16.1
270	0.4	1.1	3.3	3.4	4.3
277	2.0	5.0	9.8	12.6	17.4
284	1.5	3.3	7.9	10.1	15.6
291	1.8	5.0	9.0	13.2	17.1
303	1.0	2.9	5.4	6.3	8.3
317	0.8	3.0	4.2	6.4	11.9
321	1.5	3.4	6.3	9.9	12.5
321	1.3	3.4	7.9	10.8	14.6
329	1.8	4.7	9.5	14.1	17.0
336	1.2	2.4	4.3	8.6	10.8
341	1.7	4.4	8.7	14.1	16.9
348	1.5	3.1	6.0	10.9	15.1
350	0.5	1.3	2.0	4.7	4.9

SET 3

30 RANDOM POINTS

RANDOM	RANDOM DISTANCES				
AZIMUTH	2 m	5 m	10 m	15 m	20 m
006	0.5	1.6	3.9	5.2	6.2
006	1.9	4.8	8.0	12.2	18.2
033	2.0	4.8	8.2	13.6	16.2
042	2.0	4.4	8.3	14.5	19.6
048	1.5	3.9	6.9	9.2	12.1
063	1.1	2.0	5.2	6.3	11.2
065	1.5	3.7	6.9	9.1	15.1
072	1.9	4.4	9.4	12.6	18.4
084	0.6	1.6	2.2	4.1	7.5
095	1.0	2.5	4.7	7.4	10.7
102	1.5	3.8	6.5	9.2	13.1
112	0.1	0.5	0.7	1.9	3.9
157	0.5	1.0	2.3	4.6	6.1
160	1.5	3.8	7.5	11.4	12.7
169	1.4	3.4	7.9	9.9	13.6
174	0.5	1.4	2.7	5.2	5.5
180	1.2	3.2	7.6	9.4	15.8
186	1.7	4.8	9.4	14.8	16.0
195	1.8	4.5	8.4	14.5	17.5
200	1.3	3.5	7.7	9.5	14.3
250	1.1	2.7	4.9	7.2	9.7
257	1.0	2.8	5.1	7.3	11.1
257	1.9	4.0	8.0	14.6	18.5
258	1.1	2.8	4.4	8.6	12.0
263	1.0	2.1	4.7	6.1	10.4
265	1.9	4.1	9.8	13.7	19.5
307	1.9	4.8	9.3	12.7	19.8
313	1.9	4.9	8.3	12.9	17.2
316	1.7	4.8	8.5	13.7	17.7
333	1.3	3.1	6.3	11.4	13.1

SET 4

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
007	1.9	4.7	9.9	12.6	17.6
014	1.0	2.6	4.9	8.0	9.7
027	1.3	3.3	6.8	9.4	12.5
029	1.6	4.5	9.3	14.3	17.6
034	1.1	2.2	5.9	8.8	8.2
072	1.6	4.5	8.0	13.9	17.2
093	1.5	3.2	7.3	9.1	13.3
118	1.6	3.0	6.0	11.2	15.4
132	1.8	4.4	9.5	14.2	18.2
144	1.7	4.1	9.1	15.0	16.6
162	1.4	3.2	8.0	10.8	14.5
200	1.0	2.0	5.5	7.5	9.9
204	0.4	1.0	3.0	4.5	7.6
223	0.8	1.2	3.9	4.6	6.5
233	1.4	3.5	7.9	10.7	15.9
251	0.6	1.8	3.6	4.3	4.1
252	1.7	4.9	9.2	14.2	18.7
254	1.4	3.8	7.1	9.0	12.5
257	1.0	2.6	5.6	6.1	9.3
266	1.1	3.0	6.0	8.6	8.2
283	0.1	0.3	1.2	0.3	2.5
294	1.7	4.8	8.2	12.8	16.5
296	0.7	1.4	3.5	5.0	4.0
298	1.8	4.2	9.4	12.2	17.9
321	1.1	2.7	5.2	6.8	10.0
331	0.6	3.7	6.9	9.9	15.7
332	1.6	4.8	9.1	14.5	19.6
333	1.9	4.7	8.7	13.1	18.3
336	1.7	4.9	9.2	12.4	18.3
344	1.5	3.8	6.8	11.9	13.1

