



Monitoring Ozone and Associated Foliar Injury at Cumberland Piedmont Network Parks

Protocol Narrative—Version 2.0



A foliar injury monitoring site at Cowpens National Battlefield (top image), and ozone-caused foliar injury on common milkweed at Carl Sandburg Home National Historic Site in 2012 (bottom image).

NPS/AMY WASHUTA

Monitoring ozone and associated foliar injury at Cumberland Piedmont Network parks: Protocol narrative—version 2.0

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Abstract

Ozone is a naturally occurring gas that can also be generated in reactions with anthropogenically created nitrogen oxides. While stratospheric ozone prevents ultraviolet radiation from making its way to the Earth's surface, tropospheric ozone that is in higher than naturally occurring concentrations can have harmful effects on the health of people and plants. Not all plant species are susceptible to ozone injury, but the several species that are susceptible do exist in Cumberland Piedmont Network Parks. This protocol presents sampling designs and methods for assessing ozone impacts to susceptible plants in Cumberland Piedmont Network parks.

Acknowledgments

The investigators wish to thank Dr. Robert Woodman for his guidance and input during the early stages of this protocol's development. Dr. Woodman was a USGS-BRD ecologist stationed at Mammoth Cave National Park during the early development of the Cumberland Piedmont Network. It was Dr. Woodman's task to assist network personnel with the design and testing of monitoring protocols. The designs and methodologies presented in this protocol are based upon early designs and methodologies proposed by Dr. Woodman. Thanks to "Woody" and the USGS-BRD. Without their assistance, this protocol could not have been written.

Thanks also to Dr. John Ray and Ellen Porter from the Air Resources Division of the National Park Service. Stationed in Lakewood, Colorado, Dr. Ray and Ms. Porter have provided data access and input on the designs presented in this protocol. Ms. Porter also coordinated the external review process.

The Cumberland Piedmont Network thanks Dr. Robert Kohut of the Boyce Thompson Institute for his assistance in the development of the foliar injury assessment portion of this protocol. Dr. Kohut met with the network and MACA staff at CUPN's MACA office to help prepare for the 2006 pilot study. He also traveled to each of the participating CUPN parks to assist with implementation in July and August 2006.

Thanks also to the U.S. Forest Service and their staff for their assistance in establishing this protocol early on and for help inspecting the pressed leaves that we sent to them to determine if ozone injury was present on them. Dan Stratton came to Mammoth Cave and helped us with our first injury survey. He also provided on-site training in assessment procedures. Gretchen Smith reviewed our pressed leaf samples for the first decade or so of our executing this protocol.

For the last few years, Howard Neufeld, of Appalachian State University, has picked up where Gretchen Smith left off with the examination of our pressed leaf samples. Thanks so much to Dr. Neufeld for helping us these last several years.

The investigators would like to thank the many CUPN parks and their staff who participated in the pilot studies initiated by Dr. Woodman and the network as well as the initial monitoring undertaken from 2008 to 2010. There were many participants (including the primary author's nephew) who helped with servicing and the installation or removal of ozone monitoring stations and passive ozone samplers. Unfortunately, for brevity's sake, their names cannot all be listed here. Without their help, the data necessary to arrive at the designs and methodologies presented here could not have been collected.

When version 2.0 of this protocol was written, Bobby Carson had been retired for a few years. Bobby was a coauthor for version 1.0 of the protocol. The authors would like to thank Bobby for his assistance in creating version 1.0 of this document.

Version 2.0 of this protocol has incorporated many edits and changes in approaches, and we would like to thank a couple of individuals for their assistance in moving toward those edits and changes.

Brenda Wells authored the text about how voucher specimens are processed when back in the office. Thanks to Brenda for her contributions. Also, Ellen Cheng has provided analyses of existing data to help us decide on potential approaches to use for long-term analyses included in synthesis reports. Thanks so much to Ellen for her assistance.

