Section 20. Ozone Bioindicator Plants

20.1 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).

20.1.2 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 INJURY LOCATION codes are recorded to fully describe the injury observed in the field. Additional quality control measures include field revisits and audits of 10 percent of the biomonitoring sites.

The implementation of an ozone survey 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.

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Ozone site maps are used by audit and regular crews in subsequent visits to the plot (see fig. 20-1) 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.

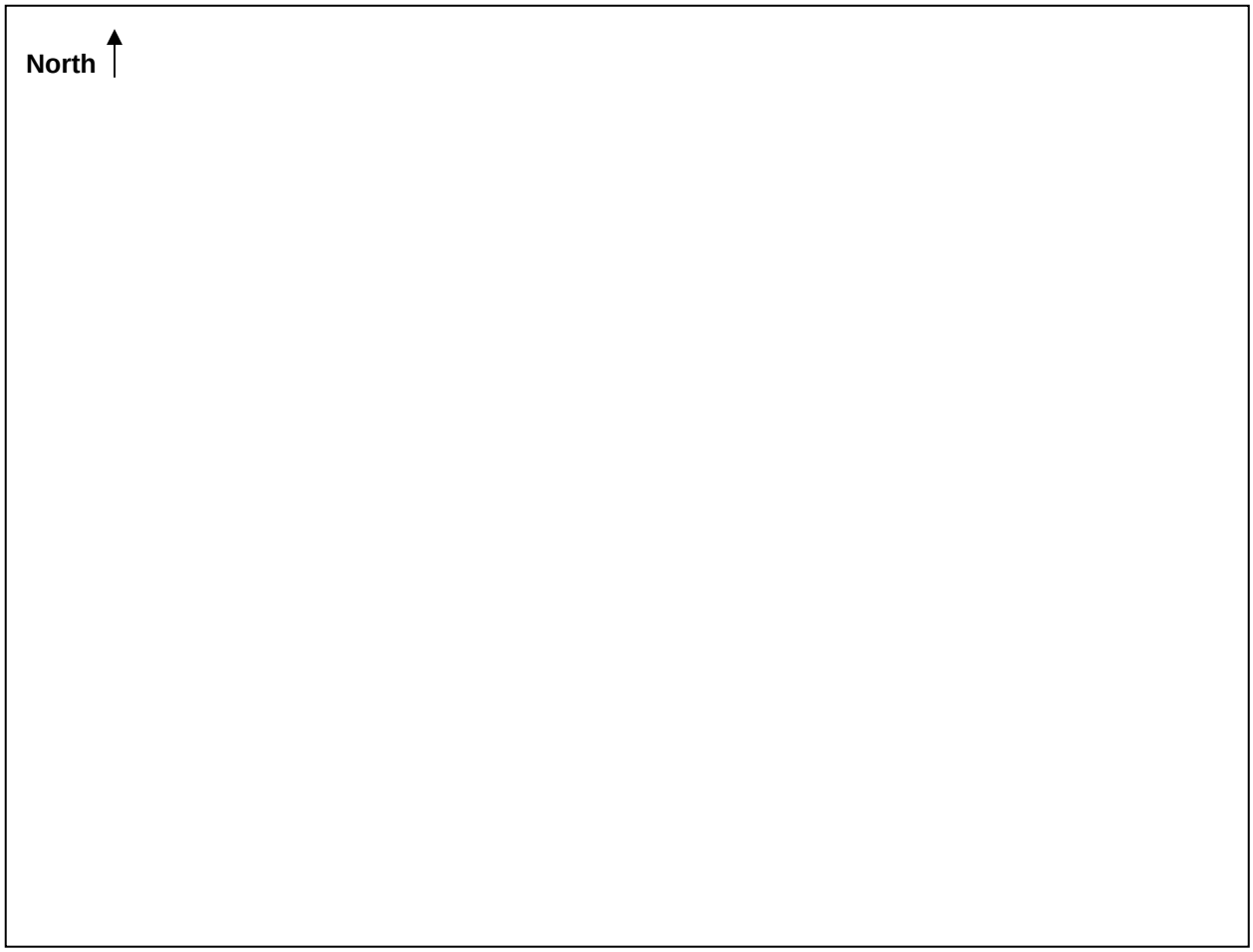

Figure 20-1. Example of a well-drawn map of an ozone biomonitoring site showing the location of the site relative to an obvious and permanent marker (road intersection and house number), road names and distances, North arrow, species codes and approximate location of plant groupings, location and distance to two major roads, distance and direction to two major towns, and Gazetteer reference page.

