

Cumberland Piedmont Network

National Park Service
Inventory and Monitoring Division



Standard Operating Procedure 4

Sampling—Version 2.0

Please cite this as:

Cumberland Piedmont Network. 2024. Ozone and Foliar Injury Monitoring. Sampling—Version 2.0. Standard Operating Procedure. NPS/CUPN/SOP—4. Cumberland Piedmont Network, Mammoth Cave, Kentucky.

Summary

This standard operating procedure (SOP) explains the procedures used for foliar injury assessments that are followed by all project crews. Included are step-by-step instructions that cover details such as sketching a site map, collecting foliar injury data, and collecting voucher leaf samples. Foliar injury sampling is completed during the sampling window (currently late July through late August) as prescribed by the U. S. Forest Service protocol (USFS 2008).

Revision Log

Revision Date	Author	Changes Made	Reason for Change	New Version #
June 2020	Johnathan Jernigan	QA/QC procedures were added and field forms were updated. There are numerous other clarifications and minor edits/updates as well.	Version 1.0 did not include QA/QC procedures except as described by USFS. Since the USFS ozone program is no longer active, we needed to design our own QA/QC processes. Version 1.0 also used USFS field forms. We have modified those here to better reflect how we have adopted USFS processes. Many of the fields they collected were not consistent with our needs.	2.0

Procedures

Foliar Injury Sampling Procedure

1. Pre-sampling activities (see SOP 2 *Pre-Sampling—Version 2.0* [CUPN 2024]) must have been completed prior to any field sampling.
2. Depart for the sampling site. Be aware of and schedule around any inclement weather. The USFS (2008) specifies that sampling does not take place during rain events.
3. Most of the sites are accessible by vehicle. Some, however, must be accessed on foot. Most of these sites are accessible by a short walk from a parking lot or road. However, a few sites involve hiking longer distances across rough terrain. Caution is needed during the hike as prescribed in this protocol's Job Hazard Analysis (JHA).
4. Collect foliar injury data. Data are recorded on the current year's field form. The mockup of a 2023 field form is shown in Figure 1. Based loosely upon site sketches from previous years, the sampler walks systematically throughout the plot, looking at each of the selected plant species (see section below on preferred plant species). Each plant is visually inspected (with a hand lens where necessary) to determine whether any foliar injury is evident. As prescribed by the USFS (2008), only leaves on the plant that are exposed to direct sunlight are considered. As is stipulated in the USFS protocol, clonal individuals (those plants originating vegetatively, rather than sexually, from a single ancestor) are avoided. To avoid this, the USFS protocol advises the sampler to "take several steps between each plant selected for evaluation." Continue to move from one individual to the next until all individuals in the site have been examined. Ideally, 30 individuals from each of at least two species will be examined.
5. At QA/QC sites, conduct QA/QC procedures (prior to the sampling described in step 4). The network's past QA/QC field sampling at one Mammoth Cave National Park (MACA) site was expanded in version 2.0 of this protocol to include more species and sites. The primary goal of the field QA/QC is, to the highest degree possible, help the primary crew leader correctly find, identify, and quantify ozone injury. Beginning in 2024, there will be two QA/QC sites at Mammoth Cave NP and one at Abraham Lincoln Birthplace National Historic Park (ABLI). The MACA sites are advantageous because they are complex sites. This complexity is due to the size of the sites, the height of the surrounding plants in the sites, and a relatively high number of plants available for some of the plant species examined. Table 1 contains a list of species and the sites at which each will undergo QA/QC. The table lists each species and the number of plants examined at each park from 2008 to 2020, inclusively. Species with "None" listed as a QA/QC had limited numbers of plants examined between 2008 and 2020 or diminishing numbers in more recent years. The QA/QC sampling schedule is:
 - Year 1: ABLI0003

- Year 2: MACA0002
- Year 3: MACA0001
- Year 4: MACA0002
- Year 5: MACA0001
- Year 6: MACA0002

Table 1. List of QA/QC species, number of individuals found at sites in each park between 2008 and 2020, and where they will be examined for QA/QC. ABLI=Abraham Lincoln Birthplace National Historic Site; CARL=Carl Sandburg Home National Historic Site; CHCH=Chickamauga and Chattanooga National Military Park; COWP=Cowpens National Battlefield; CUGA=Cumberland Gap National Historical Park; FODO=Fort Donelson National Battlefield; GUCO=Guilford Courthouse National Military Park; KIMO=Kings Mountain National Military Park; LIRI=Little River Canyon National Preserve; MACA=Mammoth Cave National Park; NISI=Ninety Six National Historic Site; RUCA=Russell Cave National Monument; SHIL=Shiloh National Military Park; STRI=Stones River National Battlefield.

