

**Section 9. Ozone Bioindicator Plants (East)**

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*Indicator regionally revised April 2004.*

## 9.1 OVERVIEW

Air pollutants, such as ground-level ozone, are known to interact with forest ecosystems. Ozone is the only regional gaseous air pollutant that is frequently measured at known phytotoxic levels (Cleveland and Graedel 1979; Lefohn and Pinkerton 1988). Ozone pollution has been shown to have an adverse effect on tree growth and alter tree succession, species composition, and pest interactions (Forest Health and Ozone 1987; Miller and Millecan 1971; Smith 1974). In addition, we know that ozone causes direct foliar injury to many species (Skelly and others 1987; Treshow and Stewart 1973). We can use this visible injury response to detect and monitor ozone stress in the forest environment. This approach is known as biomonitoring and the plant species used are known as bioindicators (Manning and Feder 1980). Ozone bioindicator plants are used to monitor changes in air quality across a region, and to assess the relationship between ozone air quality and Phase 2 and Phase 3 indicators of forest condition (e.g., growth increment and dieback).

A useful bioindicator plant may be a tree, a woody shrub, or a nonwoody herb species. The essential characteristic is that the species respond to ambient levels of ozone pollution with distinct visible foliar symptoms that are easy to diagnose. Field studies and/or fumigation experiments have identified ozone sensitive species and characterized the ozone specific foliar response for both eastern (Davis and Umbach 1981; Duchelle and Skelly 1981; Krupa and Manning 1988) and western (Richards and others 1968; Mavity and others 1995; Brace 1996) bioindicators. Foliar injury symptoms include distinct patterns of coloration, often associated with accelerated senescence.

This section describes procedures to select field sites for ozone biomonitoring and to evaluate ozone injury on the foliage of sensitive plant species using the FIA ozone grid. Additional ozone sites, on an intensified ozone grid, may also be established by State and federal cooperators to improve the interpretive value of this indicator. This intensified sampling is done using the same methodology as the regular grid activities and is just as important.

### 9.1.1 SCOPE AND APPLICATION

The scope of this indicator is national, but procedures are amended regionally as needed, particularly with regard to suitable sites and target species. Other variables, such as number of species, number of plants, and methods of scoring are standardized nationally. The procedures, reporting, and assessment goals were developed with the following considerations:

1. Ozone plot distribution across the landscape covers both the more remote and expansive forests away from population centers and the more fragmented forests located in close proximity to urban areas;
2. Ozone plot stratification nation-wide reflects regional differences in air quality regimes and perceived risks to different forest types;
3. Sampling intensity in different regions is designed to allow links between ozone biomonitoring data and other FIA indicators;

4. Seasonal variability in ozone injury is addressed. We know that ozone injury must reach an undefined threshold within a leaf before the injury becomes visible to the human eye, and then tends to be cumulative over the growing season until fall senescence masks the symptoms.

NOTE: There are certain regions of the country where ambient ozone concentrations, during the growing season, routinely exceed levels that are known to injure sensitive plants. Other regions have relatively clean air. In regions with poor air quality, the crew data underscore the extent and severity of ozone pollution in the nation's forests. In regions with better air quality, the emphasis must be on establishing a baseline for the ozone indicator. In this regard, field crews that do not find ozone injury (zero values for the ozone injury variables) are making a significant contribution to the national FIA database.

#### 9.1.2 SUMMARY OF METHOD

Field procedures include the selection of a suitable site for symptom evaluation, identification of three or more known ozone-sensitive species at the site, and identification of ozone injury on the foliage of up to 30 plants of each species. Each plant is evaluated for the percentage of injured area and severity of injury on a five-point scale. Field crews record information on the location and size of the opening used for biomonitoring, and record injury amount and severity ratings for each plant.

In the East, to eliminate problems with seasonal variability in ozone response, all foliar evaluations are conducted during a four-week window towards the end of the growing season. In the West, due to differences in growing season, topography, target species, and other regional factors that influence plant response to ozone, the identification of an optimum evaluation window for this indicator is problematic. Nevertheless, to maintain national consistency and improve crew logistics, the western regions use a mid-season, five or six-week window for foliar injury evaluations.

In some States with a particular interest in air quality, foliar injury data are also collected from ozone sites on an intensified ozone grid. These supplementary ozone sites are standardized for certain site characteristics that influence ozone uptake by sensitive plants (Heck 1968; Krupa and Manning 1988), and are often co-located with physical air quality monitors. They are intended to improve the regional responsiveness of the ozone indicator.

Voucher specimens (pressed leaves with symptoms) are collected for each species for proper symptom identification. For each voucher, injury type and location codes are recorded to fully describe the injury observed in the field. Additional quality control measures include field audits and remeasurement of 10% of the biomonitoring sites.

The implementation of an ozone grid independent of the traditional FIA plot system allows greater flexibility in plot location on the ground and greater sampling intensity in areas believed to be at high risk for ozone impact. In addition, plots are deliberately chosen for ease of access and for optimal size, species, and plant counts, thus maximizing data quality. Ozone is a regional pollutant, understood to have regional effects on vegetation. Therefore, data collected on the ozone grid will have direct application to the FIA P2 and P3 plots within the same region

No specialized safety precautions are necessary to complete the fieldwork for the ozone indicator.

### 9.1.3 SUMMARY OF PDR SCREENS AND TALLY PROCEDURES

Ozone indicator data are recorded on portable data recorders (PDRs). For crews using the Tally application, all of the ozone bioindicator data are entered under Option 07 on the Tally main menu. There are three data entry screens for ozone data: the Bioindicator Plot Identification Screen, the Plot Notes Screen, and the Bio Species Screen. On the handheld units, the corresponding screens are Plot Data, Ozone Notes, and Species Data. The Bioindicator Plot Identification Screen (Plot Data) includes a record of plot location and status as well as detail on site characteristics that influence ozone injury expression. The Plot Notes Screen (Ozone Notes) prompts crews to record safety tips and additional information that will help analysts interpret the results or assist subsequent crews collecting data at the same location. The Bio Species Screen (Species Data) prompts crews for injury amount and severity codes on a plant by plant basis. This screen includes a pop-up menu, which keeps a running total of numbers of plants and species evaluated by the field crews. Help screens may be accessed for any variable from any of the three data entry screens.

For a written summary of the data entry procedures, definitions, and codes for the ozone measurement variables refer to section 9.4 through 9.6.

### 9.1.4 EQUIPMENT AND SUPPLIES

- A large diameter, 10X hand lens for close examination of plant leaves for ozone injury.
- Reference photographs and laminated leaf samples to aid in symptom identification.
- A forester-grade PLANT PRESS with cardboard inserts to store leaf vouchers collected in the field.
- Envelopes ready for mailing the leaf vouchers to the National Ozone Advisor.
- Stiff paper or cardboard for protecting the leaf vouchers in the mailing envelopes.
- Flagging: for temporary marking of sites or sample plants.
- Three field data sheets: (1) For documenting Foliar Injury Data in the event of a PDR failure; (2) For preparing the plot location map; and (3) For recording Voucher Leaf Samples Data for QA. (see Appendix 9.B).

### 9.1.5 TRAINING AND QUALITY ASSURANCE

Each field crew member is trained and tested for familiarity with the site selection, species selection, and data collection procedures, and their ability to recognize ozone injury and discriminate against mimicking symptoms. Field crews are certified just prior to the beginning of the evaluation window for this indicator.

The National Ozone Advisor and one or more individuals in each region assume quality control responsibilities for the field season. Regional Advisors meet during a preseason session to refine methods and establish a unified approach to training, audits, and debriefing. Their responsibilities include: (1) training and certifying the State trainers and/or field crews as needed for their region, (2) documenting hot audits of the field crews, (3) overseeing the field crew refresher session held just prior to the evaluation

window for this indicator, (4) assisting in the field with remeasurement procedures for symptom quantification, and (5) conducting a debriefing for the indicator.

A field audit crew remeasures a subsample of the ozone ground plots in each region. Auditing procedures cover species selection, symptom identification, and quantification of injury, as well as foliar sample collection, preservation and shipment.

Results of the field audits and remeasurement activities are used to determine if the measurement quality objectives are being met. Regional Advisors and Field Supervisors who are certified for the ozone indicator have the authority to implement whatever corrective action is needed in the field (e.g., retraining and retesting).

#### 9.1.6 VOUCHER SPECIMENS

Leaf samples are collected by field crews, cooperators, and all QA staff. They are to be placed in a small plant press immediately after removal from the selected plant. This is to preserve the integrity of the leaf sample and the injury symptoms until they can be validated by the National Indicator Advisor. A data sheet identifying the field crew and plot location is to be filled out and mailed with each sample.

Field crews, cooperators, and all QA staff collect leaf samples on the ozone biomonitoring sites according to procedures outlined in Subsection 9.2.7. These voucher specimens are pressed and mailed to the National Indicator Advisor for validation of the ozone symptom. If QA staff and regular field crews happen to be evaluating the same site at the same time, they collect and mail separate vouchers.

#### 9.1.7 COMMUNICATIONS

Any questions arising during the field season that cannot be answered by the Field Supervisor or State Coordinator, should be directed to the Regional Advisor for the ozone indicator. If any field crew or cooperator is uncertain about whom to call for information, or if a Regional Advisor is not indicated, they should contact the National Ozone Advisor. Keep in mind that Advisors may be in the field and, therefore, unavailable for phone calls during normal workday hours. Messages left on answering machines should clearly identify who you are and when, where, and how to return your call. Field crews should be aware of differences in time zones and use email, if possible.