20.2.4 Split Plots

Maximizing the quality of each ozone plot with respect to the number of plants and species that are evaluated for ozone injury is a priority. As indicated in the site selection Decision Table in section 20.2.2, 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 (fig. 20-2). 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 FIELD ID is assigned to each location. However, each location is assigned a different SPLITPLOT ID (1 or 2) so that each location can be uniquely identified and described. In the following example (fig. 20-2), the distance between the two open areas is less than 3 miles. The site selection criteria for a high quality ozone biosite are met as the total species and plant counts for FIELD ID XXXXXXX are black cherry = 38, white ash = 30, milkweed = 30, and dogbane = 15.

Table 20-3. Target species and codes for the Interior Region

Code	Definition	Scientific Names
0122	Ponderosa pine[1]	*Pinus ponderosa*
0116	Jeffrey pine[2]	*Pinus jeffreyi*
0960	Blue elderberry	*Sambucus mexicana*

Table 20-5. Target species and codes for the Plains States

Code	Definition – Bioindicator Species	Scientific Names
0915	Blackberry	*Rubus allegheniensis* (second year canes only)
0762	Black Cherry	*Prunus serotina*
0365	Common and Tall Milkweed	*Asclepias spp.*
0621	Yellow Poplar	*Liriodendron tulipifera*
0541	White Ash	*Fraxinus americana*
0931	Sassafras	*Sassafras albidum*
0366	Spreading Dogbane	*Apocynum androsaemifolium*
0364	Big Leaf Aster	*Aster macrophyllus: Eurybia macrophylla*
0611	Sweetgum	*Liquidambar styraciflua*
0761	Pin Cherry	*Prunus pensylvanica*
0122	Ponderosa pine	*Pinus ponderosa*
0908	Mugwort	*Artemisia douglasiana*
0746	Quaking aspen	*Populus tremuloides*
0909	Skunk bush	*Rhus trilobata*
0905	Ninebark	*Physocarpus malvaceus*
0969	Snowberry and coralberry	*Symphoricarpos* spp.
0907	Western wormwood	*Artemisia ludoviciana*
0968	Evening primrose	*Oenothera elata*

Site selection requirements for species and plant counts (section 20.2.2, Decision Table for site selection) must be met using the species listed on the preceding tables (Tables 20-2, 20-3, 20-4, and 20-5). A list of supplemental bioindicator species (e.g., *Sambucus canadensis* American elder) that may be used as the 4th, 5th, and 6th, species at a selected biomonitoring site is provided in Appendix 20.E. This list may be updated annually as new information becomes available. Species on the supplemental list 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 maintain a record of when supplemental species have been used at a site.

NOTE: New data collection software may allow supplemental species (code 0998) to be tallied electronically using the same procedures as those used for the target species for each region.

20.2.6 Plant Selection

After site and species selection, the next task is contiguously to 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 in the East are expected to complete 3 ozone sites in a ten hour work day while crews in the West are expected to complete 2 ozone sites per 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.

The most common leaf injury symptom on broadleaf bioindicator species is upper-leaf-surface ozone stipple. On ponderosa and Jeffery pine, the most common needle injury symptom is ozone chlorotic mottle.