Executive Summary

The Cumberland Piedmont Network reports ozone data and conducts long-term monitoring of ozone injury to vegetation as part of the Inventory and Monitoring Division of the National Park Service. Ozone and foliar injury in parks is monitored as a key vital sign and indicator of possible anthropogenic effects to overall ecosystem health. Ozone concentration information and the associated impacts to plant life provide managers information about impacts to those park resources. The results of ozone and foliar injury monitoring can be used by park managers to inform park planning documents (such as Natural Resource Condition Assessments and Resource Stewardship Strategies) and to work with state regulatory agencies.

Ozone foliar injury monitoring takes place within natural vegetation areas of 14 national park units within the Cumberland Piedmont Network. Parks include Abraham Lincoln Birthplace National Historical Park (ABLI), Carl Sandburg Home National Historic Park (CARL), Chickamauga and Chattanooga National Military Park (CHCH), Cowpens National Battlefield (COWP), Cumberland Gap National Historical Park (CUGA), Fort Donelson National Battlefield (FODO), Guilford Courthouse National Military Park (GUCO), Kings Mountain National Military Park (KIMO), Little River Canyon National Preserve (LIRI), Mammoth Cave National Park (MACA), Ninety Six National Historic Site (NISI), Russell Cave National Monument (RUCA), Shiloh National Military Park (SHIL), and Stones River National Battlefield (STRI). The goals of ozone reporting and foliar injury monitoring are to assess exposure to ozone and its impacts to susceptible plant life at those parks. The Cumberland Piedmont Network monitors between one and three foliar injury sites within each park. The number of foliar sites depends on availability of sites with adequate ozone-sensitive species richness and abundance living under abiotic conditions that can manifest injury. Parks are sampled on a panel design, with three to four parks sampled each year over the course of a six-year sampling rotation. That sampling is completed annually between late July and late August. Data collected in each site include individual plant assessments of symptomology, site mapping, and site conditions. Ozone exposure indices are retrieved from sources outside the Cumberland Piedmont Network.

The Cumberland Piedmont Network's approach, rationale, and required resources for ozone and foliar injury monitoring are described in this protocol narrative. Eight associated Standard Operating Procedures (SOPs) provide detailed instructions on how to collect, manage, analyze, and disseminate the project's findings.

Background and Objectives

Ground level (tropospheric) ozone is a secondary pollutant, meaning is not directly emitted by sources, but created through chemical reactions between primary pollutants emitted directly from sources such as power plants, automobiles and factories (GRSM 2006). The U.S. Environmental Protection Agency (EPA) maintains that ozone is “Good up high, bad nearby” (EPA 2003). This is because, up high (in the stratosphere), the ozone layer protects Earth from ultraviolet radiation. Down low (in the troposphere), however, higher concentrations of ozone are undesirable since breathing ozone is harmful to humans.

Ozone also injures plants, including crops (EPA 2003), which is visually displayed as foliar injury, usually on leaf surfaces. An example of an ozone-injured spreading dogbane (*Apocynum androsaemifolium*) leaf is shown in Figure 1.



Figure 1. Foliar injury on spreading dogbane. Photo was copied from Kohut (2007).

The exact visual presentation of ozone-caused foliar injury varies among plant species. Figure 2 shows a close-up of foliar injury on common milkweed (*Asclepias syriaca*). Each of the small spots on the leaf is ozone-caused foliar injury. Figure 3 shows typical injury on blackberry (*Rubus* sp.). Note that in the absence of direct sunlight, a result of shading by leaves above, portions of the leaflet do not exhibit symptoms of ozone injury.