Species	ABLI	CARL	CHCH	COWP	CUGA	FODO	GUCO	KIMO	LIRI	MACA	NISI	RUCA	SHIL	STRI	QA/QC Sites
<i>Aplos americana</i>	–	2	–	–	–	–	–	–	–	–	–	–	–	–	None
<i>Apocynum</i> sp.	19	–	–	30	60	–	76	–	–	165	30	–	–	–	MACA0002
<i>Asclepias</i> sp.	10	62	–	–	60	–	1	1	–	497	26	30	–	42	MACA0001
<i>Cercis canadensis</i>	274	–	13	–	38	109	–	–	–	–	–	24	–	30	MACA0002
<i>Echinacea purpurea</i>	–	–	–	–	–	–	–	–	–	–	–	7	–	–	MACA0002
<i>Eupatorium</i> sp.	–	–	–	–	–	–	–	–	–	–	–	22	30	–	None
<i>Fraxinus</i> sp.	221	–	–	–	9	88	–	–	–	8	–	2	4	–	ABLI0003
<i>Liquidambar styraciflua</i>	32	–	–	100	17	146	120	51	60	387	120	3	124	–	MACA0001
<i>Liriodendron tulipifera</i>	212	12	–	10	78	29	17	31	5	2	–	46	3	–	MACA0002
<i>Oxydendrum arboreum</i>	–	–	–	–	22	–	–	37	4	1	–	–	–	–	None
<i>Parthenocissus quinquefolia</i>	1	–	–	–	–	1	–	–	–	–	–	–	5	–	None
<i>Platanus occidentalis</i>	31	–	1	–	66	4	–	–	–	4	–	–	–	–	None
<i>Prunus pensylvanica</i>	–	–	–	–	–	1	–	–	–	1	–	–	–	–	None
<i>Prunus serotina</i>	50	7	–	7	7	15	–	51	12	14	–	4	15	2	ABLI0003
<i>Rhus copallinum</i>	118	30	13	117	2	70	–	120	121	5	90	30	167	–	MACA0002
<i>Robinia pseudoacacia</i>	13	–	–	–	60	11	–	–	–	–	–	–	–	–	MACA0002

Species	ABLI	CARL	CHCH	COWP	CUGA	FODO	GUCO	KIMO	LIRI	MACA	NISI	RUCA	SHIL	STRI	QA/QC Sites
<i>Rubus</i> sp.	174	40	30	120	30	30	95	120	121	510	120	60	120	60	MACA0001
<i>Sambucus nigra</i>	–	4	–	–	–	–	–	–	–	–	–	–	–	–	None
<i>Sassafras albidum</i>	75	–	–	–	59	25	–	–	3	–	–	1	5	9	MACA0002
<i>Verbesina occidentalis</i>	–	–	–	90	–	–	–	–	–	–	60	–	–	–	TBD
<i>Vernonia</i> sp.	6	60	–	–	15	–	–	–	–	5	–	1		–	TBD
<i>Vitis</i> sp.	–	–	–	–	–	–	–	–	–	–	–	–	3	–	None

There are three QA/QC sites listed in Table 1. The procedures outlined below refer to the MACA0001 (Great Onyx Job Corps) site. The same rules will be followed at other QA/QC sites.

Sites that have standard and QA/QC teams are sampled independently by both teams. The two teams consult with one another on three things: (1) identify which team will be labeled the standard and which will be labeled the QA/QC, (2) agreeing to a rough plan for what areas to search for plants (determined by consulting previous sampling event's site sketch), and (3) working together to assess the first 10 plants (QA/QC plants) that will be part of the QA/QC process.

When identifying which team is the standard team and which is the QA/QC, the teams should consider which of the crew leaders will be doing most of the sampling during that year. The standard team should be the team whose crew leader will do the most sampling.