#### National Advisor (East and West) and Regional Advisor for the Northeast and Mid-Atlantic States:

**Gretchen Smith** Phone: (413) 545-1680  
Holdsworth Hall (978) 544-7186 (before 7am; after 7pm)  
University of Massachusetts  
Department of Forestry and Wildlife Management  
Amherst, MA 01003-0130  
e-mail: gcsmith@forwild.umass.edu

#### Regional Advisor for the North Central States:

**Ed Jepsen** Phone: (608) 266-3538  
Wisconsin Department of Natural Resources  
101 South Webster Street

Madison, WI 53707  
e-mail: jepsee@dnr.state.wi.us

Regional Advisor for the South:

**Dan Stratton** Phone: (828) 257-4352  
USDA Forest Service  
P.O. Box 2680  
Asheville, NC 28802  
e-mail: dstratton@fs.fed.us

## 9.2 OZONE BIOMONITORING PROCEDURES

NOTE: In the following discussion the words site, biosite, and plot are used interchangeably to refer to the open area used for the ozone biomonitoring evaluations.

The primary objective of the field crew procedures for the ozone indicator is to establish an ozone biomonitoring site within each polygon on the FIA ozone grid using the site selection guidelines provided in the Decision Table – section 9.2.2. These sites are used to detect and monitor trends in ozone air pollution injury on sensitive species.

Procedures include the selection of a suitable site for symptom evaluation, identification of three or more known ozone-sensitive species at the site, symptom identification and scoring on the foliage of up to 30 plants of each species, and the collection of voucher leaf samples. Each individual plant with ozone injury is scored for amount and severity of injury. Plants used for the selection of leaf vouchers are also evaluated for injury location and type. If a plant does not have ozone injury, it is still tallied with zeros for the amount and severity measurements. A hardcopy map, providing directions, plot coordinates, and key characteristics of the bioindicator site, is prepared for each plot.

All foliar evaluations are conducted during the latter half of the field season. This is necessary to eliminate differences between plots that are caused by timing. During the evaluation window, all ozone sites on the ozone grid are evaluated for ozone injury. The same sites are evaluated every year.

### 9.2.1 EVALUATION WINDOW

The evaluation window for crews in the Northern Regions begins the last week of July and extends through the third week in August. In the Southern Region, the window is open from the third week in July through the third week in August.

All established biomonitoring sites are evaluated each year. The ozone injury evaluations are generally completed over a 5 to 20 day period during the window depending on the size of the State and the number of crews dedicated to the ozone survey. If possible, crews should adjust the timing of their evaluations so that the biomonitoring sites within each State are done at approximately the same time every year.

NOTE: States in the Northern Region that border the southern regions and have ozone exposure seasons more typical of the South may choose to select the evaluation window for the Southern Region. This may only be done with approval from the National Advisor.

9.2.2 SITE SELECTION PROCEDURES

Site selection procedures begin with an in-office review of the ozone grid for each State. Candidate sites must be easily accessible open areas greater than one acre in size that are more than 100 feet (30 m) from a busy (paved) road. A site must contain at least thirty individuals of at least two bioindicator species to be evaluated for ozone injury. It is preferable that all sites have three or more species. The following table may be used as a decision guide for site selection:

Decision Table	First Choice = <b>Best Site</b>	Second Choice
Access:	Easy	Easy
Location:	Single location is used.	One or two locations (split-plot).
Size of opening:	>3 acres (1.2h); wide open area; <50% crown closure.	Between 1-3 acres; long narrow or irregularly sized opening.
Species count:	More than three species.	Two or more species.
Plant count:	30 plants of 3 species; 10-30 plants of additional species.	30 plants of 2 species; 10-30 plants of additional species.
Soil conditions:	Low drought potential. Good fertility.	Moderate dry. Moderate fertility.
Site disturbance:	No recent (1-3 years) disturbance; No obvious soil compaction.	Little or no disturbance; No obvious soil compaction.

The best ozone sites are often associated with wildlife preserves on public land. Private landowners are often eager to participate in the ozone program. State and county parks and wildlife openings also provide good ozone sites. Other examples of suitable openings include old logging sites and abandoned pasture or farmland where you are reasonably certain that soil/site conditions are stable and free of chemical contaminants. Generally, if bedrock is exposed throughout an open area, then the soil conditions may be shallow, infertile, and often too dry to allow plants to respond to ozone stress. Sites that are routinely waterlogged are similarly unsuitable for biomonitoring. Avoid open areas where plants are obviously stressed by some other factor that could mimic or inhibit the ozone response. For example, the wooded edges of large parking lots in recreational areas are often highly compacted by car and foot traffic and should not be used. Do not select a site under a high-tension power line or on or near an active or reclaimed landfill. Do not select plants within 50 feet of the open edge around a cultivated field or tree plantation.

FIA crews and State Cooperators that have an established network of ozone sites may need to select and map replacement sites when previously mapped areas become overgrown or disturbed. Some sites may be split between two near-by locations to improve species and plant counts. In the case of split-plots, separate plot files (i.e., Tally files) are maintained for each location. Both have the same plot identification number (i.e., OZONE HEXAGON NUMBER) but different values for the plot number variable (i.e., PLOT NUMBER) as defined in Subsection 9.4.4. A split-plot is considered a unique ozone plot and should not be confused with grid intensification when two or more plots with different hex numbers fall in the same polygon.

No more than one half day should be spent locating a new or replacement bioindicator evaluation site. Crews must provide geographic coordinates (i.e., latitude and longitude) for all newly established ozone sites. If a site is split between two locations, the geographic coordinates for both locations are recorded.

### 9.2.3 SITE MAPPING

Once a bioindicator site is selected, the field crew records the estimated size of the site opening and other key site characteristics identified on the PDR or data sheet. The crew then maps the location of the site relative to some obvious and permanent marker such as a telephone pole, building, or property marker. Directions to the site, including road names and distances, are added to the map. Crews also mark the starting point for plant selection (see section 9.2.5) and approximate location of plant groupings used for evaluation (see section 9.2.6) on the site map. If available, a GPS unit is used to determine plot coordinates and elevation. Otherwise, this information is obtained from a USGS topographic map, generally the 7½ minute series quadrangle.

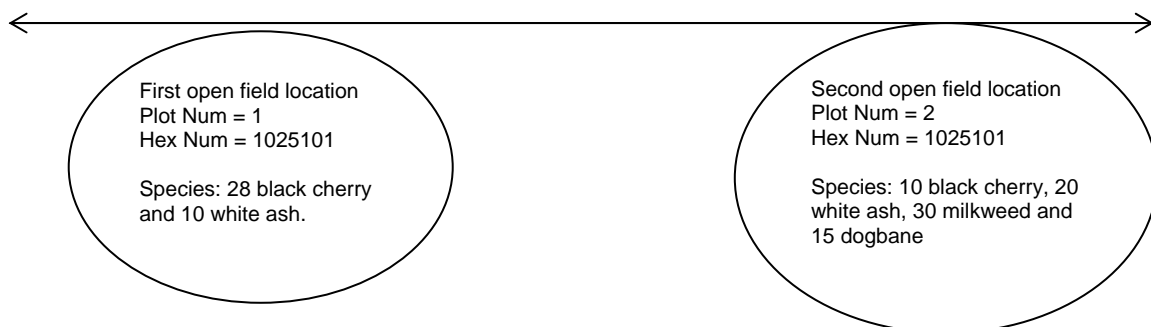
Ozone site maps are used by audit and regular crews in subsequent visits to the plot (see Figure 9-1) to ensure that the same site and the same population of plants are remeasured every year. This bioindicator site map must be kept with the appropriate state or federal cooperator so that it is readily available to whoever needs it.

### 9.2.4 SPLIT PLOTS

With the 2002 implementation of the national ozone grid, there is an emphasis on maximizing the quality of each ozone plot with respect to the number of plants and species that are evaluated for ozone injury. As indicated in the site selection Decision Table in section 9.2.2, the best sites have more than 3 species; 30 plants of 3 species and between 10 and 30 plants of 1, 2, or 3 additional species. Finding high plant counts at a single wide-open location can be challenging. Split plots are intended to address this challenge. A split-plot consists of two different locations within 3 miles of each other, preferably with similar site characteristics. Species and plant counts from one location are combined with the species and plant counts from the second location to meet the species and plant count standards for site selection. On the PDR or data sheet, the same OZONE HEXAGON NUMBER is assigned to each location. However, each location is assigned a unique PLOT NUMBER; PLOT NUMBER = 1 for the first location that is evaluated by the field crew and PLOT NUMBER = 2 for the second location. In this way, separate Tally files are maintained for each location. On the national grid, the two locations are considered a single and unique ozone plot and should not be viewed as an intensification of the grid.

In the following example, the site selection criteria for a high quality ozone plot are met as the total species and plant counts for OZONE HEXAGON NUMBER 1025101 are black cherry = 38, white ash = 30, milkweed = 30, and dogbane = 15.