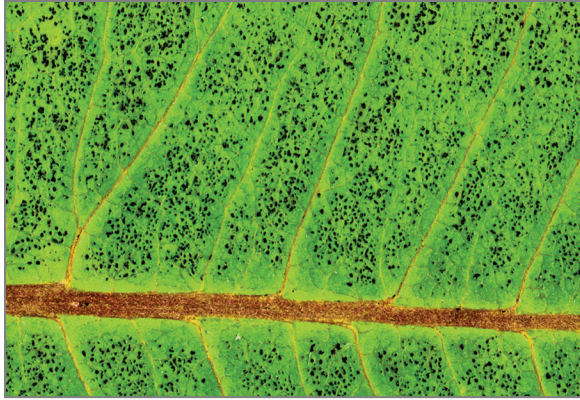


Figure 2. Close-up of ozone injury on common milkweed. Image from Smith et al. 2004.

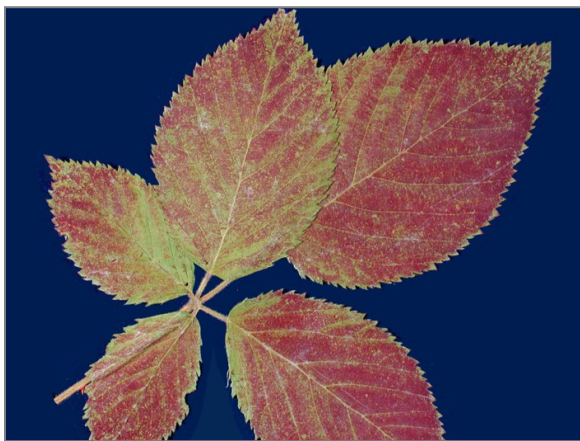


Figure 3. Ozone injury on a blackberry leaf. Note the uninjured portion on the center of some of the leaflets. That portion of the leaflets was shaded, and shaded portions of leaves tend not to show the ozone response. Image from Smith et al. 2004.

Sensitivity to ozone also varies among plant species (Rose and Coulston 2009) and runs the gamut from low to high sensitivity. For example, black cherry (*Prunus serotina*) and several blackberry species (*Rubus* spp.) are sensitive to ozone, while white oak (*Quercus alba*) is not (Smith et al. 2007).

The goal of this protocol is to provide the Cumberland Piedmont Network (CUPN) and its parks with ozone concentration and foliar injury data. Ozone concentrations for each park (which previously came from Portable Ozone Monitors (POMS) under Version 1 of this protocol) will now be derived from interpolated information provided by NPS Air Resources Division (NPS ARD). Foliar injury data collection has not changed and will come from fieldwork on parks conducted using this protocol. Broadly speaking, the data collected will primarily address whether ozone concentration is high enough to cause ozone foliar injury and whether that injury is occurring. Ancillary issues, such as whether ozone concentrations are high enough to violate human health standards, will also be explored.

Due to the discontinuation of POMS (see rationale later in this document), the procedures for directly monitoring ozone concentration using measurement methods have been removed from this protocol. The protocol now focuses primarily on procedures for measuring foliar injury and relies on ozone concentration measurements and estimations from other sources.

Issues Being Addressed and Rationale for Monitoring

Ozone is formed through chemical reactions between nitrogen oxides and volatile organic compounds (both of which are emitted by anthropogenic sources such as automobiles, power plants and factories). Volatile organic compounds are also emitted by plants (Ray 2004). The chemical reactions are catalyzed by sunlight, resulting in generally higher concentrations of ozone during summer. Ozone is naturally present in both the stratosphere and troposphere. Stratospheric ozone is beneficial since it protects life on Earth from the negative health effects of ultraviolet radiation. Ozone in the troposphere, especially when elevated by anthropogenic stressors, can have negative health effects on park visitors and park vegetation.

Ozone Effects and EPA Standards to Protect Against those Effects

Ray (2004) lists several ways that ozone negatively affects human health. Those effects are:

- Acute respiratory problems, such as aggravation of asthma, temporary decreases in lung capacity in some adults, and inflammation of lung tissue
- Impairment of the body's immune system

The American Lung Association also reports that exposure to elevated ozone can result in premature death in humans (see the [American Lung Association website](https://www.lung.org/our-initiatives/healthy-air/outdoor/air-pollution/ozone.html) [https://www.lung.org/our-initiatives/healthy-air/outdoor/air-pollution/ozone.html]).