At the MACA Great Onyx Job Corps site, the 10 plants examined by the standard and QA/QC crew are determined by walking the edge of the meadow at the park. The perimeter of the examined meadow is about 730 feet (222.5 meters) long. To determine where to look for plants examined by both teams, 15 random numbers between zero and 730 were generated in the office before going into the field. These 15 numbers are the distances, in feet, along the perimeter at which plants will be examined. The first 10 perimeter distances are the preferred distances. The remaining five perimeter distances are backups, used when a preferred perimeter distance has no ozone sensitive plants along its orthogonal transect. At each distance, the samplers move orthogonally into the meadow, looking for the first suitable ozone-sensitive plant. That plant is examined, first by the standard team and second by the QA/QC team. Neither team is told what the other's assessment was, and avoids knowing the deliberations of the other team. On their field forms, each team writes their values for amount and severity. Both teams highlight these plants on the field forms as QA/QC plants by circling their amount and severity assignments. If a perimeter distance does not have ozone sensitive plants along a transect orthogonal to the meadow's perimeter, then backup perimeter distances are used sequentially as necessary. After both teams have assessed each QA/QC plant, that plant is marked with flagging tape. This flagging tape will prevent those same plants from being reexamined by either team once independent sampling is being conducted.

After the first 10 QA/QC plants have been examined, independent sampling begins. Teams do not sample the same plants at the same time. Ideally (and assuming that a linear sampling path is used), the two teams start at opposite ends of the sampling path. Each team begins by moving toward the other. This results in each team ending where the other started, so that they pass each other somewhere in the middle of the path. Sampling along roughly the same path by both teams is a goal, but some sampling of different areas by the two teams is allowed. Sampling procedures detailed in subsequent steps of this SOP are followed by both teams. Examples of properly filled out foliar injury data sheets (both standard and QA/QC teams) are shown in Figures 1 and 2.

2023 NPS-CUPN OZONE AND FOLIAR INJURY – FOLIAR INJURY DATA

STATE	COUNTY	PARK/SUBPARK CODE - PLOT NUMBER	SITE NAME	START DATE (M/D/YYYY)	Circle QA STATUS
KY	Edmonson	MACA-B001	GREAT UNYX JOB Corps	1/1/2023	STANDARD QA BLIND CHK

Species (underline is code)
<u>Rubus spp.</u> (Blackberry)
<u>Asclepias spp.</u> (Milkweed)
Prunus serotina (Black Cherry)
Liriodendron tulipifera (Yellow Poplar)
Fraxinus spp. (Ash)
Sassafras albidum (Sassafras)
Apocynum spp. (Dogbane)
Liquidambar styraciflua (Sweetgum)
Prunus pensylvanica (Pin Cherry)

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%
4	51-75%
5	>75%

Species Code			Species Code			Species Code			Species Code			Species Code		
LIQUID STY			RUBUS SPP			APOCYNUM SPP			ASCLEPIAS SPP					
Plant	Amount	Severity	Plant	Amount	Severity	Plant	Amount	Severity	Plant	Amount	Severity	Plant	Amount	Severity
1	0	0	1	0	0	1	0	0	1	0	0			
2	0	0	2	0	0	2	0	0	2	0	0			
3	0	0	3	0	0	3	0	0	3	0	0			
4	0	0	4	0	0	4	0	0	4	0	0			
5	0	0	5	0	0	5	0	0	5	0	0			
6	0	0	6	0	0	6	1	2	6	1	2			
7	0	0	7	0	0	7	0	0	7	0	0			
8	0	0	8	0	0	8	0	0	8	0	0			
9	0	0	9	0	0	9	1	1	9	0	0			
10	0	0	10	0	0	10	0	0	10	0	0			
11	0	0	11	0	0				11	0	0			
12	0	0	12	0	0				12	0	0			
13	0	0	13	0	0				13	0	0			
14	0	0	14	0	0				14	0	0			
15	0	0	15	0	0				15	0	0			
16	0	0	16	0	0				16	0	0			
17	0	0	17	0	0				17	0	0			
18	0	0	18	0	0				18	0	0			
19	0	0	19	0	0				19	0	0			
20	0	0	20	0	0				20	0	0			
21	0	0	21	0	0				21	0	0			
22	0	0	22	0	0				22	0	0			
23	0	0	23	0	0				23	0	0			
24	0	0	24	0	0				24	1	1			
25	0	0	25	0	0				25	0	0			
26	0	0	26	0	0				26	0	0			
27	0	0	27	0	0				27	1	1			
28	0	0	28	0	0				28	0	0			
29	0	0	29	0	0				29	2	1			
30	0	0	30	0	0				30	0	0			

Did you collect 3 leaves that clearly show ozone stipple, for each injured species? Enter injury location and type codes for the collected leaf vouchers.