Example: Road access to the biomonitoring plot – Distance between locations is about 2 miles



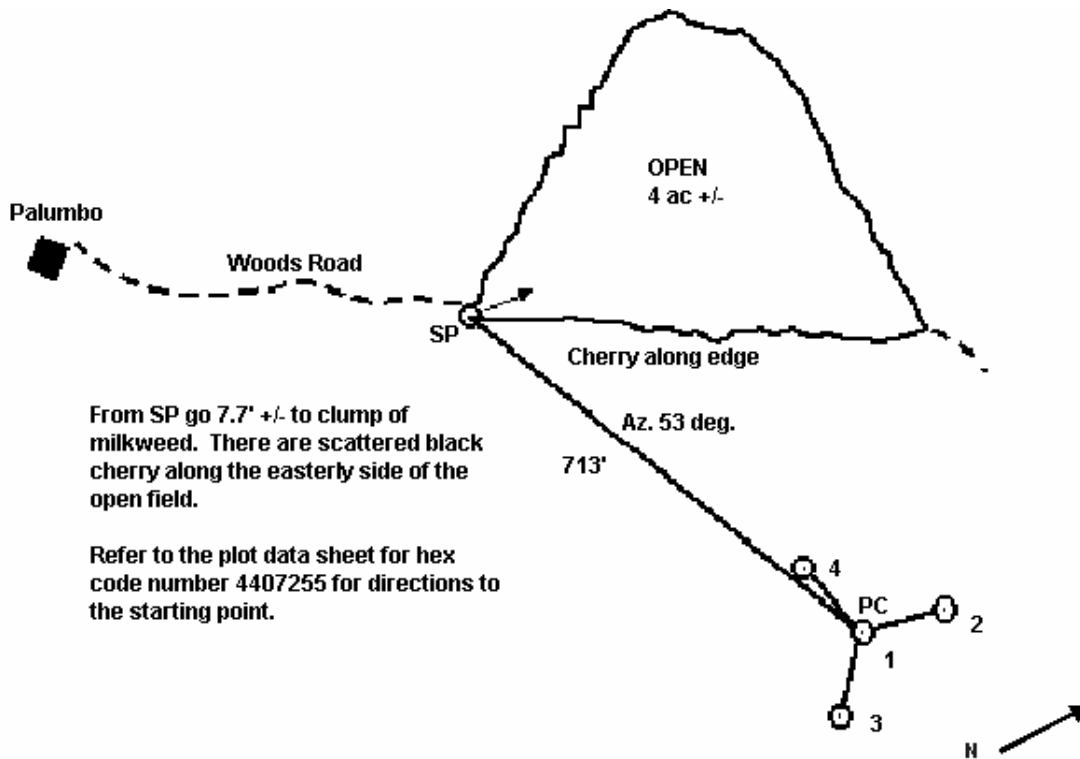


Figure 9-1. Example of a well-drawn map showing the location of the biosite and the approximate location of the bioindicator species and other key landmarks. Road names and north arrow are also included.

### 9.2.5 SPECIES SELECTION

At the selected bioindicator site, the crew evaluates 30 individuals of three or more bioindicator species. If three species cannot be found at the site, then two species are still evaluated. Crews may combine species and plant counts from neighboring locations to obtain the required plant counts for each site. If 30 plants of two or more species cannot be found at the site, then a new site or additional location must be selected. A prioritized list of species is provided to the field crews for each region. The top three species in each list are the most common throughout the sampling region and should be selected for evaluation whenever possible. Species with 30 or more individual plants should be a first priority for choice of species, regardless of their position on the list. Key identifying characteristics of each species are provided in the Appendix 9.A.

Field crews record the species code number for each selected species in the PDR or on the data sheet. The target species and codes for the North and South Regions are presented in the following table.

Code	Definition – Bioindicator Species	Scientific Names
0915	Blackberry	<i>Rubus allegheniensis</i> (second year canes only)
0762	Black Cherry	<i>Prunus serotina</i>
0365	Common and Tall Milkweed	<i>Asclepias spp.</i>
0621	Yellow Poplar	<i>Liriodendron tulipifera</i>
0541	White Ash	<i>Fraxinus americana</i>
0931	Sassafras	<i>Sassafras albidum</i>
0366	Spreading Dogbane	<i>Apocynum androsaemifolium</i>
0364	Big Leaf Aster	<i>Aster macrophyllum</i>
0611	Sweetgum	<i>Liquidambar styraciflua</i>
0761	Pin Cherry	<i>Prunus pensylvanica</i>

NOTE: Site selection requirements for species and plant counts (section 9.2.2, Decision Table for site selection) must be met using the species listed on the preceding table. Field crews may receive supplemental lists of regional species (e.g., Paw-paw, *Asimina triloba* in the South) that may be used as additional species for a selected biomonitoring site. Species on supplemental lists are for field trials only as they have not yet been adequately tested for ozone sensitivity under controlled conditions. Use the Plot Notes screen to make a record of when supplemental species have been used at a site.

SPECIAL NOTE: Field crews in the Plains States including North Dakota, South Dakota, Nebraska, Kansas, Oklahoma and Texas should refer to Appendix 9.D for a special insert on western bioindicator species that can be used in addition to the target species and codes presented in the preceding table. **This special insert is not included in the Northeast revised section.**

### 9.2.6 PLANT SELECTION

After site and species selection, the next task is to contiguously sample 30 individual plants of each species. Thirty plants of a target species must be sampled, if they are available on site. In fact, crews are strongly encouraged to evaluate 150 plants at each site (30 plants of five species), if possible. The value of the bioindicator data increases significantly with increased numbers of plants evaluated. This is true even if the crew records 30 consecutive zeros on three different species.

NOTE: The borders of some biomonitoring sites are difficult to determine and crews may be uncertain how much ground area to cover to complete the plant selection procedures. Specific guidelines are not set because the constraints on crew time and resources vary considerably from one State to the next. Time and safety concerns should take priority. Each crew must make every effort to maximize the number of plants and species evaluated for ozone injury at each plot location. Generally, ozone injury evaluations take 1 hour to complete and, assuming routine travel, crews are expected to complete 3 ozone sites in a ten hour work day.

The following procedures help crews to collect the bioindicator data in as systematic or unbiased a way as possible.

1. Identify a starting point at the edge of the opening. This point is mapped on the site data sheet so that audit and regular crews evaluate roughly the same population of plants in subsequent visits to the plot.
2. Move away from the starting point, towards the center of the opening.

3. Begin locating individuals in a sweeping pattern, selecting plants that are growing under the same or similar growing (microhabitat) conditions. Do not skip plants with little or no injury.
4. Select the more exposed plants (high sunlight exposure) and avoid suppressed and shaded individuals. Plants along the edge of an opening may be used if, in your judgment, they receive direct sunlight for three to four hours each day.
5. Avoid plants under 12 inches in height or so tall that you cannot see or touch at least half of the crown area.
6. Evaluate the foliage that you can see and touch on 30 plants of each species in the opening.
7. Record the amount and severity of injury for each plant evaluated (with or without symptoms) on the PDR, personal data assistant, or data sheet.

NOTE: A pop-up menu keeps track of the plant counts by species. For any one species, stop when the pop-up display indicates you have tabulated 30 plants, or when no additional plants of that species can be found on site. You can tabulate 30 plants of 5 species or any combination of species and plants that adds up to 150 data line entries.

Several bioindicator species (e.g., milkweed and blackberry) can spread vegetatively. This means that neighboring plants are often genetically identical. To avoid repeat sampling of clonal material, take several steps between each plant selected for evaluation. This same approach should be used for all species to minimize bias in the plant selection process. For example, select the plant closest to your left side then take several steps and select the plant closest to your right side and repeat. In addition, it is often difficult to distinguish individual plants or stems for species, like blackberry, that grow in clumps. In this case, use an approximate 2-foot square area to represent a single plant.

#### 9.2.7 SYMPTOM IDENTIFICATION AND SCORING

The bioindicator species selected for each region are those that have been determined through field and laboratory studies to be highly sensitive to ozone air pollution. However, within a species, differences in genetics between individuals result in differential sensitivities to ozone. This means that you often find an individual of a species with severe air pollution injury growing immediately adjacent to another individual of the same species with few or no symptoms.

In addition to genetics, the age of the leaves (position on the stem, branch, or rosette) affects a plant's susceptibility to ozone air pollution. In general, leaves at 75% full expansion are the most sensitive and tend to show symptoms most definitively toward the center of the leaf. Older leaves show symptoms more widespread over the leaf surface, while younger leaves show symptoms more commonly near the leaf tip. If leaves on one branch are affected, then leaves at a similar leaf position on another branch should be affected, especially for branches on the same side of the plant under similar environmental conditions (sun or shade leaves).

When scoring foliar symptoms on bioindicator plants check for the following characteristics of ozone injury.

- X Symptoms are more severe on mid-aged and older leaves. New leaves will have no or very little injury.
- X Symptoms are most likely confined to the upper leaf surface, and are typically visible as tiny purple-red to black spots (stippling).
  - X Check leaves covering each other. Overlapped leaves will have no injury on the bottom leaf.
  - X There will be some uniformity to size and shape of the lesions (stippling) on a leaf.
  - X Later in the growing season, stippling may be associated with leaf yellowing or premature senescence. Check the ground for fallen leaves.

Each plant with ozone injury is evaluated for the percent of the plant that is injured and the average severity of injury. For each plant located, the percentage of injured area and the severity of injury are both rated on a scale of 0 to 5 (see below). Both injury AMOUNT and injury SEVERITY estimates are confined to the exposed portion of the plant. If a plant does not have injury, it is still tallied with zeros for these measurements.

Percent Scale for injury AMOUNT: Estimate and record the percentage of leaves on the plant with ozone injury symptoms relative to the total number of leaves on the plant.

<u>CODE</u>	<u>DEFINITION</u>
0	No injury; the plant does not have any leaves with ozone symptoms.
1	1 to 6 percent of the leaves have ozone symptoms.
2	7 to 25 percent of the leaves are injured.
3	26 to 50 percent of the leaves are injured.
4	51 to 75 percent of the leaves are injured.
5	>75 percent of the leaves have ozone symptoms.

Percent Scale for SEVERITY of injury: Estimate and record the mean severity of symptoms on injured foliage.