Vegetation can also be negatively affected by elevated ozone concentrations. Extensive research (such as exposing plants to various concentrations of ozone in gas chambers and observing the plants' responses) has shown that ozone causes the stipple shown in Figure 1 as well as the other effects listed in the overview (Negra et al. 2009). Several species have been examined and it has been determined that different species (and even different individuals within the same species) have different tolerances to ozone. That is, some species are generally more able than others to withstand higher ozone exposure.

While many studies of plant response to ozone have been undertaken in controlled laboratory settings, plant response has also been studied in natural settings. Ray (2004) notes studies have shown that foliar injury occurs at ozone concentrations well below the EPA's primary (human health) standard for ozone at that time. In addition, Ray (2004) notes that studies have documented foliar injury in national parks. Some of CUPN's assessments (conducted using the draft version of this protocol) in 2008 and 2009 also show that foliar injury is occurring in CUPN parks (Jernigan et al. 2009; Jernigan et al. 2010).

Ray (2004) states that ozone enters leaf stomata and oxidizes plant tissue. This exposure results in changes to biochemical and physiological processes. Ray (2004) also states that severe cases of foliar injury can lead to growth effects in particular plants and that long-term exposure of sensitive plants

can lead to shifts in species composition wherein more sensitive species are replaced by less sensitive species. For example, note the estimated biomass loss in black cherry shown in Figure 4. Grantz (2009) further indicates that the exact nature of changes in species composition is reliant not only on ozone concentration, but also on other complicating factors.