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

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

When scoring foliar symptoms on pines, check for the following characteristics of ozone injury:

Symptoms are visible as diffuse yellow areas (chlorotic mottle) without sharp borders between green
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WEST NOTE: Missowinwhorlszo(On ponderosn piashluielpl)Tj235T58 0 TD-.0013 Tc.0006 T(e tal brfr bordle af ozone injua) w

n bro

INJURY LOCATION: Specify the leaf age or position of the leaves with ozone injury (West – broadleaf

20.2.9 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. The STATE, COUNTY, FIELD ID, SPLITPLOT ID, DATE, QA STATUS, and crew identification codes must be entered correctly on the voucher data sheet before mailing. 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. Additional guidance on voucher preparation and mailing procedures is provided in Appendix 20.C.

WEST NOTE: Western crews are encouraged to add information on the biosite location to the voucher data sheet such as the uncoded name of the county or closest town. This helps the Western Regional Trainer map the initial findings from the leaf vouchers and alert FIA staff to high ozone areas.

The lower half of the voucher data sheet is filled out by the National Field Ozone Advisor (East) or Western Regional Trainer (West) 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 Field Advisor (East) or Western Regional Trainer (West). Manila folders and newspaper may also be used for voucher mailings. Do not tape the leaves or needles 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.

WEST NOTE: The Western Regional Trainer will make every effort to provide immediate feedback on the leaf vouchers. To facilitate this, crews must fill in the contact information on the voucher data sheet.

20.2.10 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.

20.2.11 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

20.4.3 FIELD ID

Record the unique code assigned to each ozone polygon. In some cases this will be a former FHM or P3 polygon number or special use plot number.

When collected: All plots
Field width: 7 digits
Tolerance: No errors
MQO: At least 99% of the time
Values: 0000001 - 9999999

20.4.4 SPLITPLOT ID

Record the SPLITPLOT ID 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 biosite. The FIELD ID is the same for both locations.

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

1 The ozone biosite consists of a single location or this is the first location of a biosite split between two locations.
2 The ozone biosite 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 polygon number.

20.4.5 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 to 2 and 4 to 7

1 Standard ozone plot
2 Cold ID 1 off griinelot 5[(1)-15BoteIedone plf3 mlo

20.4.7 OZONE SAMPLE KIND

Record the code that describes the kind of plot being visited.

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

1 Initial plot establishment on the base grid or on a newly intensified grid.
2 Remeasurement of a previously established plot, or replacement biosite within 3 miles of the previously established plot.
3 Replacement when the replacement biosite is more than 3 miles from the previously established plot.

20.4.8 CURRENT DATE

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

20.4.8.1 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

20.4.8.2 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: 01 to 12

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

20.4.8.3 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

20.4.9 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: At least 99% of the time
Values: 1 to 2

1 Greater than or equal to three acres.
2 Greater than one acre, but less than three acres.

20.4.10 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 - 360

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

20.4.11 TERRAIN POSITION

20.4.13 SOIL DRAINAGE (East only)

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: No error
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

Values: 0 to 1

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.

20.4.16 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: -00100 to +20000

20.4.17 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 the Datum used. 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.

20.4.17.1 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

20.5 GPS Coordinates

Use a global positioning system (GPS) unit to determine the plot coordinates and elevation of all field visited ozone plot locations, even if GPS has been used to locate the plot in the past. GPS readings are collected according to procedures outlined in the FIA National Core Field Guide for Phase 2 & 3 Plots, Version 5.0. 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.

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.

20.5.1 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 use the NAD83 Datum to collect coordinates.

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.

20.5.2 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. Record the azimuth and horizontal distance as described in Sections 1.19.14 and 1.19.15.

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. Record the azimuth and horizontal distance as described in Sections 1.19.14 and 1.19.15.

In all cases try to obtain at least 180 positions before recording the coordinates. Coordinates not collected by automatic means shall be manually double-entered into the data recorder, both forward and backward.

20.5.3 GPS UNIT

Record the kind of GPS unit used to s is far less).

20.5.5 GPS ENTRY METHOD

Identify the method used to record GPS data. If GPS data are manually entered, record 0. If GPS data are transferred electronically from the GPS receiver to the data recorder, record 1.

Upon entering a 1 the following variables are automatically populated in accordance with the GPS receiver setup in 1.19.1 (coordinates LATITUDE, LONGITUDE or UTM, GPS ELEVATION, GPS ERROR, and NUMBER OF READINGS). All other GPS variables must be populated via manual key-entry.