<u>CODE</u>	<u>DEFINITION</u>
0	No injury; the plant does not have any leaves with ozone symptoms.
1	On average, 1 to 6 percent of the leaf area of injured leaves have ozone symptoms.
2	On average, 7 to 25 percent of the leaf area of injured leaves have ozone symptoms.
3	On average, 26 to 50 percent of the leaf area of injured leaves have ozone symptoms.
4	On average, 51 to 75 percent of the leaf area of injured leaves have ozone symptoms.
5	On average, >75 percent of the leaf area of injured leaves have ozone symptoms.

NOTE: Blackberry and white ash have compound leaves. Use the whole leaf, not each leaflet, to estimate injury AMOUNT and injury SEVERITY. A typical clump of blackberry plants will have both current year (vegetative) and second year (flower and fruit bearing) canes available for evaluation. The injury AMOUNT and injury SEVERITY measurements are confined to the foliage on the second year canes. The foliage on the current year canes is naturally resistant to ozone injury. Do not use blackberry if you can find only current year canes.

NOTE: The recognition of ozone injury symptoms in the field is not an exact science, and mimicking symptoms can make field diagnosis difficult. Crews are expected to

record AMOUNT and SEVERITY estimates for injury that they are unsure of as well as the more obvious or classic injury symptoms.

Proceed as follows:

1. Record the injury AMOUNT and the injury SEVERITY ratings for each plant on the PDR or data sheet.
2. Use the notes section on the PDR or data sheet to add other information that will help interpret the results (e.g., below average rainfall for the area).
3. Collect a voucher leaf sample (three leaves of each injured species evaluated at each location) and mail them to the National Advisor using the guidelines presented in Subsection 9.6.7.

NOTE: Do not take measurements in steady rain. Foliar symptoms are easiest to see under overcast skies. Bright sun will make it difficult to see the ozone stipple. Stand so that you reduce the glare on the leaf surface. Long periods without rain will inhibit symptom development even on the most sensitive plants. If you are experiencing below average rainfall for your area, please note this in the PDR or on the data sheet.

#### 9.2.8 COLLECTION OF LEAF SAMPLES AND VOUCHER DATA

The voucher leaf samples are a critical aspect of the data collection procedures as they provide the necessary validation of the ozone injury symptom observed in the field by the field crews. A plant press is essential to the collection of useable leaf samples and must be taken into the field by the field crews. Crew data that do not include a useable voucher leaf sample with a completed voucher data sheet are removed from the FIA database.

During the evaluation window, a voucher leaf sample must be collected for each injured species evaluated on the bioindicator site. For each injured species, the voucher consists of three leaves that clearly show the ozone injury symptom. For example, if a field crew records ozone injury on blackberry, black cherry, and milkweed then a minimum of one voucher (3 LEAVES) from each of the three species (9 LEAVES IN ALL) is collected and mailed, with the corresponding voucher data sheet(s), to the National Indicator Advisor.

The most useful voucher leaf samples show obvious foliar injury symptoms. If injury symptoms are not obvious and severe, send whatever leaf sample is available even if it is only one leaf with faint symptoms. Cut the leaf at the petiole, shake off any excess moisture, and place the leaf on blotter paper in the plant press. Each leaf is placed in the press so that it does not overlap another leaf. Include a label with each leaf sample placed into the plant press that identifies which plot the sample came from (i.e., OZONE HEXAGON NUMBER) and the date. Petiole labels are provided for this purpose. Record the information on the labels with indelible ink and then wrap them around the petiole of at least one leaf per sample

NOTE: Blackberry and white ash have compound leaves. Select the whole leaf (not individual leaflets) when preparing a voucher sample.

NOTE: If QA staff and regular field crews happen to be evaluating the same site at the same time, they collect and mail separate vouchers.

NOTE: The recognition of ozone injury symptoms in the field is not an exact science, and many other foliar injury symptoms can be mistaken for ozone injury. Crews are encouraged to collect and mail in voucher specimens of both known and suspected ozone injury for verification by the National Advisor.

The voucher data sheet must be completed for plot identification codes (e.g., STATE, COUNTY, OZONE HEXAGON NUMBER and PLOT NUMBER), CURRENT DATE, CREW ID, CREW TYPE, and SPECIES code(s). This sheet is filled out at the bioindicator site on the same day the sample is collected. In addition, the plants from which the leaf vouchers are selected must be evaluated by the field crews for INJURY LOCATION and INJURY TYPE (defined below), and for the amount of injury present on the leaf that is not ozone stipple. This information, together with the visible injury symptoms on the leaf samples, is used to validate the ozone injury data observed and recorded in the field by the field crews. For each species, the INJURY LOCATION and INJURY TYPE codes are intended to represent what the crew observed on the majority of the injured plants in the sample population. In contrast, the recorded estimates of percent injury caused by some stress other than ozone are based on what the crew observed on the injured leaf samples mailed in with the voucher data sheet.

The INJURY LOCATION and INJURY TYPE codes are recorded on the upper half of the voucher data sheet as follows:

INJURY LOCATION: Specify the leaf age or position of the leaves with ozone injury.

CODE	DEFINTION
1	>50% of the injured leaves are younger leaves. Younger leaves are usually located towards the branch tip (e.g., blackberry, black cherry, yellow poplar, white ash, sassafras, sweetgum, pin cherry, and spreading dogbane) or top of the plant (e.g., milkweed and big-leaf aster).
2	>50% of the injured leaves are mid-aged or older leaves. Mid-aged and older leaves are located halfway along the branch (e.g., blackberry, black cherry, yellow poplar, white ash, sassafras, sweetgum, pin cherry, and spreading dogbane), or main stem of the plant (e.g., milkweed and big-leaf aster), or more towards the base of the branch or stem.
3	Injured leaves are not concentrated in any one location, leaf age or position. Injury may be spread more or less evenly over the plant or is, otherwise, difficult to describe.

INJURY TYPE: Specify the visible injury symptom.

CODE	DEFINITION
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1	The injury on >50% of the injured leaves is best described as upper-leaf-surface stipple, i.e., tiny purple-red to black spots occurring between the veins. Stippling may be associated with leaf yellowing and leaf drop late in the evaluation window; When injury is severe, stipples may coalesce and appear as uniform discoloration of the leaf surface.
2	The injury on >50% of the injured leaves is something other than upper-leaf-surface stipple. For example, small white to tan flecks occurring between the veins, or injury that is clearly visible on both leaf surfaces, or a general discoloration of the leaf that resembles early fall coloration.
3	The visible injury is varied or, otherwise, difficult to describe.

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NOTE: Not all location and type codes are indicative of ozone injury. Certain combinations of location and type codes, considered with a questionable leaf voucher, may invalidate the injury data. Other combinations provide quality assurance for the injury assessment. Crews should describe any unusual or questionable symptoms on the upper half of the voucher data sheet.

### 9.2.9 VOUCHER MAILING PROCEDURES

Vouchers are mailed in bulk at the end of the evaluation window, or earlier, depending on the crew's work schedule. It is very important to mail only dry, pressed leaf samples. Before mailing, make sure the upper half of the voucher data sheet is filled out. This sheet is filled out on the same day the sample is collected even if the sample is not mailed on that day. Please comment on the weather or general plot conditions that might help interpret the injury data. For example, *"It's been 14 days now without rain,"* *"Every plant showed the same response and it was very obvious,"* or *"This was a highly disturbed site."* Avoid noting whether the crew thinks the leaf sample shows ozone injury or a mimicking symptom, and referring to the amount and severity ratings so as not to influence the validation process.

The lower half of the voucher data sheet is filled out by the National Ozone Advisor to whom the sample is being sent. Place the voucher data sheet and the leaf sample between two pieces of stiff paper or cardboard before placing into a mailing envelope addressed to the National Advisor. Manila folders and newspaper may also be used for voucher mailings. Do not tape the leaves to the folders, paper or cardboard. Taped samples often break apart when they are handled, making evaluation difficult. Include as many samples as fit easily into each mailing envelope. There must be a unique voucher data sheet for each sample or species, unless the form is being used for multi-species. Keep leaf samples and the corresponding leaf voucher data sheets together. Leaf samples that are separated from the corresponding leaf voucher data sheets may be mislaid, especially if the petiole leaf labels are missing or incomplete.

### 9.2.10 CREW MEMBER RESPONSIBILITIES

1. Although one or two crew partners may be trained for this indicator, one person typically takes the lead responsibility for site selection, plant selection, and ozone injury evaluations. All procedures can be successfully completed by one person. Two person crews are recommended for safety reasons.
2. All members of the field crew may assist each other in the site selection process. Once a site is selected, one crew member is responsible for mapping the site and the location of bioindicator species on the field data sheet.

3. Only the crew member trained and certified in ozone injury evaluations may collect the amount and severity data and the leaf voucher. Other crew members may assist by recording the injury scores on the PDR or data sheet and by getting the plant press supplies ready.
4. The crew member that evaluates the plants for injury is responsible for collecting and mailing the voucher sample with air pollution symptoms.

#### 9.2.11 FIELD PROCEDURES FOR UNTRAINED FIELD CREWS

There are certain procedures for the ozone indicator that may be performed by individuals that have not attended the ozone training and been certified to collect ozone data. These procedures still require some explanation and oversight by the certified crew member. Untrained personnel may assist in the selection and mapping of the ozone biomonitoring site and in the location and identification of bioindicator species on the selected site. They may not rate plant injury. It may also be helpful for the untrained crew person to act as the data recorder for the certified crew member, thus speeding up the data collection process.