SET 5

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	5 m	20 m
006	1.2	2.5	4.9	7.1	9.5
040	1.0	2.3	4.1	8.3	10.2
041	0.9	2.9	5.3	9.0	10.6
061	2.0	4.6	9.1	12.2	18.3
084	1.0	2.4	5.4	6.2	9.7
091	1.2	4.0	6.9	10.1	12.2
102	1.3	3.0	6.9	11.2	14.3
123	1.5	3.5	6.9	10.2	13.4
146	0.7	1.6	2.1	3.8	6.0
164	1.9	4.3	9.5	13.8	16.6
165	1.2	2.6	5.3	9.0	8.9
174	1.8	4.4	8.2	14.0	18.7
184	0.9	2.7	4.3	7.6	8.1
188	1.2	3.2	6.9	11.9	15.0
195	0.7	1.1	3.9	5.1	7.8
204	1.8	5.0	8.7	14.4	16.9
206	1.4	3.7	7.0	10.6	12.5
207	0.6	1.6	2.3	5.4	7.4
208	1.7	4.3	9.3	14.4	19.9
242	1.4	3.7	6.7	9.7	14.6
242	1.9	4.7	9.1	13.9	17.9
261	1.7	4.3	8.8	14.1	18.5
283	0.1	0.3	0.3	1.6	0.9
288	0.5	1.4	2.8	3.0	5.4
294	2.0	4.1	9.7	14.3	19.4
300	1.2	3.7	6.1	11.9	14.5
322	1.6	4.4	9.3	14.5	16.3
329	1.5	3.8	7.3	10.1	12.4
330	1.9	4.3	10.0	13.4	18.7
339	2.0	4.4	8.2	14.9	19.0

SET 6

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
007	0.2	0.5	0.4	2.9	0.1
011	1.6	3.9	6.5	11.2	12.8
027	1.6	3.4	7.4	9.9	13.4
031	1.9	4.3	8.9	13.7	16.8
040	0.9	2.9	5.3	6.1	10.9
044	1.3	3.6	6.2	11.2	12.5
055	1.4	3.5	7.4	9.3	12.9
083	1.8	4.8	9.7	14.7	17.7
084	2.0	4.4	9.6	14.4	19.9
096	0.5	1.4	2.8	5.9	6.1
104	1.7	4.8	8.9	14.6	16.7
111	0.9	2.5	5.9	8.8	11.8
132	1.7	4.1	9.8	12.2	18.8
139	1.7	4.6	8.4	13.2	18.8
163	1.6	5.0	9.2	14.7	18.2
198	0.8	2.4	4.7	8.3	10.2
200	1.5	3.0	6.0	9.5	13.6
202	1.7	4.4	8.7	13.8	19.7
207	1.3	3.6	7.7	10.1	15.5
216	1.9	4.9	9.8	14.8	20.0
217	0.7	1.8	2.0	4.0	5.7
221	1.5	3.7	7.4	11.4	14.6
230	1.6	3.7	6.1	9.0	12.9
237	1.8	4.1	8.1	13.0	19.6
258	0.8	1.8	2.8	6.0	4.1
267	0.7	1.8	3.3	3.4	7.4
321	1.2	2.7	5.6	7.7	11.2
337	1.0	2.8	5.3	8.6	11.5
346	2.0	4.6	9.4	14.1	16.7
356	0.9	2.6	4.1	7.5	9.3