✓=yes	Location =	Type =	Location =	Type =	Location = 2	Type = 2	Location =	Type =	Location =	Type =
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Early Leaf Drop (Y/N)? N Early Leaf Drop (Y/N)? N Early Leaf Drop (Y/N)? N Early Leaf Drop (Y/N)? Y Early Leaf Drop (Y/N)?

Notes:

Data are mock-up. Not real.

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Page 1 of 5
Form version 20230927

Figure 1. Mockup of a 2023 foliar injury field form for the standard team. This example is a QA/QC plot (note the 10 circled plants). Note that USFS species codes are no longer used.

2023 NPS-CUPN OZONE AND FOLIAR INJURY – FOLIAR INJURY DATA

STATE	COUNTY	PARK/SUBPARK CODE - PLOT NUMBER	SITE NAME	START DATE (M/D/YYYY)	Circle QA STATUS
KY	Edmonson	NACA-0001	GREAT OXYGEN JOB CORP	1/1/2023	STANDARD QA BLIND CHK

Species (underline is code)
<u>Rubus spp.</u> (Blackberry)
<u>Asclepias spp.</u> (Milkweed)
Prunus serotina (Black Cherry)
Liriodendron tulipifera (Yellow Poplar)
Fraxinus spp. (Ash)
Sassafras albidum (Sassafras)
Apocynum spp. (Dogbane)
Liquidambar styraciflua (Sweetgum)
Prunus pensylvanica (Pin Cherry)

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%
4	51-75%
5	>75%

Species Code			Species Code			Species Code			Species Code			Species Code		
LIQUID STY			RUBUS SPP			APOCYNUM SPP			ASCLEPIAS SPP			PRUN SER		
Plant	Amount	Severity	Amount	Severity	Amount	Severity	Amount	Severity	Amount	Severity	Amount	Severity	Amount	Severity
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Did you collect 3 leaves that clearly show ozone stipple, for each injured species? Enter injury location and type codes for the collected leaf vouchers.

✓=yes	Location =	Type =	✓	Location = 2	Type = 2	✓	Location =	Type =	✓	Location = 1	Type = 1	✓	Location =	Type =
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Early Leaf Drop (Y/N)? N Early Leaf Drop (Y/N)? N Early Leaf Drop (Y/N)? N Early Leaf Drop (Y/N)? Y Early Leaf Drop (Y/N)? N

Notes:

Data are mock-up. Not real.

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Page 2 of 5
Form version 20230927

Figure 2. Mockup of a 2023 foliar injury field form for the QA/QC team. This example is a QA/QC plot (note the ten circled plants). Note that USFS species codes are no longer used.

6. Plant species are selected from Table 2 with preference given to the bolded species. For a complete list of ozone sensitive species by park, see the spreadsheet in the supplemental materials directory. Some of the commonly encountered sensitive plants that are difficult to identify at the species level may be recorded at the genus level. We currently have seven genera used in this way: *Apocynum* (dogbane), *Asclepias* (milkweed), *Eupatorium* (boneset), *Fraxinus* (ash), *Rubus* (blackberry), *Vernonia* (ironweed), and *Vitis* (grapevine).
7. Evaluate the amount of foliar injury. In the USFS protocol, injury amount is defined as “the percentage of leaves on the plant with ozone injury symptoms relative to the total number of leaves on the plant.” The Cumberland Piedmont Network will use the scale for injury amount that was developed by the USFS shown in Table 3.

Table 2. Target species for the North and South regions (USFS 2008). Bold species names indicate preferred species. These indicator species are based upon USFS guidelines.

Definition – Bioindicator Species	Scientific Names
Blackberry	<i>Rubus</i> spp. (second year canes only)
Common and Tall milkweed	<i>Asclepias</i> spp.
Black cherry	<i>Prunus serotina</i>
Yellow poplar/Tuliptree	<i>Liriodendron tulipifera</i>
Ash (including white ash)	<i>Fraxinus</i> spp.
Sassafras	<i>Sassafras albidum</i>
Dogbane	<i>Apocynum</i> spp.
Big leaf aster	<i>Aster macrophyllus</i> ; <i>Eurybia macrophylla</i>
Sweetgum	<i>Liquidambar styraciflua</i>
Pin cherry	<i>Prunus pensylvanica</i>
Supplemental species	Refer to “3_2008 Supplemental Species ID_PDF.pdf” in training materials directory

Table 3. Codes used for the “amount” index.