### 9.3 SITE INTENSIFICATION

In addition to the unique ozone plots that are identified by the base ozone grid, some Cooperators have established additional biomonitoring sites to represent the local plant populations and environmental conditions. This is not an auxiliary effort, but an integral part of the monitoring activities for this indicator. In some States, additional biomonitoring sites are limited in number and are deliberately located close to weather and air quality monitoring stations. In other States, the ozone grid is intensified to allow for an unbiased allocation of additional biomonitoring sites. It is recommended that additional sites, whether few or many in number, be located on public land to facilitate the annual measurement activities.

Ozone biomonitoring sites added to the base grid typically possess attributes of an ideal site for evaluating ozone injury on sensitive species. They are larger than three acres, contain the maximum number of indicator species, and have soil/site conditions with low drought potential and adequate fertility. They are evaluated for ozone injury using the same methods and during the same time frame as described above in section 9.2.

### 9.4 PLOT LEVEL DATA

All plot-level measurement codes for the ozone indicator are defined below. The codes and definitions are the same whether the crew is entering data using Tally (Paravant or Husky) or a personal data assistant (Handspring or Palm).

Ozone plots vary in size and do not have set boundaries. When describing plot-level characteristics, use the predominant characteristics where most of the plant species are located. If conditions vary markedly across the site, or by species, then describe this in the plot notes or on the site map. Specify the elevation, aspect, terrain position, soil depth, soil drainage, and disturbance for the highest priority species (Subsection 9.6.4) found on the site. The soil depth, soil drainage, and disturbance variables are intended to describe general conditions on the plot and are not based on actual measurements. For a complete explanation of the procedures associated with these measurement codes, refer to section 9.2.

9.4.1 STATE

Record the unique FIPS (Federal Information Processing Standard) code identifying the State where the plot center is located.

When collected: All plots  
Field width: 2 digits  
Tolerance: No errors  
MQO: At least 99% of the time  
Values: See Appendix 1

9.4.2 COUNTY

Record the unique FIPS (Federal Information Processing Standard) code identifying the county, parish, Borough (or unit in AK) where the plot center is located.

When collected: All plots  
Field width: 3 digits  
Tolerance: No errors  
MQO: At least 99% of the time  
Values: See Appendix 1

9.4.3 OZONE HEXAGON NUMBER

Record the unique code assigned to each ozone hexagon. In some cases this will be a former FHM or P3 hexagon.

When collected: All plots  
Field width: 7 digits  
Tolerance: No errors  
MQO: At least 99% of the time  
Values:

9.4.4 PLOT NUMBER

Record the plot number that describes whether an ozone plot consists of one or two locations. If two locations are selected, they must be within 3 miles of each other. Two locations are selected as needed to obtain optimal species and plant counts for each ozone plot. The OZONE HEXAGON NUMBER is the same for both locations. Note: The PLOT NUMBER value is not related to the GRID DENSITY value.

When collected: All plots  
Field width: 1 digit  
Tolerance: No errors  
MQO: At least 99% of the time  
Values:

- 1 The ozone plot consists of a single location or this is the first location of a plot split between two locations.
- 2 The ozone plot is split between two locations. This code identifies the second location added by the field crew to increase species and plant counts for a single hexagon number.

#### 9.4.5 QA STATUS

Record the code to indicate the type of plot data collected.

When collected: All plots

Field width: 1 digit

Tolerance: No errors

MQO: At least 99% of the time

Values:

- 1 Standard ozone plot
- 2 Cold check
  
- 4 Training/practice plot (off grid)
- 5 Botched plot file
- 6 Blind check
- 7 Hot check (production plot )

#### 9.4.6 CREW TYPE

Record the code to specify what type of crew is measuring the plot.

When collected: All plots

Field width: 1 digit

Tolerance: No errors

MQO: At least 99% of the time

Values:

- 1 Standard field crew
- 2 QA crew (any QA crew member present collecting remeasurement data)

#### 9.4.7 OZONE SAMPLE KIND

Record the code that describes the kind of plot being visited. Note: OZONE SAMPLE KIND has a value of 1 only when an ozone plot is established in a previously empty polygon.

When collected: All plots

Field width: 1 digit

Tolerance: No errors

MQO: At least 99% of the time

Values:

- 1 Initial plot establishment on the base grid or on a newly intensified grid..
- 2 Remeasurement of a previously established plot.
- 3 Replacement of a previously established plot that was replaced because the original plot could not be relocated or because it no longer met ozone plot measurement criteria.

#### 9.4.8 CURRENT DATE

Record the year, month, and day that the current plot visit was completed as follows:

##### 9.4.8.1 YEAR

Record the year that the plot was completed.

When collected: All plots  
Field width: 4 digits  
Tolerance: No errors  
MQO: At least 99% of the time  
Values: Beginning with 1998, constant for a given year

#### 9.4.8.2 MONTH

Record the month that the plot was completed.

When collected: All plots  
Field width: 2 digits  
Tolerance: No errors  
MQO: At least 99% of the time  
Values:

January	01	May	05	September	09
February	02	June	06	October	10
March	03	July	07	November	11
April	04	August	08	December	12

#### 9.4.8.3 DAY

Record the day of the month that the plot was completed.

When collected: All plots  
Field width: 2 digits  
Tolerance: No errors  
MQO: At least 99% of the time  
Values: 01 to 31

#### 9.4.9 OZONE GRID DENSITY

Record the code that identifies whether the plot is on the base ozone grid or on an intensified ozone grid.

Note: The OZONE GRID DENSITY value = 2 when there are two ozone plots with different OZONE HEXAGON NUMBERS in the same polygon. The two plots may be located in different States.

When collected: All plots  
Field width: 1 digit  
Tolerance: No errors  
MQO: At least 99% of the time  
Values:

- 1 Unique ozone plot within a polygon. (1 site:1polygon)
- 2 One of two or more ozone plots within the same polygon.

#### 9.4.10 PLOT SIZE

Record the code that indicates the size of the opening used for biomonitoring.

When collected: All plots  
Field width: 1 digit  
Tolerance: No errors

MQO: Repeatable estimate

Values: 1 to 2

- 1 Greater than three acres.
- 2 Greater than one acre, but less than three acres.

#### 9.4.11 ASPECT

Record the code that identifies the direction of slope for land surfaces with at least 5 percent slope as measured with a hand compass to the nearest degree.

When collected: All plots

Field width: 3 digits

Tolerance: +/- 30°

MQO: At least 99% of the time

Values:

000	No aspect, slope < 5 percent
001	1 degree
002	2 degrees
.	.
.	.
360	360 degrees, due north

#### 9.4.12 TERRAIN POSITION

Record the code that identifies the position of the plot in relation to the surrounding topography.

When collected: All plots

Field width: 1 digit

Tolerance: Repeatable estimate

MQO: At least 99% of the time

Values:

- 1 Ridge top or upper slope
- 2 Bench or level area along a slope
- 3 Lower slope
- 4 Flat land unrelated to slope
- 5 Bottom land with occasional flooding

#### 9.4.13 SOIL DEPTH

Record the code that indicates the general depth of the soil where most of the bioindicator species are growing.

When collected: All plots

Field width: 1 digit

Tolerance: Repeatable estimate

MQO: At least 99% of the time

Values:

- 1 Bedrock is not exposed.

2 Bedrock is exposed; Soil is generally shallow.

#### 9.4.14 SOIL DRAINAGE

Record the code that identifies the general soil drainage conditions where most of the bioindicator species are growing.

When collected: All plots

Field width: 1 digit

Tolerance: Repeatable estimate

MQO: At least 99% of the time

Values: 1 to 3

1	Soil is well drained
2	Soil is generally wet
3	Soil is excessively dry

#### 9.4.15 DISTURBANCE

Record the code that identifies the presence and kind of disturbance where most of the bioindicator plants are growing. The area affected by any human caused or natural disturbance must be clearly visible and recent enough to influence plant health and condition. Disturbance that results in significant soil compaction is especially significant.

When collected: All plots

Field width: 1 digit

Tolerance: Repeatable estimate

MQO: At least 99% of the time

Values:

0	No recent or significant disturbance.
1	Evidence of overuse; Human activity causing obvious soil compaction or erosion.
2	Evidence of natural disturbance including fire, wind, flooding, grazing, pests, etc.

#### 9.4.16 INJURY CHECK

Record the code that indicates whether ozone injury was observed on non-tallied plants or species. This variable allows a plot to be identified as impacted by ozone even though there is no quantitative data on injury severity for trend analyses. A leaf voucher must be collected to validate the injury.

When collected: All plots

Field width: 1 digit

Tolerance: No error

MQO: At least 99% of the time

Values:

0	No injury was observed on non-tallied plants or species.
1	Ozone injury was observed on non-tallied plants or species and a leaf voucher collected.

#### 9.4.17 ELEVATION

Obtain elevation data from USGS topographic maps, generally the 7½ minute series quadrangle. Locate the area where most of the bioindicator species are growing and record elevation to the nearest foot.

When collected: When GPS UNIT = 0

Field width: 6 digits

Tolerance: +/-200 feet

MQO: At least 99% of the time

Values:

#### 9.4.18 Plot Notes

Use these fields to record notes pertaining to the entire plot. If the notes apply to a specific aspect of the plot, then make that clear in the notes. Record the location where GPS coordinates were collected, and GPS file name, as needed. If no GPS Unit was available, record the geographic coordinates (i.e., latitude and longitude) of the plot center in Degrees, Minutes, and Seconds using USGS topographic maps, generally the 7½ minute series quadrangle.

##### 9.4.18.1 REMARK1 and REMARK2

Record any information on site characteristics, use of supplemental species, safety, plant location, injury patterns, or recent rainfall amounts that will assist subsequent crews visiting the site or help interpret the results.