SET 7

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
005	1.8	4.7	9.8	14.6	17.1
010	1.0	2.5	5.8	7.2	10.9
010	1.8	4.9	9.7	13.1	20.0
036	1.9	4.1	8.1	13.1	18.5
081	0.8	2.9	5.2	8.9	8.3
098	1.3	3.1	7.7	10.6	14.1
104	1.6	3.9	7.7	10.7	13.4
109	1.1	3.0	5.1	6.7	11.3
129	1.3	3.5	7.3	10.6	15.3
137	0.8	3.0	4.1	7.4	11.3
146	1.3	3.8	8.0	9.7	16.0
165	1.3	3.6	6.3	10.3	13.0
168	0.3	0.3	1.6	1.3	0.6
172	1.7	4.6	9.2	15.0	19.4
182	1.9	4.8	9.3	12.4	17.9
186	0.8	1.6	3.2	3.3	5.4
230	1.5	3.5	7.8	10.8	14.7
236	0.9	2.1	4.5	7.0	8.2
251	1.5	3.4	6.4	11.6	14.3
259	1.9	4.7	9.1	13.4	18.1
265	0.7	1.2	2.8	5.0	6.3
276	0.8	1.8	3.5	4.9	7.3
276	1.0	2.1	4.8	8.7	10.9
281	0.5	1.3	3.2	5.0	5.5
288	1.6	3.7	7.9	9.8	14.7
300	2.0	4.4	9.3	14.7	18.6
309	1.9	4.8	9.8	13.2	19.5
316	1.8	4.2	8.6	13.4	19.2
350	1.7	4.5	9.7	12.7	19.9
355	1.9	4.2	9.0	12.6	17.7

SET 8

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
006	0.7	1.7	3.7	5.5	6.4
013	1.2	2.4	5.5	7.6	11.2
013	1.7	4.3	8.4	12.5	17.1
026	1.9	4.9	8.7	14.3	17.3
048	1.2	3.8	7.5	11.8	12.3
083	0.4	1.5	2.7	5.7	6.8
100	1.3	3.9	6.5	10.7	13.4
104	0.2	0.2	0.5	1.8	0.1
106	1.4	3.4	6.4	10.5	13.2
107	1.5	3.9	7.0	11.0	12.3
112	1.7	4.3	8.9	13.9	19.1
145	1.6	3.5	6.9	11.5	12.2
166	1.0	3.0	5.4	6.7	10.2
182	1.6	3.9	7.8	11.1	15.0
191	1.4	3.6	7.1	9.3	12.3
195	2.0	4.9	8.7	13.0	17.7
198	1.8	4.8	9.7	13.8	18.3
204	0.9	2.7	4.3	7.1	9.1
225	0.8	2.6	5.4	6.5	9.6
242	0.9	2.5	5.8	7.0	11.4
248	1.7	4.9	8.2	13.7	18.1
279	1.8	4.1	9.5	14.2	19.4
282	1.4	3.9	7.6	11.1	13.2
284	1.9	4.0	8.9	13.2	17.3
285	0.5	1.2	3.8	4.2	6.2
297	0.7	1.5	2.7	5.1	5.8
309	1.1	2.8	5.9	6.4	10.4
341	1.8	4.6	8.3	12.4	19.6
346	1.8	4.9	9.7	12.2	16.3
359	1.7	5.0	8.1	13.1	16.6