Code	Definition
0	No injury; the plant does not have any leaves with ozone symptoms.
1	1 to 6% of the leaves have ozone symptoms
2	7 to 25% of the leaves have ozone symptoms
3	26 to 50% of the leaves have ozone symptoms
4	51 to 75% of the leaves have ozone symptoms
5	> 75% of the leaves have ozone symptoms

8. Evaluate the severity of foliar injury. Severity is based on a pool of injured leaves on each plant and does not consider uninjured leaves. In the USFS protocol, injury severity is defined as “on average, ... [the] percent of the leaf area of injured leaves [that] has ozone symptoms.” As with the amount index, the severity index is estimated for each plant. The USFS scale for severity is a 0–5 scale, similar to that used for the amount index. The severity scale is shown in Table 4.

Table 4. Codes used for the “severity” index.

Code	Definition
0	No injury; the plant does not have any leaves with ozone symptoms.
1	On average, 1 to 6% of the leaf area of injured leaves has ozone symptoms
2	On average, 7 to 25% of the leaf area of injured leaves has ozone symptoms
3	On average, 26 to 50% of the leaf area of injured leaves has ozone symptoms
4	On average, 51 to 75% of the leaf area of injured leaves has ozone symptoms
5	On average, > 75% of the leaf area of injured leaves has ozone symptoms

Notes on filling out the foliar injury field form. Some general rules apply to filling out this and all other field forms. These include using dark pencils or pens, writing legibly, and striking through corrected information rather than erasing it. Using the notes field is also a recommended location for inserting any clarifying comments, including comments about any corrections that are made to the forms.

The foliar injury field form (Figure 1) contains two sections. The first section captures information on the sampling event (where, when and who collected the data). The second section captures information on the species observed and documents any injury found.

Most fields in the first section are self-explanatory (e.g., county, state, date). The field titled “Park/Subpark Code – Plot Number” consists of the four-character park abbreviation and a four-digit site identifier (e.g., MACA-0001). The site identifier for each site will remain unchanged from year-to-year. For a full list of site names and IDs by park see SOP 3 *Establishment and Location of Sampling Sites—Version 2.0* (CUPN 2024b).

The “Circle QA Status” field is used to identify the type of crew filling out the field form. Teams decide prior to sampling which will be the “standard,” and which will be the “QA Blind Check” team.

The second section details the species, amount of ozone injury, and severity of ozone injury for each of the plants observed at the site. This part of the form is arranged in columns with each column pair devoted to one species. The box at the top of each column pair is where the species name is entered. As each plant from a given species is encountered in the field, the amount and severity indices are recorded for that plant using the next available plant number (1–30). More details on the fields on the form may be found in the USFS protocol.

Below each column of species is a field to document whether each species at the site is showing signs of early leaf drop. If the sampler estimates that more than 25% of the plants have dropped more than 25% of their leaves, then the sampler should indicate yes. Otherwise, the sampler should indicate no. Leaf drop above this threshold would indicate a biomass loss of at least approximately 6%. As biomass loss increases, the likelihood that ozone-injured leaves have dropped also increases, leading to potential false negative evaluations.

9. **As appropriate, collect leaf vouchers.** For each species showing foliar injury, at least one voucher specimen is collected at each site. In cases where both the standard and the QA/QC team suspects injury on a species at a site, both teams collect specimens. Then, at the end of the sampling session, a decision can be made about whose specimens will be kept and sent to the regional expert. It is allowed for both the standard and the QA/QC teams’ voucher specimens to be retained and forwarded to the regional expert. The general procedures for collecting voucher leaf samples are as follows.
 - a. **Collect a voucher specimen that is representative in appearance and severity of what has been observed at the site.** Three leaves are considered one voucher and at least one voucher is collected for each species showing foliar injury. Ideally, leaves come from multiple plants and are not abnormally large or small. However, field conditions and damaged leaf availability may dictate that complete or ideal vouchers cannot be collected. Field crews need to collect whatever is available to collect full vouchers. Also, QA/QC teams collect vouchers independently of the standard field team.
 - b. **Immediately label the specimen using a petiole label.** Site, date, and species are noted on the label as shown in Figure 3. Vouchers belonging to the standard team

will have “STD” circled on the label and vouchers collected by the QA/QC team will have “QA” circled on the label. The species name is written in on the right of the label. Images of a properly attached petiole label and an updated label are shown in Figure 3.