When collected: All plots

Field width: Unlimited alphanumeric character field

Tolerance: N/A

MQO: N/A

Values: English language words, phrases and numbers

#### 9.5 GPS COORDINATES

Use a global positioning system (GPS) unit to determine the plot coordinates and elevation of all ozone plot locations. GPS readings are collected according to procedures outlined in the FIA National Core Field Guide for Phase 2 & 3 Plots, Version 2.0. The ozone data entry applications accept GPS readings obtained using a geographic coordinate system (not UTM). If you are using UTM, record readings on the field data sheet for mapping and on the PDR Plot Notes screen. If GPS coordinates cannot be collected, elevation and plot coordinates are obtained from USGS topographic maps, generally the 7½ minute series quadrangle. Record elevation on the Plot ID screen and approximate latitude and longitude on the Plot Notes screen.

Use a global positioning system (GPS) unit to determine the plot coordinates and elevation of all field-visited plot locations.

NOTE: For several of the following GPS variables, the term plot center is used. There may be no obvious center to the ozone plots. Coordinates are collected as close as possible to a central location or marker that clearly locates the plot for returning crews. Explanatory notes are added to the plot map and Plot Notes screen as needed.

#### 9.5.1 GPS Unit Settings, Datum, and COORDINATE SYSTEM

Consult the GPS unit operating manual or other regional instructions to ensure that the GPS unit internal settings, including Datum and Coordinate system, are correctly configured.

Each FIA unit will determine the Datum to be used in that region. Most will use the NAD 27 Datum (also known as NAS-C or NA 27 CONUS/CLK66), but coordinates collected using any appropriate datum can be converted back to a national standard for reporting purposes. **The Northeast uses NAD 27 Datum.**

Each FIA unit will also determine which coordinate system to use. Regions using a Geographic system will collect coordinates in Degrees, Minutes, and Seconds of Latitude and Longitude; the regions using the UTM coordinate system will collect UTM Easting, Northing, and Zone. **The Northeast uses the Geographic coordinate system.**

#### 9.5.2 Collecting Readings

Collect at least 180 GPS readings at the plot center (see Note above). These may be collected in a file for post-processing or may be averaged by the GPS unit. Each individual position should have an error of less than 70 feet if possible (the error of all the averaged readings is far less).

Soon after arriving at plot center, use the GPS unit to attempt to collect coordinates. If suitable positions (180 readings at error less than or equal to 70 feet) cannot be obtained, try again before leaving the plot center.

If it is still not possible to get suitable coordinates from plot center, attempt to obtain them from a location within 200 feet of plot center. Obtain the azimuth and horizontal distance from the "offset" location to plot center. If a PLGR unit is used, use the Rng-Calc function in the PLGR to compute the coordinates of the plot center. If another type of GPS unit is used, record the azimuth and horizontal distance as described in Sections 1.14.12 and 1.14.13.

Coordinates may be collected further away than 200 feet from the plot center if a laser measuring device is used to determine the horizontal distance from the "offset" location to plot center. Again, if a PLGR unit is used, use the Rng-Calc function in the PLGR to compute the coordinates of the plot center. If another type of GPS unit is used, record the azimuth and horizontal distance as described in Sections 1.14.12 and 1.14.13.

In all cases try to obtain at least 180 positions before recording the coordinates.

#### 9.5.3 GPS UNIT

Record the kind of GPS unit used to collect coordinates. If suitable coordinates cannot be obtained, record 0.

When collected: All field visited plots

Field width: 1 digit

Tolerance: No errors

MQO: At least 99% of the time

Values:

0	GPS coordinates not collected
1	Rockwell Precision Lightweight GPS Receiver (PLGR)
2	Other brand capable of field-averaging
3	Other brands capable of producing files that can be post-processed
4	Other brands not capable of field-averaging or post processing

#### 9.5.4 GPS SERIAL NUMBER

Record the last six digits of the serial number on the GPS unit used.

When collected: When GPS UNIT >0

Field width: 6 digits

Tolerance: No errors

MQO: At least 99% of the time

Values: 000001 to 999999

#### 9.5.5 GPS LATITUDE

Record the latitude of the plot center to the nearest hundredth second, as determined by GPS.

When collected: When GPS UNIT = 1, 2, 3 or 4

Field width: 8 digits (DDMMSSSS)

Tolerance: +/- 140 ft

MQO: At least 99% of the time

Values:

#### 9.5.6 GPS LONGITUDE

Record the longitude of the plot center to the nearest hundredth second, as determined by GPS.

When collected: When GPS UNIT = 1, 2, 3 or 4

Field width: 9 digits (DDDMMSSSS)

Tolerance: +/- 140 ft

MQO: At least 99% of the time

Values:

#### 9.5.7 GPS ELEVATION

Record the elevation above mean sea level of the plot center, in feet, as determined by GPS.

If no GPS Unit is available, record elevation from the appropriate USGS topographic map.

When collected: When GPS UNIT = 1, 2 or 4

Field width: 6 digits

Tolerance:

MQO: At least 99% of the time

Values: -00100 to +20000

#### 9.5.8 GPS ERROR

Record the error as shown on the GPS unit to the nearest foot.

When collected: When GPS UNIT = 1 or 2

Field width: 3 digits

Tolerance: No errors

MQO: At least 99% of the time

Values: 000 to 070 if possible; 071 to 999 if an error of less than 70 cannot be obtained

#### 9.5.9 NUMBER OF GPS READINGS

Record a 3-digit code indicating how many readings were averaged by the GPS unit to calculate the plot coordinates. Collect at least 180 readings if possible.

When collected: When GPS UNIT = 1 or 2

Field width: 3 digits

Tolerance: No errors

MQO: At least 99% of the time

Values: 001 to 999

#### 9.5.10 GPS FILENAME (CORE OPTIONAL)

Record the filename containing the GPS positions collected on the plot.

When collected: When GPS UNIT = 3

Field width: 8 characters.3 characters e.g. R0171519.ssf

Tolerance: No errors

MQO: At least 99% of the time

Values: Letters and numbers

### 9.6 FOLIAR INJURY DATA

All measurement codes for the foliar injury data are defined below. Plants selected for ozone injury evaluations are rated for the percent of injured area and the severity of injury on a scale of 0 to 5 (see section 9.2.6). If a plant does not have injury, it is tallied with zeros for these measurements. A pop-up menu keeps track of plant counts by species. The plot is complete only when you have tallied 30 plants of at least 3 species, or when no additional plants can be found on the plot. Ozone plots vary in size and do not have set boundaries. Time and safety concerns should dictate how much ground area to cover to complete the foliar injury evaluation procedures.

#### 9.6.1 SPECIES

Record the three-digit code that identifies each species on the plot. Species codes may be entered in the order they are encountered as you walk through the plot evaluating plants. A pop-up menu keeps a running total of numbers of plants and species evaluated.

When collected: All plots

Field width: 4 digits

Tolerance: No error

MQO: At least 90% of the time

Values: See 9.2.5

### 9.6.2 AMOUNT

Record the code that identifies the percentage of leaves on the plant with ozone injury symptoms relative to the total number of leaves on the plant. The percent scale code and definitions are fully described in Subsection 9.2.7.

When collected: All plots

Field width: 1 digit

Tolerance: +/- 1 class

MQO: At least 90% of the time

Values:

0	No injury; The evaluated plant does not have any leaves with ozone symptoms.
1	1 to 6 percent of the leaves have ozone symptoms
2	7 to 25 percent of the leaves are injured.
3	26 to 50 percent of the leaves are injured.
4	51 to 75 percent of the leaves are injured.
5	Greater than 75 percent of the leaves have ozone symptoms.

### 9.6.3 NUMBER OF PLANTS

Record the number of plants tallied so far with no injury. When 0 is entered for AMOUNT, the PDR prompts for the NUMBER OF PLANTS with no injury. When a number greater than zero is entered for AMOUNT, the PDR prompts for the associated SEVERITY value. Zero and non-zero values for any species can be entered as they are encountered on the plot. The pop-up menu keeps track of plant counts by species.

When collected: When AMOUNT = 0

Field width: 2 digits

Tolerance: No error

MQO: At least 90% of the time

Values: 1 to 30

### 9.6.4 SEVERITY

Record the code that identifies the mean severity of symptoms on injured foliage. The percent scale code and definitions are fully described in Subsection 9.2.7.

When collected: When AMOUNT > 0

Field width: 1 digit

Tolerance: +/- 1 class

MQO: At least 90% of the time

Values:

0	No injury. The evaluated plant does not have any leaves with ozone symptoms.
1	On average, 1 to 6 percent of the leaf area of injured leaves has ozone symptoms.
2	On average, 7 to 25 percent of the leaf area of injured leaves has ozone symptoms.
3	On average, 26 to 50 percent of the leaf area of injured leaves has ozone symptoms.
4	On average, 51 to 75 percent of the leaf area of injured leaves has

- 5 ozone symptoms.  
On average, greater than 75 percent of the leaf area of injured leaves has ozone symptoms.

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## **9.8 ACKNOWLEDGEMENTS**

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## Appendix 9.A Key Identifying Characteristics of the Ozone Bioindicator Species

1. **Blackberry** is an upright or arching shrub; greenish to greenish-red stems are ridged with stout prickles. Alternate leaves have 3-7, mostly 5, leaflets, sparingly pubescent above, velvety beneath, green on both sides. Flowers white, May-July. Fruits black, July-September. Dewberry is very similar to common blackberry, but it is a vine with prickly stems trailing over the ground. Raspberry has smaller leaves and rounded stems covered with a whitish bloom. Blackberry is found in dry fields, clearings, and sunny thickets.