When Collected: GPS UNIT > 0
Field width: 1 digit
Tolerance: No errors
MQO: at least 99% of the time
Values: 0 to1

0 GPS data manually entered
1 GPS data electronically transferred

20.5.6 GPS DATUM

Record the acronym indicating the map datum that the GPS coordinates are collected in (i.e., the map datum selected on the GPS unit to display the coordinates).

When collected: When GPS UNIT >0
Field width: 5 characters (cccnn)
Tolerance: No errors
MQO: At least 99% of the time
Values:

NAD83 North American Datum of 1983

20.5.7 Latitude

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

NOTE: The following can be customized at the region level (e.g., decimal minutes to the nearest thousandth) as long as the final results recorded are within the specified tolerance to the nearest hundredth of a second or +/- 1.01 ft.

20.5.7.1 LATITUDE DEGREES

Record the latitude degrees of the plot center as determined by GPS.

When collected: When GPS UNIT = 1, 2, 3 or 4
Field width: 2 digits
Tolerance: When GPS ENTRY METHOD = 0, No errors in data entry
When GPS ENTRY METHOD = 1, not applicable
MQO: When GPS ENTRY METHOD = 0, at least 99% of the time
When GPS ENTRY METHOD = 1, not applicable
Values: 00-90

20.5.8.3 LONGITUDE SECONDS

Record the longitude decimal seconds of the plot center to the nearest hundredth place as determined by GPS.

When collected: When GPS UNIT = 1, 2, 3 or 4
Field width: 4 digits
Tolerance: When GPS ENTRY METHOD = 0, No errors in data entry
When GPS ENTRY METHOD = 1, not applicable
MQO: When GPS ENTRY METHOD = 0, At least 99% of the time
When GPS ENTRY METHOD = 1, not applicable
Values: 0.00 – 59.99

20.5.9 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: No errors
MQO: At least 99% of the time
Values: -00100 to 20000

20.6.4 SEVERITY

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20.8 Acknowledgements

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Appendix 20.A Information and Data Sheets for the East North and South regions, and Plains States

Includes: (1) species key for eastern bioindicators, (2) species key for Plains States bioindicators, (3) data sheet for biosite characteristics, (4) data sheet for mapping biosite location, (5) foliar injury data sheet for eastern bioindicators, (6) voucher leaf sample data sheet for eastern bioindicators, and (7) voucher leaf sample data sheet for Plains States bioindicators.

Species Key for eastern bioindicators:

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.

Species Key for bioindicators used in the Plains States (e.g., North Dakota, South Dakota, Nebraska, Kansas, Oklahoma, and Texas). This key combines the 10 eastern bioindicator species with 8 western 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. De

and dark-hairy. Cones with a prickle at the tip of each scale. May be confused with Jeffrey pine which differs by having non-resinous, light-brown buds, and grayish blue-green glaucous needles.

12. **Quaking Aspen** is a medium sized tree up to 36 meters in height. Bark is smooth, greenish-white. Buds shiny but not resinous. Leaf petiole is strongly flattened. The leaf blade is broadly ovate (almost round) with a tapering tip and finely toothed margins, upper surface smooth, lower surface covered with a bloom. Aspen could be confused with black cottonwood which differs in its resinous buds, rough bark and round leaf petioles.

13. **Ninebark** is an erect, loosely branched shrub with maple-like leaves and shreddy bark. May be up to 2 meters in height. Leaves and flowers similar to Pacific ninebark except the ovaries are densely hairy. May be confused with Douglas maple which has opposite leaves, or sticky currant, which has leaves that are sticky to the touch. Often associated with ponderosa pine and Douglas-fir forests at low to mid-elevation.

14. **Western Wormwood** is an aromatic perennial herb, 0.3 to 1.0 meter in height. Leaves mostly 3-11 cm long, variable in shape but most often with 3-5 narrow lobes, white hairy beneath, sometimes above as well. Flowers small and arranged in a loose, narrow flower cluster, 5-30 cm long. May be confused with Douglas' wormwood which has wider leaves and is usually found in moister habitats. Also similar to Riverbank wormwood which occurs only near streams and outwash areas.