SET 9

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
014	1.7	4.1	9.0	12.2	17.6
044	1.2	3.1	7.9	10.9	14.3
081	2.0	4.5	8.1	14.2	17.8
082	0.8	2.9	5.5	7.4	8.4
090	1.8	4.5	9.4	14.5	17.5
093	1.1	2.7	4.1	6.3	11.8
096	1.8	4.7	9.4	12.7	19.5
103	1.8	4.8	9.0	12.7	16.9
107	0.7	1.2	2.1	4.8	5.3
107	1.4	3.4	7.6	11.0	12.3
128	1.0	2.3	4.7	7.7	9.9
133	1.4	3.6	6.1	9.5	13.3
133	1.5	3.1	7.7	10.2	13.3
157	1.7	4.7	8.4	13.4	19.2
165	1.1	2.9	4.5	7.4	9.2
181	1.9	4.2	8.7	14.1	20.0
185	1.8	5.0	9.9	14.2	19.8
187	0.6	1.6	3.6	5.8	4.4
218	0.5	1.4	2.9	3.0	6.1
220	0.9	2.4	5.4	8.7	11.2
227	0.5	1.8	2.4	4.4	7.5
231	1.4	3.9	7.0	12.0	12.4
241	1.3	3.6	7.4	10.2	12.4
282	1.7	4.1	9.7	13.8	19.6
304	1.7	4.2	8.1	14.3	17.3
309	1.2	3.8	6.7	10.9	12.1
320	1.1	2.4	4.2	6.9	9.7
325	1.3	3.8	7.7	9.6	15.8
347	0.3	0.5	1.3	0.4	2.0
356	1.8	4.9	8.6	14.2	18.8

SET 10

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
006	1.2	2.4	5.9	7.9	8.4
078	1.4	3.5	7.6	11.4	12.5
090	0.9	2.3	5.9	6.3	10.8
108	1.9	4.8	8.8	14.1	17.6
115	1.5	3.8	6.6	9.8	14.0
127	1.0	2.2	5.1	8.3	10.3
128	1.5	3.7	6.9	10.2	13.9
134	1.9	4.4	8.3	12.8	17.5
156	1.7	4.5	8.4	12.7	17.2
164	0.2	0.2	1.9	0.0	3.8
165	1.7	4.6	8.5	14.1	17.2
172	0.8	1.7	3.6	5.1	6.3
174	1.7	4.8	9.2	13.0	19.3
183	2.0	4.3	9.2	13.8	17.1
213	1.9	4.9	8.3	12.7	19.2
225	1.1	2.9	5.7	8.6	10.2
255	1.0	2.5	4.0	8.0	9.6
257	1.5	3.9	6.3	10.2	15.0
257	1.3	3.6	7.8	9.1	12.1
258	0.6	1.4	3.0	3.1	6.7
264	1.9	4.5	9.7	15.0	19.4
287	0.6	1.7	3.3	4.0	6.6
296	1.4	3.6	7.6	9.9	12.4
297	1.0	2.5	4.5	6.9	10.8
320	1.5	3.6	6.9	11.8	14.0
332	1.8	5.0	9.3	13.4	17.8
334	1.3	3.1	7.6	10.1	12.6
334	1.7	4.9	9.2	12.6	18.4
339	0.4	1.3	2.3	3.5	6.2
355	1.7	4.5	9.4	12.8	18.3

SET 11

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
022	1.5	3.9	6.1	9.4	13.3
029	1.3	3.5	7.8	10.2	12.5
034	1.8	4.8	8.1	12.8	16.3
040	1.9	4.7	8.8	14.0	19.3
043	1.6	4.1	8.3	13.0	17.8
060	1.6	4.9	9.3	14.1	17.6
075	0.6	1.5	3.8	4.7	5.1
079	0.5	1.7	3.2	4.0	5.6
092	1.7	4.2	8.8	13.5	17.6
112	1.0	2.4	5.8	7.3	8.6
118	1.5	3.1	7.7	9.2	13.2
128	0.3	0.2	1.2	2.3	0.8
149	1.7	4.6	9.8	13.5	19.0
161	0.7	1.6	2.6	5.0	5.4
165	1.7	4.2	9.3	12.7	19.6
166	1.6	3.3	7.4	12.0	12.7
178	2.0	4.7	8.6	12.1	16.6
183	1.1	2.1	4.3	8.0	10.1
196	1.7	4.3	9.5	13.4	19.6
224	1.0	2.6	5.1	6.3	9.1
239	1.3	3.1	6.4	9.3	14.9
274	1.1	2.0	5.6	8.9	9.1
288	1.5	3.2	7.9	11.4	15.2
295	1.0	2.5	5.0	6.2	10.6
305	1.8	4.3	8.4	12.2	19.3
313	0.6	1.7	2.9	5.5	7.4
314	1.7	4.9	8.0	13.4	19.2
322	1.3	3.7	6.2	10.4	12.5
344	1.2	2.6	5.3	7.4	11.4
349	1.4	3.6	7.7	11.1	13.3