2. **Black Cherry** is a small to large tree. Twigs have a bitter-almond smell and taste. The alternate leaves are narrow, shiny, 2-6 inches long, and blunt-toothed, with the midrib prominently fringed beneath with white to brown hair. Leaves of choke cherry, a similar species, have a hairless midrib beneath and are sharp toothed. Leaves of pin cherry are longer and narrower with finely serrated margins. Black cherry is found on a variety of forest soils, deep and moist to dry and gravelly, and along the edges of disturbed areas.

3. **Common Milkweed** is recognized by a solitary, simple stem 1-6 feet tall that may or may not be covered with hair. The opposite or whorled leaves are twice as long (2 to 12 inches) as they are wide, have smooth margins, and stems with milky juice. The surface of the leaf is hairy below and smooth above. The petioles are short and thick. Flowers are borne in large clusters on stalks in the upper nodes. They appear rose or greenish-white, from June to August. You may see developmental stages of the Monarch butterfly or feeding injury on the plants. Milkweed is common along roadsides, in fields and meadows.

4. **Yellow Poplar** is a tall, straight, forest tree found on good sites with many hardwoods and loblolly pine in the South. Leaves are 4 to 6 inches in diameter, squarish at base, mostly 4-lobed, with smooth margins. Twigs stout, bitter to taste, with diaphragmed pith. Bud shaped like a duck's bill.

5. **White ash** is characterized by opposite, compound leaves; leaflets 5-9, stalked, green above and white or pale beneath, usually with smooth margins, slightly toothed near the leaf tips. Buds are inset in the leaf scar. Twigs are round, shiny, and mostly hairless. White ash is difficult to distinguish from green ash; Green ash leaves tend to be narrower, with more teeth, and hairy beneath; buds are set above the leaf scar and branch stems are usually hairy. Ash is sometimes confused with hickory, but can be readily distinguished by its opposite leaves and buds.

6. **Sassafras** has a characteristic odor and taste, spicy. Leaves are simple, narrowly lobed (mitten shaped) or entire. Twigs are green. Found from southwestern Maine, south to Florida, north to central Michigan, and west.

7. **Sweetgum** has star shaped leaves, deeply 5-7 lobed, margin finely serrate, bright green above, hairy in the axils of the leaf veins below. Twigs shiny and green to yellowish brown, somewhat fragrant when crushed. Fruit a spiny ball, often hanging. Common on bottomland soils and old fields from southern Connecticut, south to Florida and west.

8. **Pin Cherry** is a small, shrubby tree often found on cut over, burned, or abandoned sites. Leaves are long, narrow, finely serrate, and yellow-green; less shiny than those of black cherry. Pin cherry leaves may look like black cherry leaves, but they have no hair beneath. Maine to northern Georgia and west.

9. **Spreading Dogbane** is a perennial herb characterized by its opposite leaves with smooth margins and red stems with milky juice. The simple leaves are oblong or egg-shaped, dark green above and pale beneath; 2-3 inches long. The plant grows 1-4 feet high and has wide-spreading branches that give the plant an awkward appearance. It flowers throughout the summer; pinkish with a pink stripe in the center. Pods are long and narrow, in pairs. Young milkweed may be confused with dogbane, but differs in having larger, thicker leaves, hairy on the under surface. If evident, milkweed flowers are showy and the pods are large. Dogbane prefers the edges of dry woods from Canada to Mexico, but is also found in dry fields and thickets.

10. **Bigleaf Aster** is a perennial wild flower commonly found as an understory plant in dry woods. The leaves of this aster are heart shaped, 3 or more inches wide, with unevenly toothed margins, and have a stem nearly as long as the length of the leaf. Near the flat-topped flower cluster, the leaves become smaller and the stems are margined by a wavy leaf portion called a wing. Flowers may be violet, lavender, or light blue; evident in August and September. The plant grows 1-4 feet high and is native over eastern U.S. and south to North Carolina, west to Illinois.

**Appendix 9.B Ozone Data Sheets.****OZONE BIOINDICATOR PLANTS - 2003****Site Characteristics**

This sheet must be completed only if you have *not* entered this same information on the Bioindicator Plot ID screen.

To be filled out by the FIELD CREW or Cooperator: Refer to Field Guide 1.6 for code definitions.

State	County	Ozone Hexagon Number	Plot Number <sup>1</sup>	Month	Day	Crew ID	Crew Type
							regular QA

<sup>1</sup>Plot Number refers to the number of locations (1 or 2) used for each hexagon number. A separate sheet should be used for each location.

√ Please put a check mark beside the correct information. Please complete all data fields.

Ozone Sample Kind:	
<input type="checkbox"/>	Initial plot establishment on the FIA ozone grid.
<input type="checkbox"/>	Remeasurement of a previously established plot.
<input type="checkbox"/>	Replacement of a previously established plot that was replaced to meet the site selection guidelines (or lost site).

Ozone Grid Density: (Is the grid intensified, or not?)	
<input type="checkbox"/>	This hex number identifies a unique ozone plot within a polygon (1 site:1 polygon)
<input type="checkbox"/>	One of two or more ozone plots within the same polygon, each with their own hexagon number.

Plot size:		Terrain position:	
<input type="checkbox"/>	> 1.2 hectares (3.0 acres)	<input type="checkbox"/>	Ridge top or upper slope
<input type="checkbox"/>	0.4 – 1.2 hectares (1 to 3 acres)	<input type="checkbox"/>	Bench or level area along a slope
<input type="checkbox"/>	Other: please describe	<input type="checkbox"/>	Lower slope
<input type="checkbox"/>		<input type="checkbox"/>	Flat land unrelated to slope
<input type="checkbox"/>		<input type="checkbox"/>	Bottom land with occasional flooding

Aspect: 000° = no aspect; 360° = N aspect		Elevation: record estimate in feet or meters	
Record to nearest degree =		Feet =	Meters =

Soil Drainage:		Soil Depth:	
<input type="checkbox"/>	Well-drained	<input type="checkbox"/>	Bedrock not exposed
<input type="checkbox"/>	Wet	<input type="checkbox"/>	Bedrock exposed
<input type="checkbox"/>	Excessively dry		

Disturbance: Disturbance on the site or in localized areas where the bioindicator plants are growing.	
<input type="checkbox"/>	No recent or significant disturbance; Do not count disturbance >3 years old.
<input type="checkbox"/>	Evidence of overuse; Human activity causing obvious soil compaction or erosion.
<input type="checkbox"/>	Evidence of natural disturbance including fire, wind, flooding, grazing, pests, etc.

Fill in below all that apply. Check here if geographic coordinates were obtained from a topographic map:

GPS Type:		GPS Serial Number:	
Latitude =		GPS Error =	
Longitude =		Number of Readings =	
Elevation =		GPS File Name =	

<sup>1</sup>If no GPS Unit is available, please use a map and record estimated latitude, longitude, and elevation for each plot location.

**Comments:** Include information on additional species in the area, safety, directions, or additional site characteristics that may be useful.

**File this completed data sheet with the sheet used for mapping the Bioindicator Site Location and then store it in the appropriate Ozone Plot Folder for your State or Region.**

### OZONE BIOINDICATOR PLANTS - 2003

**Foliar Injury Data – Use this sheet *only* if no PDR is available for data entry!**

State	Cty	Hexagon No.	Plot No.	Month	Day	Measurement Type (check one):
						<input type="checkbox"/> Regular crew <input type="checkbox"/> QA crew

<sup>1</sup>Plot Number refers to the number of locations (1 or 2) used for each hexagon number. A separate sheet should be used for each location.

Record species code number (use additional sheets for >3 species at one site): **0915** Blackberry    **0762** Black cherry  
**0365** Milkweed    **0621** Yellow Poplar    **0541** White ash    **0931** Sassafras    **0611** Sweetgum    **0366** Dogbane spp.  
**0364** Big leaf aster    **0761** Pin cherry. Record the percent of the leaves injured relative to the total leaf number  
**(Amount)** and the average severity of symptoms on the injured leaves **(Severity)**. Add notes to the back of this sheet.

**0 = No injury;    1 = 1-6%;    2 = 7-25%;    3 = 26-50%;    4 = 51-75%;    5 = >75%**

Species Code			Species Code			Species Code		
Plant	Amount	Severity		Amount	Severity		Amount	Severity
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
Enter codes	Injury Location	Injury Type		Injury Location	Injury Type		Injury Location	Injury Type

**Did you collect 3 leaves that clearly show ozone stipple, for each injured species?**

**OZONE BIOINDICATOR PLANTS**  
**Data Sheet for Mapping the Bioindicator Site Location**

To be filled out by the FIELD CREW or State Cooperator: Refer to Field Guide 1.6 for code definitions.

State	County	Ozone Hexagon Number	Plot Number	Month	Day	Year	Crew ID

<sup>1</sup>Plot Number refers to the number of locations (1 or 2) used for each hexagon number. A separate mapping sheet should be used for each location.

PLGR Information: (Please fill in this information, if available)

Easting	Northing	+/- Error (ft.)	Grid Zone

Include the following information on the map:

- (1) Location of the site relative to some obvious and permanent marker
- (2) Road names and distances as needed
- (3) North arrow
- (4) Starting point for plant selection
- (5) Species codes and approximate location of plant groupings used for the ozone injury evaluations.