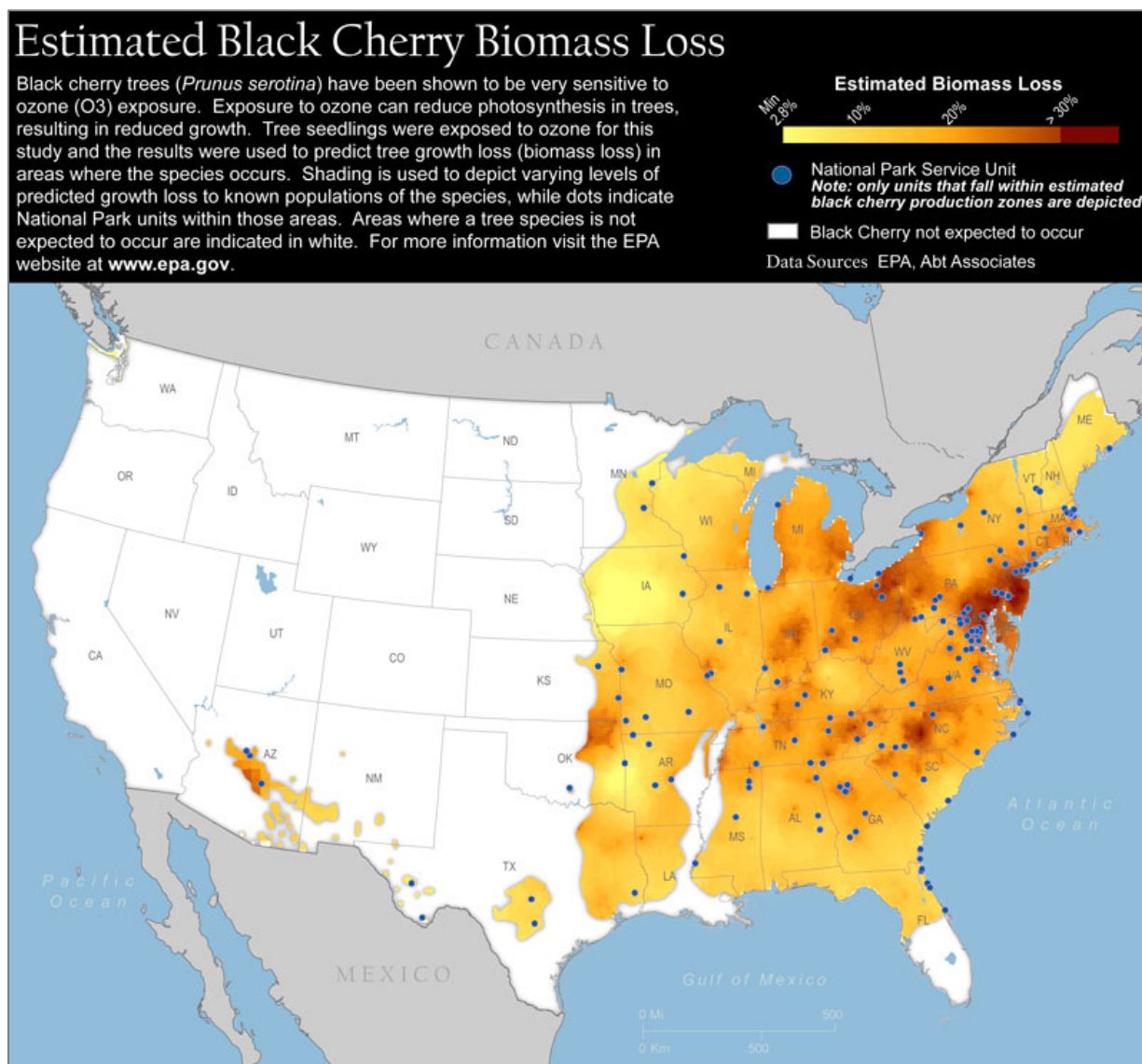


Figure 4. Map showing estimated biomass loss in black cherry seedlings due to ozone injury. Image downloaded from Ozone Effects on Tree Growth on nps.gov (<https://www.nps.gov/subjects/air/nature-trees.htm>).

To protect human health and welfare from effects such as those just discussed, the EPA developed two National Ambient Air Quality Standards (NAAQS) for ozone. The primary standard is designed to be protective of human health and the secondary standard is designed to be protective of human welfare. The secondary standard for all air pollutants addressed in the NAAQS is defined as ([EPA NAAQS Table](https://www.epa.gov/criteria-air-pollutants/naaqs-table) [https://www.epa.gov/criteria-air-pollutants/naaqs-table]):

“Secondary standards provide public welfare protection, including protection against decreased visibility and damage to animals, crops, vegetation, and buildings.”

The EPA determines whether these standards are being met through the evaluation of exposure indices that are generally based in the science behind the subjects of each standard. For ozone, the primary standard is evaluated using the index defined as the “Annual fourth-highest daily maximum eight-hour concentration, averaged over 3 years.” The secondary standard for ozone currently uses the same index as the primary standard.

Factors Affecting Plant Response to Ozone

Simply having sensitive plants in the presence of high levels of ozone does not guarantee that those plants will be injured by ozone. There are conditions under which sensitive plants may not be significantly affected even with high ozone exposure. This is a consequence of the need for ozone gas to enter the interior of the leaf through the stomata for injury to occur. If environmental conditions are not favorable for gas exchange, foliar injury is less likely to occur. Two conditions that lead to a decrease in gas exchange are high air temperature and drought. Plants in hot and dry (low soil moisture) conditions may close their stomata, resulting in less ozone within the leaf interior and, therefore, less exposure of the plant to ozone (Kohut 2005).