SET 12

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
009	1.8	4.1	8.8	14.5	17.8
013	1.0	2.4	5.2	7.5	10.1
015	0.5	1.9	2.9	5.4	4.9
026	1.9	4.9	9.3	13.9	18.2
029	1.7	4.3	9.7	13.2	17.7
039	1.5	3.1	6.4	11.5	13.5
050	1.7	4.8	9.2	14.8	16.4
061	1.6	3.6	7.0	10.8	13.6
070	1.6	4.6	9.3	12.4	16.2
073	1.8	4.9	9.3	12.5	18.0
111	1.8	4.8	9.6	12.8	18.9
114	1.5	3.5	6.9	11.8	15.3
123	0.8	1.3	2.3	4.7	4.6
124	0.2	0.7	0.5	2.1	2.3
127	0.5	1.9	2.1	4.8	5.9
129	1.7	4.9	8.0	12.5	19.3
136	1.3	3.8	6.1	10.9	15.1
153	0.5	1.1	2.8	5.8	4.8
186	1.2	3.8	7.5	11.2	13.9
251	1.0	2.7	4.6	8.0	8.6
270	0.9	2.1	4.5	8.6	9.2
289	1.2	2.0	4.5	6.7	10.0
310	1.1	3.0	4.9	6.3	8.2
313	1.7	4.5	9.9	13.7	17.5
314	1.5	3.2	6.2	9.4	12.7
318	1.8	4.7	8.5	15.0	18.5
324	1.0	2.2	4.3	6.3	11.3
328	1.6	3.3	7.9	10.0	12.3
335	1.7	4.8	9.3	12.1	19.2
338	1.2	3.4	7.3	10.7	12.4

SET 13

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
004	1.7	4.2	8.7	14.7	19.7
005	2.0	4.8	8.1	12.1	18.6
007	1.7	4.3	8.7	13.0	19.9
014	1.8	4.6	8.6	14.4	19.0
016	1.1	2.3	5.0	7.7	9.0
019	1.4	3.3	6.9	11.0	12.3
025	1.8	4.6	9.4	13.8	16.3
030	0.7	2.0	2.3	5.7	7.2
041	0.7	1.9	2.4	3.4	6.9
043	0.9	2.0	4.1	8.1	10.2
044	0.6	1.6	3.8	3.2	4.5
061	0.2	0.8	0.9	2.5	0.6
093	1.8	4.2	8.2	14.8	16.4
095	1.8	4.9	8.6	12.8	18.5
096	1.3	3.8	7.2	11.9	15.5
107	1.5	3.3	7.4	9.9	12.0
110	1.5	3.6	7.7	10.6	14.0
113	1.6	4.5	9.1	12.6	19.9
136	1.0	2.0	5.1	8.8	9.3
138	1.8	4.1	8.5	14.4	18.4
145	1.7	4.0	9.3	13.2	19.7
204	2.0	4.2	8.1	14.1	18.5
216	0.8	2.7	5.4	7.9	11.3
220	1.4	3.2	7.1	9.8	12.6
230	1.6	3.5	7.5	9.5	14.3
252	1.4	3.8	6.8	10.3	13.8
268	1.2	3.9	7.6	10.2	15.8
338	0.9	2.6	6.0	6.1	9.0
339	0.6	1.3	2.5	4.2	4.3
354	0.9	2.9	5.6	7.1	11.7