**Return the original of this map to the corresponding Plot Folder so that it can be used by audit and regular crews in subsequent visits to the plot. Mail a copy to the National Indicator Advisor the year that the site is established.**

Geographic coordinates	
GPS Latitude	GPS Longitude
Latitude estimated from a topographic map:	
Longitude estimated from a topographic map:	

## OZONE BIOINDICATOR PLANTS

### Summary Sheet on Field Methods

Decision Table	First Choice = <b>Best Site</b>	Second Choice
Access:	Easy	Easy
Location:	Single location is used.	One or two locations (split-plot).
Size of opening:	>3 acres (1.2h); wide open area; <50% crown closure.	Between 1-3 acres; long narrow or irregularly sized opening.
Species count:	More than three species.	Two or more species.
Plant count:	30 plants of 3 species; 10-30 plants of additional species.	30 plants of 2 species; 10-30 plants of additional species.
Soil conditions:	Low drought potential; good fertility.	Moderate dry; Moderate fertility.
Site disturbance:	No recent (1-3 years) disturbance; No obvious soil compaction.	Little or no disturbance; No obvious soil compaction.

#### Unbiased Plant Selection:

1. Identify a starting point at the edge of the opening and record this on the site map.
2. Move towards the center of the opening, locating individuals in a sweeping pattern and selecting plants that are growing under similar growing conditions.
3. Select the more exposed plants and avoid suppressed and shaded individuals. Do not skip plants with little or no injury.
4. Evaluate the foliage on each plant for the amount and severity of injury. Record it electronically.
5. Collect a leaf voucher (3 leaves) showing ozone injury on each species. Store it in a leaf press.

#### Characteristics of ozone injury on broad-leaf plants:

1. Symptoms are usually more severe on mid-aged and older leaves.
2. New leaves will have no or very little injury.
3. Symptoms are most likely confined to the upper leaf surface as tiny purple-red to black spots.
4. Check leaves covering each other. Overlapped leaves will have no injury on the bottom leaf.
5. There will be some uniformity to size and shape of the lesions (stippling) on a leaf.
6. Stippling may be associated with leaf yellowing or premature senescence.

**INJURY AMOUNT:** Estimate and record the percentage of leaves on the plant with ozone injury symptoms relative to the total number of leaves on the plant.

<u>CODE</u>	<u>DEFINITION</u>
0	No injury; the plant does not have any leaves with ozone symptoms.
1	1 to 6 percent of the leaves have ozone symptoms.
2	7 to 25 percent of the leaves are injured.
3	26 to 50 percent of the leaves are injured.
4	51 to 75 percent of the leaves are injured.
5	>75 percent of the leaves have ozone symptoms.

**INJURY SEVERITY:** Estimate and record the mean severity of symptoms on injured foliage.

<u>CODE</u>	<u>DEFINITION</u>
0	No injury; the plant does not have any leaves with ozone symptoms.
1	On average, 1 to 6 percent of the leaf area of injured leaves have ozone symptoms.
2	On average, 7 to 25 percent of the leaf area of injured leaves have ozone symptoms.
3	On average, 26 to 50 percent of the leaf area of injured leaves have ozone symptoms.
4	On average, 51 to 75 percent of the leaf area of injured leaves have ozone symptoms.
5	On average, >75 percent of the leaf area of injured leaves have ozone symptoms.

0915 Blackberry 0762 Black cherry 0365 Milkweed 0621 Yellow poplar 0541 White ash 0931 Sassafras  
0366 Spreading dogbane 0364 Bigleaf aster 0611 Sweetgum 0761 Pin cherry.

The best sites are large open areas with 3, 4, or 5 species and close to 30 plants of each species.

**fiaozone.net**

**OZONE BIOINDICATOR PLANTS**  
**Data Sheet for the Voucher Leaf Samples**

To be filled out by the FIELD CREW or State Cooperator: Refer to Field Guide 1.6 for code definitions.

State	County	Ozone Hexagon Number	Plot Number	Month	Day	Year	Crew ID

<sup>1</sup>Plot Number refers to the number of locations (1 or 2) used for each hexagon number. A separate mapping sheet should be used for each location.

To be filled out by the Cooperator (only needed when the hex number and tally numbers are not known).

Ozone plot name or identification number	Name and e-mail address of data collector

Fill in the required codes. Code definitions are in the Field Guide. For quick reference, see below.

Bioindicator Species	Injury Location	Injury Type	Is the leaf sample injury close to 100% ozone stipple, or is some other upper-leaf-surface injury also present?
			Close to 100% _____ Estimated percent other _____
			Close to 100% _____ Estimated percent other _____
			Close to 100% _____ Estimated percent other _____
			Close to 100% _____ Estimated percent other _____

Notes: Add notes on the leaf samples, supplemental species, plot conditions, safety, and weather as needed.

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0915 Blackberry 0762 Black cherry 0365 Milkweed 0621 Yellow poplar 0541 White ash 0931 Sassafras  
 0611 Sweetgum 0761 Pin cherry 0366 Spreading dogbane 0364 Bigleaf aster.  
 Injury Location codes: 1 = greater than 50% of the injured leaves are younger leaves; 2 = greater than 50% of the injured leaves are mid-aged or older; 3 = injured leaves are all ages.  
 Injury Type codes: 1 = greater than 50% of the injury is upper-leaf-surface stipple; 2 = greater than 50% is not stipple (tan flecks, bifacial or general discoloration); 3 = injury is varied or difficult to describe.

**Mail this sheet with the leaf samples to :**

Gretchen Smith  
 Department of Natural Resources Conservation  
 160 Holdsworth Way  
 University of Massachusetts  
 Amherst. MA 01003

QA/QC PERSON: To be filled out by the National Ozone Advisor or Regional Expert. ✓

Date checked	Date rechecked	Sample Condition			Plot Status
		Good:	Fair:	Poor:	

Bioindicator Species	Positive for Ozone	Negative for Ozone	Explanation

## Appendix 9.C Detailed Procedures for Handling Leaf Vouchers

### Leaf Collection in the Field

1. Collect 3 leaves from each species showing ozone injury symptoms
  - These 3 leaves should be from different plants, if possible
  - These 3 leaves should show obvious injury rather than the range of different symptoms you may have observed
2. Once the leaf vouchers are cut from a plant they should be placed immediately into a plant press
  - Each leaf should have its own space on the blotter paper – do not overlap leaves
  - Each leaf should be marked with the date and the hex number in case they get shuffled
  - Leaves that are not put into a plant press immediately will wrinkle and break easily when handled
  - Leaves that are laid on top of each other will “bleed” such that all overlapped leaves become murky and the ozone injury symptom is no longer visible
3. Before you leave the plot where you have collected voucher leaves, fill out the leaf voucher data sheet and complete the Tally PlotID screen on the PDR. There is important information on the voucher sheet and on the PDR screen that you need to record while you are standing on the biosite. You will not remember these details later on, so take the time to get it right while you are on the plot.
4. Pressed leaves can be removed from the plant press after 36 hours. Once they are flat and dry they can be kept in mailing envelopes, folders, or newspaper until you have time to mail them in to be validated.

### Leaf Preparation for Mailing

1. First, label each mailing envelope with the 7-digit hex numbers that you insert into each envelope. Mark the outside of the mailing envelope on the side where the list of numbers will not interfere with the address information.
2. Each mailing envelope may contain the leaves from ONE or SEVERAL hexes (biosites). Use common sense to decide how many leaves will fit comfortably into each envelope. Don't forget to mark the outside with each OZONE HEXAGON NUMBER that has been included.
3. Each pile of leaves from each plot should be placed on top of the corresponding voucher data sheet. It is very helpful to include an additional blank piece of recycled paper or newspaper to help keep larger piles of leaves separated from each other.
4. At least 1 of the 3 leaves (and preferably all 3 leaves) you have selected for each species should have a petiole label with the hex number written on it. This will prevent data loss, if the leaf pile is dropped and separated from its corresponding voucher data sheet when the leaves are removed from the envelope.
  - Do not put large piles of leaves into a single mailing envelope. Help minimize human error by mailing the leaves and data sheets 1 plot per envelope or, up to 5 plots per envelope, depending on how many species were injured and how many leaves and data sheets must be mailed in.
  - Use the 10"x12" size mailing envelopes that are provided at training. If you make a substitution, make sure it is approximately the same size or larger.
  - If you choose to mail larger piles in a single large mailing container, please use newspaper or manila folders to separate vouchers and their corresponding data sheets by OZONE HEXAGON NUMBER.
  - The only way it is safe and acceptable to mail unmarked leaves (no petiole label), is if each group of leaves from each biosite is contained in a separate mailing envelope (10"x12") that contains the corresponding voucher data sheet and is clearly marked with the appropriate OZONE HEXAGON NUMBER.

5. If you have the time and the resources, supply a cover sheet that lists all of the hex numbers you have included in your mailing(s). It is also extremely helpful if the leaves and vouchers are organized by OZONE HEXAGON NUMBER in increasing or decreasing order, e.g., 4207328, 4207422, 4307521, etc.
6. Feel free to ask for the return of your leaves and a copy of the voucher data sheets. This will only be done on request. Remember that the validation process begins in mid-September and may take until December to complete. If you have a time constraint and need a quick response, please note this on the OUTSIDE of the mailing envelope so that it will be noticed upon arrival.
7. If you have mailed extra leaves (>3 per species) for any purpose, please attach a handwritten note explaining what you have done. Clearly mark which 3 leaves should be used to validate the ozone injury at each site. Explain clearly what additional review of leaf samples is of interest to you and include a separate voucher data sheet for this purpose.
8. If you have mailed in samples of supplementary species that are not on the official bioindicator list, please keep these separated by hex number or off grid location and provide a separate data sheet for these extra species, 1 sheet per species. If possible, provide GPS coordinates of any off-grid sampling locations. Species found within approximately 3 miles of the established biosite are still on the grid and do not require additional GPS data.