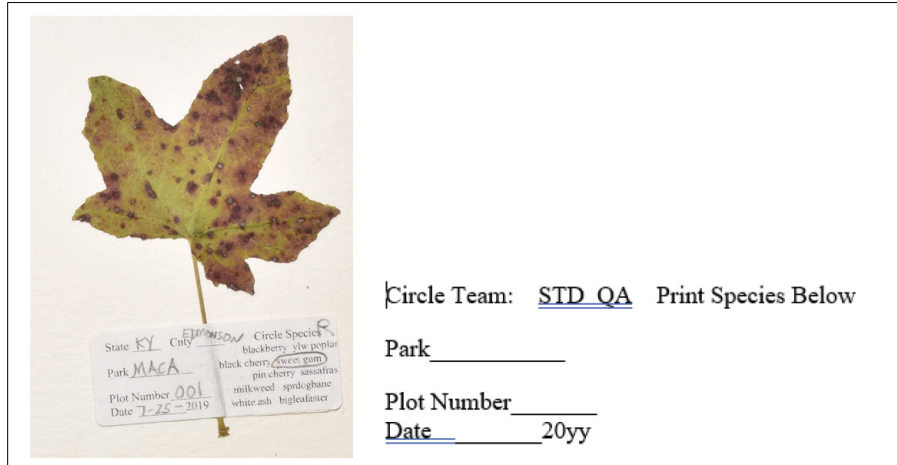


Figure 3. Properly attached petiole label (left) and updated petiole label (right).

- c. **Immediately place the specimen in a plant press.** Specimens must remain in the press for at least 36 hours.
- d. **Fill out the voucher specimen field form.** An example of a voucher specimen field form is shown in Figure 4. One field form is completed for each site at which injury is found. QA teams do not need to fill out additional forms. Specimens are listed and identified using scientific or common name as shown in the figure. The field form is divided into three sections: a general information section, a voucher specimen and site condition section, and a QA/QC section. The general information section contains fields as described above in “Notes on filling out the foliar injury field form.”

2020 NPS-CUPN OZONE AND FOLIAR INJURY – VOUCHER SPECIMENS

To be filled out by the CREW LEADER.

State	County	Park/Subpark Code – Plot Number	Site Name	Start Date (M/D/YYYY)
KY	Edmonson	MACA-0201	GREAT ONYX JOB CORPS	1/1/2020

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

Bioindicator Species Code or Common Name	Injury Location	Injury Type	TEAM (STD or QA)	Leaves taken from 1, 2, or 3 plants?	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)?
1 st 998- DOGBANE	2	2	STD	2	Close to 100% _____ Estimated percent other 20
2 nd 998- BLACKBERRY	2	2	QA	3	Close to 100% ✓ Estimated percent other _____
3 rd 998- MILKWEED	2	1	STD	3	Close to 100% _____ Estimated percent other 15
4 th 998 MILKWEED	1	1	QA	1	Close to 100% _____ Estimated percent other 65

Species codes:

998 Blackberry
762 Black cherry
998 Milkweed
621 Yellow poplar
998 Ash
931 Sassafras
611 Sweetgum
761 Pin cherry
998 Dogbane
998 Supplemental
(write out common name)
999 Unknown

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 gen. discoloration).
3 = injury is varied or difficult to describe.

Biosite notes, especially things that might affect ozone response (safety issues, excessively wet or dry, extreme disturbance of any kind, etc.)

Regional Expert Review Results:

Date Comments Received
11-30-2020

Bioindicator Species	Voucher + or – for ozone injury	Explanation – To be completed upon receipt of comments from regional expert.
DOGBANE STD	Positive	Stippling is classic.
BLACKBERRY QA	Negative	No comment received
MILKWEED STD	Positive	Stippling is classic
Milkweed QA	Negative	Symptoms are visible on veins, so not likely to be caused by ozone.

DATA ARE NOT REAL.
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Page 5 of 5

Figure 4. Mockup of a voucher specimen field form. Note that species codes are no longer used.