SET 14

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
003	1.1	3.0	4.9	6.4	10.9
004	1.9	4.6	8.6	13.1	17.3
013	1.9	4.5	8.9	12.4	16.2
019	1.9	4.2	8.2	13.2	19.9
025	1.8	4.7	8.2	12.4	19.4
051	0.6	1.9	3.3	4.8	5.3
066	1.9	4.8	8.6	12.6	17.6
095	1.7	4.4	8.6	14.6	16.6
104	0.7	1.6	3.1	5.4	6.5
108	1.1	2.2	4.1	7.7	8.9
111	1.7	4.6	8.6	12.0	19.4
121	1.7	4.5	8.9	13.6	17.9
141	1.3	3.1	6.3	9.0	14.8
143	1.4	3.0	7.1	11.7	15.2
167	0.8	2.4	5.5	7.6	10.5
212	1.9	4.5	9.6	14.0	17.0
218	1.3	3.1	6.7	10.1	13.8
219	0.7	1.1	3.7	5.8	5.5
224	1.1	2.0	5.2	8.3	11.3
227	1.5	3.6	6.7	9.2	12.0
230	1.4	3.6	6.4	9.5	12.1
231	1.6	3.0	6.5	11.7	12.5
242	1.6	5.0	9.0	14.4	19.0
243	0.6	1.8	3.2	5.7	7.4
257	0.3	0.8	0.9	0.8	1.0
278	1.5	3.2	6.3	11.7	15.3
306	1.0	2.3	5.3	7.1	8.2
312	1.8	4.7	9.2	13.7	18.6
325	1.6	3.2	7.0	10.5	12.1
343	0.9	2.4	4.6	6.8	11.6

SET 15

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
005	1.8	5.0	9.3	14.6	16.3
008	1.0	2.6	5.6	7.9	8.3
019	0.1	0.2	1.7	1.9	3.8
023	0.5	1.2	3.2	4.3	6.2
027	1.4	3.7	6.9	10.5	12.9
065	1.7	4.2	9.4	12.5	18.1
104	1.5	3.1	7.8	10.7	15.1
160	1.0	2.1	5.9	6.7	10.0
160	1.6	3.5	7.0	10.9	13.5
184	1.6	4.4	9.3	12.5	17.5
190	1.4	3.6	6.4	11.6	13.4
196	1.9	4.1	9.0	14.9	16.1
200	1.1	2.6	6.0	8.9	10.4
213	1.9	4.3	8.9	13.2	19.4
221	1.2	2.5	4.8	8.4	11.7
244	1.7	4.4	9.3	14.5	20.0
247	1.1	2.2	4.1	7.5	11.4
282	0.5	1.7	3.1	5.4	6.5
285	2.0	4.6	8.4	13.3	16.3
293	0.8	1.9	3.3	4.8	5.8
297	1.4	3.3	7.6	9.7	13.8
303	1.6	4.3	9.5	13.2	17.7
317	2.0	4.0	9.0	14.4	16.7
320	0.8	2.9	5.4	7.9	11.6
329	0.7	1.7	3.3	4.8	7.1
333	1.5	3.1	6.2	9.1	12.5
352	1.3	3.2	7.9	9.3	12.2
355	1.9	4.7	8.4	14.1	18.9
356	1.6	4.3	8.9	12.1	17.4
358	1.2	3.1	6.0	11.1	14.6