Conversely, ozone injury can be exacerbated under certain conditions. For example, if moisture availability is above average when ozone concentration is only average, injury to a plant can be similar to injury under average moisture availability and above average ozone concentration. Ray (2004) lists the following as factors that can enhance the risk of ozone injury:

- Existence of sensitive species
- Ozone concentrations high enough to cause injury
- Environmental conditions that support gas exchange in plant leaves

As part of its annual resource briefs, the Cumberland Piedmont Network reports ozone exposure indices as reported by NPS-ARD interpolated values that are reported via its website [Air Quality Conditions & Trends](https://www.nps.gov/subjects/air/park-conditions-trends.htm) (<https://www.nps.gov/subjects/air/park-conditions-trends.htm>). The network will also report moisture availability to plants, the most relevant environmental condition that affects plant response to ozone via historical maps showing the Palmer Drought Severity Index at the time of the foliar injury survey: [U.S. Drought Monitor](https://droughtmonitor.unl.edu/Maps/MapArchive.aspx) (<https://droughtmonitor.unl.edu/Maps/MapArchive.aspx>).

Computing Exposure Indices

Ray (2004) notes that the SUM06, W126 and N100 indices have been developed to quantify the likelihood that a plant will be injured by ozone. In the past, the Cumberland Piedmont Network has used the SUM06 and the W126 indices. We now prefer the W126 index since it emphasizes (through weighting) higher ozone concentrations that are more damaging to plants. Ray (2004) defines the W126 index as “a cumulative index that is calculated as the maximum running 90-day sum of the 0800–2000 weighted hourly ozone concentrations, where a sigmoidal weighting function is used to

give increasing significance (weights between 0 and 1) to concentrations of ozone greater than 0.04 ppm, and no weight to concentrations below 0.04 ppm.”

The W126 index may be computed using only values from 8 am to 8 pm (the hourly values reported for 8 am to 7 pm, inclusively). It may also be computed using all data available, including nighttime data. The W126 over a given period of time is defined by the equation:

$$W126 = \sum_{i=1}^n \frac{c_i}{1 + (4403e^{-126c_i})}$$

where c_i is the hourly ozone concentration expressed in ppb and n is the number of hours with valid ozone measurements. The equation is based on the information in the presentation found in Taylor (2017). Frequently, the W126 is computed for each possible three consecutive month period in the growing season and the largest of those values is taken as the W126 for the season.

Thresholds for Exposure Indices

Ray (2004) and National Park Service-Air Resources Division ([NPS Air Quality Analysis Methods 2020](https://www.nps.gov/articles/analysis-methods2020.htm) [https://www.nps.gov/articles/analysis-methods2020.htm]) provide estimates for W126 threshold values above which effects to plant health can be expected. NPS-ARD’s W126 thresholds are:

- Significant Concern: > 13 ppm-hr
- Moderate: 7–13 ppm-hr
- Good: < 7 ppm-hr

In 2010, the EPA proposed that the secondary standard no longer be the same as the primary standard. According to NPS-ARD (2010), that secondary standard would prescribe a W126 value between 7 and 15 ppm-hours. The EPA was also proposing a method for computing the W126 that would be based on a three-year average rather than an annual value. However, in 2015, the EPA officially decided to keep the primary and secondary standards the same (EPA 2015). Further discussion of ozone, ozone exposure indices and foliar injury may be found in U.S. Forest Service et al. (2010).

Established Methods Used by this Protocol

The literature available on ozone and associated foliar injury is myriad. A comprehensive review of this literature is beyond the scope of this protocol. As such, the authors will primarily discuss only those documents which are directly influential to our sampling designs and methods. Those are Kohut (2007), Ray (2004) and the U.S. Forest Service’s (USFS) standard operating procedure for conducting foliar injury surveys (USFS 2008). In version 1.0 of this protocol, our ozone monitoring Standard Operating Procedures (SOPs) were based upon work by Ray (2004). Since we are no longer collecting ozone concentration data, those SOPs have been deleted in version 2.0. Our foliar injury sampling SOPs are based upon the USFS (2008) protocol and are influenced by the Kohut (2007) protocol.

Brief Description of the Cumberland Piedmont Network (CUPN)

The Cumberland Piedmont Network Inventory and Monitoring Program was initiated in January 2001 to conduct biological inventories and examine the status and trends of ecosystem health for its cohort of 14 national parks, battlefields, and historic sites (Figure 5).