15. **Mugwort** is a large perennial herb 0.5 to 1.5 meters tall, usually found in large colonies in wet areas, ditches, or drainages. Leaves are evenly-spaced, 1 to 10 cm long, the upper leaves are narrowly elliptical, the lower widely oblanceolate, often coarsely 3 to 5 lobed near the leaf tip, 2 to 3 cm wide, green above, covered with dense white hair beneath. Differs from western wormwood in having wider lower leaves and in its generally damp habitat.

16. **Evening Primrose** is a large biennial with elliptical leaves up to 25 cm long in a dense rosette the first year. The large (>1m) flowering stalk with long red-tinged elliptical leaves and large bright yellow four-petaled flowers forms in the second year. Both the leaves and stem are densely hairy, and the hairs often have red, blister-like bases. Usually found in moist, sunny habitats, like seeps or meadows.

17. **Snowberry** is a shrub, 0.5 to 1.5 meters in height with a solid brown pith. Bark: shreddy, brownish. Young twigs: hairy. Leaves opposite, elliptical, 1.0 to 3.5 cm long and half as wide. Flowers

OZONE BIOINDICATOR PLANTS
Data Sheet for Mapping the Bioindicator Site Location
Add mileage information on reverse side.

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

STATE	COUNTY	FIELD ID	SPLITPLOT ID[1]	MONTH	DAY	YEAR	CREW ID

[1]SPLITPLOT ID refers to the number of locations (1 or 2) used for each FIELD ID. A separate sheet should be used for each location.

Include the following information on the map. Use a legend that indicates distances between obvious points 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) Species codes and approximate location of plant groupings used for the ozone injury evaluations.
(5) Starting point
(6) Location of and distance to two major roads
(7) Distance and direction to two major towns
(8) Gazetteer reference page if available.

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

Geographic coordinates	GPS DATUM =
GPS LATITUDE =	GPS LONGITUDE =
Latitude estimated from a topographic map =	
Longitude estimated from a topographic map =	

REDRAW THE MAP AND ADD NEW INFORMATION EACH YEAR AS NEEDED!

STATE	COUNTY	FIELD ID	SPLITPLOT ID	MONTH	DAY	YEAR	QA STATUS

OZONE BIOINDICATOR PLANTS

Data Sheet for the Voucher Leaf Samples – PLAINS STATES

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

STATE	COUNTY	FIELD ID	SPLITPLOT ID[1]	MONTH	DAY	YEAR	CREW ID	QA STATUS
								Standard QA Check

[1]SPLITPLOT ID refers to the number of locations (1 or 2) used for each FIELD ID. Separate sheets should be used for each location.

Fill in the required codes. ONE SPECIES PER LINE. Code definitions are in the Field Guide.

Bioindicator Species Code or Common Name		Injury Location	Injury Type	Is the leaf sample injury close to 100% ozone stipple ( ) , or is some other upper-leaf-surface injury also present (e.g., insect injury or fungal lesions)?
1st				Close to 100% _____ Estimated percent other _____
2nd				Close to 100% _____ Estimated percent other _____
				Close to 100% _____ Estimated percent other _____
4th				Close to 100% _____ Estimated percent other _____

Species codes: **Injury Location codes:**

Appendix 20.B Information and Data Sheets for the West
Includes: (1) species key for western bioindicators, (2) data sheet for biosite characteristics, (3) data sheet for mapping biosite location, (4) foliar injury data sheet for western bioindicators, and (5) voucher leaf sample data sheet for western bioindicators.

11. ***Mugwort*** is a large perennial herb 2 to 5 feet tall, usually found in large colonies in wet areas, ditches, or drainages. Leaves are evenly-spaced, 0.4 to 4.0 inches long, the upper leaves are narrowly elliptical, the lower widely oblanceolate, often coarsely 3 to 5 lobed near the leaf tip, 0.8 to 1.0 inches wide, green above, covered with dense white hair beneath. Differs from western wormwood in having wider lower leaves and in its generally damp habitat.