SET 16

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
007	1.8	4.8	9.0	13.1	18.8
011	1.5	3.7	6.7	11.8	12.1
019	1.9	4.9	8.6	14.7	18.0
020	1.3	3.8	6.5	9.4	12.6
021	0.8	1.2	3.5	4.7	5.7
027	1.5	3.1	7.8	9.2	12.3
030	1.7	4.5	9.9	13.2	17.1
069	0.6	1.4	3.4	3.7	6.6
075	1.7	4.9	8.6	13.9	19.6
079	1.6	3.9	7.0	11.7	13.2
085	2.0	4.1	8.6	12.9	19.8
107	0.9	2.9	4.1	8.1	9.6
127	0.6	1.9	2.8	5.9	7.1
133	1.4	3.3	6.7	11.8	12.7
136	0.5	2.0	2.6	5.3	6.6
138	1.0	2.7	5.9	8.6	10.0
146	1.1	2.4	4.8	7.0	9.5
156	2.0	4.0	8.1	13.5	17.2
171	1.8	4.1	9.5	14.1	19.9
192	1.7	4.9	8.0	12.7	19.3
208	2.0	4.4	9.5	14.4	17.6
220	0.8	2.6	5.4	8.3	9.2
227	1.6	3.8	7.5	9.7	15.9
252	1.0	2.3	4.6	7.7	9.8
262	1.9	4.9	9.2	12.2	17.9
265	1.5	3.9	6.5	9.4	14.2
281	1.3	3.5	6.4	9.9	14.3
295	1.0	2.4	4.8	6.2	9.6
309	1.7	4.7	8.8	14.8	18.5
323	0.4	0.4	0.8	0.4	2.6

SET 17

30 RANDOM POINTS

RANDOM	RANDOM DISTANCES				
AZIMUTH	2 m	5 m	10 m	15 m	20 m
0	1.4	3.1	7.8	10.7	13.8
3	1.1	2.4	5.4	8.9	8.1
9	1.9	4.6	8.7	13.7	16.5
23	0.7	1.9	3.9	3.8	4.8
24	1.4	3.4	7.8	10.1	15.0
51	1.5	3.0	6.5	12.0	14.4
79	1.8	4.4	9.5	13.3	16.6
88	1.9	4.9	9.8	13.9	18.5
96	1.2	3.1	6.5	10.6	13.5
104	1.9	4.4	8.5	12.3	16.6
111	1.6	4.4	9.2	12.8	16.6
125	1.0	2.8	4.6	8.6	9.1
128	1.3	3.1	6.3	9.2	16.0
137	0.7	1.8	2.3	5.9	7.7
155	1.9	4.7	9.1	13.2	19.1
158	2.0	4.4	9.9	12.6	19.0
169	0.0	0.1	1.2	2.2	0.5
211	0.9	2.1	4.5	8.5	10.9
248	2.0	4.0	9.9	12.6	18.5
261	1.8	4.4	9.3	13.8	17.2
265	0.5	1.2	2.5	5.7	7.1
266	1.4	3.8	7.3	9.7	15.3
286	1.3	4.0	7.1	11.3	14.1
288	0.7	1.8	3.7	4.9	7.8
292	1.6	4.8	8.4	12.2	18.2
312	1.0	2.6	5.9	6.1	8.6
312	1.3	3.1	6.9	10.6	12.8
315	0.9	2.4	4.4	8.4	9.5
329	1.9	4.5	8.2	14.2	16.9
345	0.9	2.8	5.7	8.0	11.2

SET 18

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
006	1.9	4.6	8.2	13.9	19.2
010	0.8	1.4	3.5	4.3	6.1
010	1.7	4.7	9.5	15.0	18.8
017	1.9	4.2	9.2	13.8	19.6
028	0.6	1.2	3.5	6.0	4.8
040	1.4	3.1	7.5	11.3	14.2
047	0.9	2.1	5.9	8.3	8.2
069	0.3	0.9	1.0	0.9	1.5
078	1.3	3.3	7.8	10.3	15.3
096	0.8	2.6	4.5	7.6	9.9
096	1.4	3.1	7.1	9.9	13.9
162	1.1	2.7	5.0	6.8	9.5
165	1.4	3.5	7.5	11.6	15.8
175	1.7	4.8	9.7	13.7	19.2
190	1.8	4.9	9.2	13.2	16.6
191	2.0	4.7	8.4	12.5	18.2
195	1.2	2.3	4.2	7.6	11.3
206	0.5	1.9	3.3	3.6	4.6
247	1.7	4.2	9.8	13.8	19.7
248	1.3	3.6	7.5	11.1	14.9
250	1.7	4.3	8.5	12.9	18.7
251	1.3	3.6	6.6	9.3	13.4
260	1.4	3.7	6.1	9.9	14.0
280	1.7	4.9	8.3	12.9	18.8
286	0.6	1.6	2.6	5.9	5.3
307	1.4	3.6	6.6	10.1	12.6
318	1.0	2.5	5.2	8.5	9.3
322	1.2	2.8	5.6	6.6	9.1
324	1.6	4.6	8.3	12.5	17.6
340	1.6	5.0	9.0	14.2	16.2