The voucher and site section contains information on injury parameters as observed across the site and the specimen(s) collected, as well as the condition of the site during the visit. The table in this section contains information on voucher specimens and the plant population from which they were taken. The “injury location” datum describes where, on each plant, ozone injury tended to be located. The “injury type” datum describes the mix of ozone injury (such as stipple) and non-ozone injury typical of leaves at the site. The codes in Table 5 are used for each datum (these codes and text are taken from the USFS protocol). The next field lists the number of plants from which voucher leaves were collected (that is, did the three leaves come from either 1, 2, or 3 plants?). The last field in the table addresses percent of injury on the leaves that is ozone stipple. This field is concerned with the voucher specimen and not the population of plants from which the voucher was collected.

The QA/QC section is not completed in the field; it is completed in the office by the project lead based on email notes on each leaf provided by the regional expert. More information on fields contained in this field form may be found in the USFS protocol (a copy of which is in the supplemental materials directory).

Table 5. The codes for “injury location” and “injury type” data.

Injury Location	Injury Type
1 – Greater than 50% of the injured leaves are younger leaves	1 – Greater than 50% of the injury is upper-leaf-surface stipple
2 – Greater than 50% of the injured leaves are mid-aged or older	2 – Greater than 50% is not stipple (tan flecks, bifacial or general discoloration)
3 – Injured leaves are all ages	3 – Injury is varied or difficult to describe

e. Once all specimens have been collected for the site, secure the plant press.

10. **Fill out site characteristics field form.** The site characteristics field form (Figure 5) includes information about the estimated acreage of the opening and GPS coordinates/elevation at the approximate center of the opening. This field form is also divided into three sections. The first is the general information section, as in the foliar injury data sheet. The second section contains fields that provide general data about site conditions, a discussion of which may be found in USFS (2008). The final section contains geographic information as collected by a GPS unit. Details for all field entries may be found in the USFS protocol. Only the standard team (not the QA team) needs to fill out a site characteristics form.

2020 NPS-CUPN – OZONE AND FOLIAR INJURY – SITE CHARACTERISTICS

Crew Reminders: Take this sheet to the Biosite. Complete it in the field. This sheet must be completed in the field.

To be filled out by the CREW LEADER: Refer to the USFS Ozone Field Guide for code definitions.

State	County	Park/SubPark Code – Plot Number	Site Name	Start Date (M/D/YYYY)
KY	Edmonson	MACA 0001	GREAT OMYX JOB CORP	1/1/2020

Crew Members (circle crew leader) JONATHAN JERNIGAN, SAMMI JO DOYLE, TERESA LEIBFRED

QA Person (if applicable) CLARE BLEDSOE

Protocol Version Used 2.0

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

Ozone Sample Kind:	
☐	Initial biosite establishment on the FIA ozone grid. (Data collection in a previously empty polygon)
☒	Remeasurement of a previously established biosite, or a replacement site within 3 miles of last year's GPS location.
☐	Remeasurement when the replacement biosite is more than 3 miles away from last year's GPS location.

Biosite size (Plot Size):	Terrain position:
☒ > 3.0 acres (1.2 hectares)	☒ Ridge top or upper slope
☐ 1 to 3 acres (0.4 – 1.2 hectares)	☐ Bench or level area along a slope
☐ Other: please describe	☐ Lower slope
	☐ Flat land unrelated to slope
	☐ Bottom land with occasional flooding

Aspect: 000° = no aspect; 360° = N aspect	Elevation: record estimate meters
Record to nearest degree = <u>000</u>	Meters = <u>216</u>

Soil Drainage:	Soil Depth:
☒ Well-drained	☒ Bedrock not exposed
☐ Wet	☐ Bedrock exposed
☐ Excessively dry	

Disturbance: Disturbance on the site or in localized areas where the bioindicator plants are growing.	
☒	No recent or significant disturbance; Do not count disturbance > 3 years old.
☐	Evidence of overuse; Human activity causing obvious soil compaction or erosion.
☐	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 Unit: <u>GISMAP 785</u>	GPS Datum: <u>NAD83</u>	GPS Serial Number: <u>MACA 4844</u>
UTM Zone = <u>16S</u>	GPS Error = <u>3 m</u>	
UTM Easting = <u>0567762</u>	Percent Confidence = <u>100 %</u>	
UTM Northing = <u>4122484</u>	GPS File Name = <u>MACA 0001-20200101-F01N</u>	

¹If no GPS Unit is available, please use a map and record estimated coordinates and elevation for each biosite location.

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

DATA ARE A MOCK-UP. NOT REAL

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Page 3 of 5

Figure 5. Mockup of a 2020 site characteristics form.