12. ***Evening Primrose*** is a large biennial with elliptical leaves up to 10 inches long in a dense rosette the first year. The large (>3ft) flowering stalk with long red-tinged elliptical leaves and large bright yellow four-petaled flowers forms in the second year. Both the leaves and stem are densely hairy, and the hairs often have red, blister-like bases. Usually found in moist, sunny habitats, like seeps or meadows.

13 ***Snowberry*** is a shrub, 1.5 to 5 feet in height with a solid brown pith. Bark: shreddy, brownish. Young twigs: hairy. Leaves opposite, elliptical, 0.4 to 1.4 inches long and half as wide. Flowers (May-June), the petals white with a pink tube. Fruit a white berry. Common snowberry differs by having a hollow pith. Trailing snowberry is a trailing shrub; and Utah honeysuckle has larger leaves and a solid white pith. Coralberry is similar to common snowberry except the fruit is a red berry. All are members of the honeysuckle family.

14 ***Red Alder*** is a deciduous tree up to 65 feet tall with dark green leaves 2.4 to 4.7 inches long. The leaves are coarsely toothed, with smaller teeth on the leaf margins, and the leaf veins are also tightly inrolled. Red alder is a common tree in damp situations and is a frequent colonizer of clearings, especially following clearcuts in coniferous forests.

15 **Skunkbush** is a small, diffusively-branched shrub, 1.6 to 3.3 feet tall. The tips of the branches often droop down almost to ground level. The leaves are alternate, compound, with three leaflets, each of which is 3-lobed. The leaves resemble those of poison oak, but the leaflets of skunkbush are smaller, more hairy, and much more deeply-lobed. The leaves of skunkbush also emit a strong, ill-scented odor when crushed. However, if unsure, DO NOT crush the leaves of a shrub with three leaflets to determine the odor. Skunkbush is usually found on dry, open, brushy hillsides, while poison oak prefers damp or shaded forested areas and riparian habitats. Skunkbush is found throughout the southwest, from California and Arizona north to Colorado and Idaho.

16 **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 in 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.

OZONE BIOINDICATOR PLANTS
BioSite Characteristics – West

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

OZONE BIOINDICATOR PLANTS
Data Sheet for Directions and Mapping for the Bioindicator Site Location

Use the table on the back of this sheet to document directions (mileage and key landmarks) to the ozone biosite.

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

STATE	COUNTY	FIELD ID	SPLITPLOT ID[1]	MONTH	DAY	YEAR	CREW ID

[1]SPLITPLOT ID refers to the number of locations (1 or 2) used for each FIELD ID. A separate sheet should be used for each location.

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. **Species codes and approximate location of plant groupings used for the ozone injury evaluations.**
5. **Location and distance to two major roads; distance and direction to two major towns.**
6. **Gazetteer reference page if available.**

GPS UNIT:	**GPS DATUM =**	**GPS SERIAL NUMBER:**	
Latitude =		**GPS ERROR =**	
Longitude =		**NUMBER OF READINGS =**	
Elevation =		**GPS FILE NAME =**	
EASTING:	**NORTHING:**	**+/-Error(ft):**	**Grid Zone:**

Return the original of this map to the corresponding Biosite 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 Field Advisor the year that the site is established

Code	Species
122	Ponderosa pine
746	Quaking aspen
924	Scouler's willow
116	Jeffrey pine
351	Red alder
969	Snowberry spp.
905	Ninebark

Amount of Injury – % of leaves injured relative to the total leaf number

Severity of Injury – Average severity of symptoms on the injured leaves

Code	Scale
0	No Injury
1	1-6%
2	7-25%
3	26-50%

Appendix 20.C Detailed Procedures for Handling Leaf Vouchers

Detailed Procedures For Handling Leaf Vouchers For Broadleaf Species

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 FIELD ID 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 Bioindicator Plot Identification Screen on the PDR. There is important information on the

10. 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.

11. If you have mailed in samples of supplementary species that are not on the official bioindicator list, please keep these separated by FIELD ID 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.