SET 19

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
007	0.9	2.4	4.7	6.9	11.3
030	1.9	4.7	9.8	13.3	19.0
052	1.7	4.2	8.3	12.7	19.5
061	1.1	2.3	4.8	6.1	9.7
075	1.8	4.9	8.3	12.1	18.2
082	0.5	1.7	2.1	5.3	5.2
084	1.6	4.3	9.1	13.2	19.3
096	1.0	2.5	5.3	8.3	11.1
103	1.3	3.3	6.3	11.6	13.5
113	0.5	1.6	3.6	5.8	4.4
117	1.0	2.2	4.8	6.8	10.2
118	1.2	2.1	5.0	6.7	11.7
118	1.8	4.6	9.9	12.4	17.6
120	0.6	1.4	2.1	3.5	6.0
133	1.7	4.1	8.0	12.7	17.0
180	1.8	4.5	9.2	12.8	19.1
180	1.9	4.6	9.3	13.8	16.8
186	1.7	5.0	9.6	13.0	18.9
197	1.3	3.5	7.3	9.0	14.0
215	1.8	4.7	8.6	12.8	16.7
229	0.8	2.4	5.0	8.3	9.3
232	1.5	3.3	7.5	10.3	14.7
242	1.5	3.7	7.3	9.0	14.0
249	0.7	1.4	3.4	4.1	6.6
261	1.5	3.1	7.7	11.8	13.8
271	1.2	3.8	7.1	9.8	14.3
283	1.5	3.4	7.6	9.1	14.3
343	0.4	0.0	1.3	1.0	3.5
347	1.5	3.7	6.4	9.2	14.2
358	1.8	4.7	9.6	14.5	19.0

SET 20

30 RANDOM POINTS

RANDOM AZIMUTH	RANDOM DISTANCES 2 m	5 m	10 m	15 m	20 m
003	2.0	4.5	9.1	14.7	18.3
009	1.5	3.3	6.8	11.4	13.0
029	1.3	3.1	7.7	10.0	15.7
030	1.5	3.3	6.2	10.3	13.6
041	1.7	4.7	8.4	14.8	17.5
053	2.0	4.6	8.7	13.8	16.2
069	1.8	4.7	10.0	12.5	18.6
089	1.3	3.6	6.4	10.1	14.6
093	0.5	1.5	4.0	4.2	5.3
101	0.4	1.9	3.2	3.5	7.3
103	0.8	1.1	2.8	5.5	6.6
125	1.0	2.4	4.9	8.7	11.8
128	1.5	3.3	7.6	12.0	14.1
128	2.0	4.2	8.6	13.7	16.3
135	1.5	3.7	6.1	9.6	14.7
151	0.6	1.4	3.8	4.3	5.1
152	1.1	2.8	4.0	8.1	10.7
155	1.6	4.4	8.2	12.5	16.2
161	1.2	3.8	7.4	10.8	12.6
162	1.1	2.4	5.3	7.8	8.7
188	0.9	2.2	4.6	8.1	11.7
189	0.2	1.0	1.4	1.9	1.6
194	1.7	4.6	8.3	13.8	18.6
196	1.6	4.8	9.2	14.7	19.5
204	1.7	4.8	9.1	12.0	17.8
211	1.6	3.6	6.5	9.6	12.7
239	1.2	2.5	5.1	7.8	11.5
260	0.9	2.7	4.0	6.9	11.7
351	1.6	4.4	9.1	13.5	19.8
359	1.9	4.7	9.3	12.3	19.3