11. **Sketch a map of the site.** Sketches are completed each time a site is sampled. The sketch shows the approximate shape of the opening, locations of plant groupings, and the location of the site relative to some landmark such as a town or geographical landmark. The field form is divided into three sections. The first section contains general information on where, when, and by which team the site map was sketched. This section is like the corresponding section of the foliar injury data sheet (Figure 1). The second section is a rectangle in which to draw the site sketch. Lastly, details of the site photography are listed. These consist of photographer initials, camera information, and image number range (as established by the camera's assigned image numbering system). For an example of a properly sketched site, see sections 9.2.3 and 9.2.4 of the USFS protocol (USFS 2008). An example site sketch is also shown in Figure 6. Only the standard team (not the QA team) needs to fill out a site sketch form.

2020 NPS-CUPN – OZONE AND FOLIAR INJURY – SITE MAP

To be filled out by the CREW LEADER.

State	County	Park/SubPark Code - Plot Number	Site Name	Start Date (M/D/YYYY)
KY	Edmonson	MACA-0001	GREAT ONYX JOB CORP	1/1/2020

Map No. 1 of 1.

S = STANDARD TEAM SAMPLING PATH
Q = QA TEAM SAMPLING PATH

Suggested information to be included on the map follows. 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 and approximate location of plant groupings used for the ozone injury evaluations. (5) Sampling path.

Site Photographs

Photographer Initials: JS Camera Make/Model/SN: Mikon/D70/A6735
First Image Number: IMG0001 Final Image Number: IMG0015

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NOT REAL.

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Page 4 of 5

Figure 6. Mockup of a 2020 site sketch form.

12. Take site photographs. While photos must be taken at each site, there is no minimum or maximum number of photographs. Enough images are taken to convey the conditions under which plants at the site are growing. Are they shaded, in standing water? Images of individual clusters of plants or even symptoms on a particular plant are also useful. Images that show the location of structures or roads relative to plant populations that are sampled are also useful, especially when such features are shown on the site sketch.
13. Verify that the field forms have been completed to the degree possible, that the information is correct, and that voucher specimens have been properly labeled and secured in the plant press.
14. Both the standard and the QA team leads return to the 10 QA plants and remove the flagging. While doing so, the assessments of each team can be discussed with the other. While amount and severity assignments made at the beginning of the sampling session must not be changed, it is useful for the standard team to consider the judgment used by the QA team to assign those indices. If there is disagreement between the two teams, this discussion will potentially help the standard team leader better assess plants at subsequent site visits during the season. The goal of this discussion is not the change the rules used by the protocol to assess injury, but to help the standard team more correctly identify injury. As such, the standard team can disregard the QA team assessment if it is believed that the QA team is incorrectly identifying injury.
15. Remove flagging from QA/QC plants.
16. Prepare equipment, supplies, and voucher specimens for transport to next site or office.
17. Go to the next site at the park, if there are more sites, being certain to secure any gates behind you.
18. After all sites have been sampled or the day is complete, check in with park staff, sign the team and vehicle back in, per any such arrangements made with the park POC (point of contact). If a park vehicle was used, remove gear and trash and return vehicle keys. Report any non-emergency incidents that occurred during field sampling (e.g., worker's compensation filing needs to be done within 48 hours of incident).
19. After all sites have been sampled at a park, return any keys or park radios to park staff.
20. Return to the office or hotel.
21. Discuss any issues that arose with the monitoring procedures with the protocol lead.

Literature Cited

- Cumberland Piedmont Network. 2024a. Ozone and Foliar Injury Monitoring. Pre-sampling—Version 2.0. Standard Operating Procedure. NPS/CUPN/SOP—2. Cumberland Piedmont Network, Mammoth Cave, Kentucky. Available at: <https://irma.nps.gov/DataStore/Reference/Profile/2302026> (last accessed January 2024).
- Cumberland Piedmont Network. 2024b. Ozone and Foliar Injury Monitoring. Establishment and location of sampling sites—Version 2.0. Standard Operating Procedure. NPS/CUPN/SOP—3. Cumberland Piedmont Network, Mammoth Cave, Kentucky. Available at: <https://irma.nps.gov/DataStore/Reference/Profile/2302027> (last accessed January 2024).
- United States Forest Service. 2008. Phase 3 Field Guide—Section 9. Ozone Bioindicator Plants (east). Southern Research Station. Knoxville, TN. 49pp.