Proper Handling of Leaf Vouchers for Pine Species

1. Collect 3 leaves or 2 small branch samples from each species showing ozone injury symptoms.
   - The 3 leaves or 2 branch samples should be from different plants, if possible
   - The 3 leaves or 2 branch samples should show obvious injury rather than a range of different injury symptoms

2. Place the leaf or branch samples immediately into a plant press
   - Do not overlap leaves or needles on the blotter paper
   - Mark each sample with the date and FIELD_ID
   - Before leaving the site, fill out the top half of the voucher data sheet

3. Pressed leaves or branch samples can be removed from the plant press after 36 hours. Once they are flat and dry they can be stored in mailing envelopes or newspaper until they are mailed.

4. Branch samples that are not pressed immediately MUST BE KEPT COOL and MUST BE MAILED promptly so that they remain in good condition for the QA check on injury symptoms.
   - Keep samples out of the sun and other hot situations
   - Do not leave unprotected samples overnight in vehicles
   - Keeping samples in a cooler with ice provides adequate protection for up to 1 week
   - Refrigerate any samples that cannot be mailed promptly

5. Label each mailing envelope with the FIELD ID's of the samples that are placed in each envelope.
   - Double-check the mailing address before mailing – use the mailing labels provided at Training.
   - Double-check that all samples are labeled with their corresponding FIELD ID number and that the voucher data sheet is mailed with the corresponding leaf or branch samples
   - If you want the QA findings returned to you, please note this on the voucher data sheet and provide appropriate contact information

6. Make every effort to mail samples within 1 week of collection. If this is not possible, keep pressed leaves in a cool and dry location. Keep pine branch samples clean and dry in a plastic container in the refrigerator.

Appendix 20.D Site Ranking and Biosite Selection

BEST SITE

Crews are encouraged to use good judgment when selecting the best possible biosite for ozone injury evaluations. In all regions, the BEST biosite is a 1-3 acre opening with 3 or more bioindicator species and 30 plants of each species. Each biosite is identified by a unique, 7-digit field identification number (FIELD ID). Each biosite may consist of one or two locations on the ground. If two locations are selected, they must be within 3 miles of each other. The two locations have the same FIELD ID and are identified as SPLITPLOT ID = 1 and SPLITPLOT ID = 2. Crews must evaluate 3 species and up to 30 plants per species using whatever plants are be found at 1 or 2 locations. If more than 3 bioindicator species are found on a site, crews are strongly encouraged to evaluate at least 10 plants of these additional species.

ACCEPTABLE SITE

An ACCEPTABLE biosite is defined as one with 30 plants of only two species or fewer than 30 plants of 3 or more species. When crews are forced to settle for an acceptable biosite, they should take the time to look for and establish a second location in the current or subsequent year to increase species and plant counts.

MARGINAL SITE

Biosites of MARGINAL quality lack an acceptable number of species, or plants per species as described in the following table. Data are collected from marginal biosites only after the crew has invested ½ day without finding a biosite that meets BEST or ACCEPTABLE site rankings. Marginal sites must be replaced the following year.

SITE RANKING	Number Of Species At Each Site	Preferred Number of Plants of Each Species	Acceptable Number of Plants of Each Species	Minimum Number of Plants of Each Species
[redacted]				

Appendix 20.E Supplemental Bioindicator Species

The species listed in the following table may be used as the 4th, 5th, or 6th species at a biosite. With the exception of green ash (541), erect dogbane (366), thornless blackberry (915), and tall milkweed (365), these species may not be used to meet the requirement for 3 species per biosite as described in Section 20.2.5 in the Field Guide. If a location has only supplemental species, the crew must find a new site that meets the biosite selection guidelines.

[1]New ozone software may accept the 998 species code for supplemental species and allow crews to enter injury amount and severity data for trend analysis. Record the speces common name in the plot notes and on the voucher data sheet. *Fraxinus* spp. (541) includes both white and green ash; *Apocynum* spp. (366) includes both spreading and erect dogbane. *Rubus*