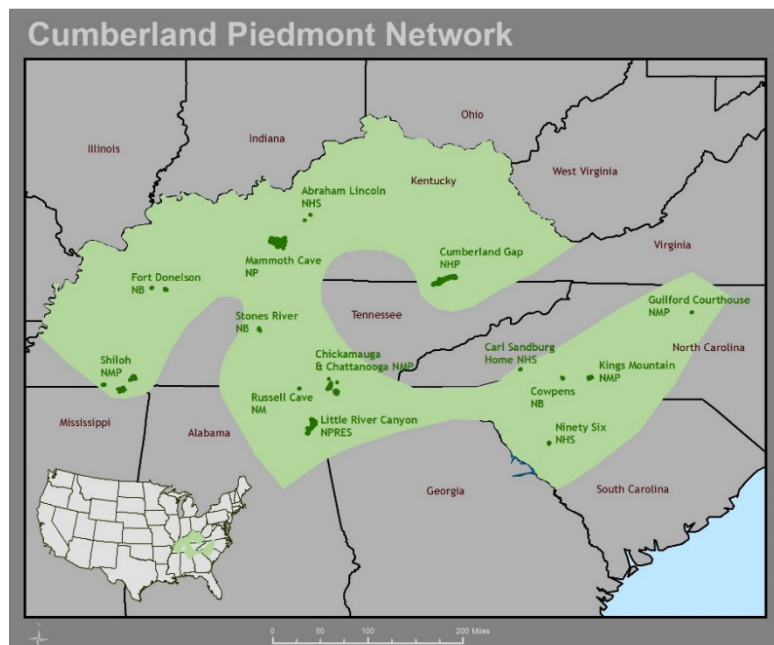


Figure 5. NPS units that make up the Cumberland Piedmont Network.

After a series of park workshops (2003–2004), the CUPN Vital Signs Monitoring Plan was approved, which included network-wide implementation of ozone and foliar injury monitoring (Leibfreid et al. 2005). Table 1 lists the parks along with abbreviations used throughout this protocol.

Table 1. List of CUPN parks and their abbreviations.

Park Name	Abbreviation
Abraham Lincoln Birthplace National Historical Park	ABLI
Carl Sandburg Home National Historic Park	CARL
Chickamauga and Chattanooga National Military Park	CHCH
Cowpens National Battlefield	COWP
Cumberland Gap National Historical Park	CUGA
Fort Donelson National Battlefield	FODO
Guilford Courthouse National Military Park	GUCO
King's Mountain National Military Park	KIMO
Little River Canyon National Preserve	LIRI

Table 1 (continued). List of CUPN parks and their abbreviations.

Park Name	Abbreviation
Mammoth Cave National Park	MACA
Ninety Six National Historic Site	NISI
Russell Cave National Monument	RUCA
Shiloh National Military Park	SHIL
Stones River National Battlefield	STRI

Sampling in Cultural and Historical Parks

All CUPN's parks have significant cultural resources, and for many of those parks, cultural and historical preservation is the primary concern. Ozone concentration is also a concern because in addition to human health issues, there are potential impacts to cultural landscapes. The NPS maintains cultural landscapes just as it preserves and protects natural ecosystems, and ozone can cause injury to vegetation that is part of a cultural landscape. For more information on cultural landscapes and NPS efforts to maintain them, see [Cultural Landscapes on nps.gov](https://www.nps.gov/subjects/culturallandscapes/index.htm) (<https://www.nps.gov/subjects/culturallandscapes/index.htm>).

In addition to the potential for direct impacts on cultural landscapes, ozone concentrations are of interest due to a connection with acid rain. Managers at most cultural and historical parks are concerned with acid rain because it can dissolve historic structures such as limestone grave markers, buildings and monuments but they do not have the resources to monitor acid rain or its causes (pollutants consisting primarily of sulfur and nitrogen compounds). Elevated ozone concentrations are indicative of poor air quality, including nitrogen pollution, since “clean” air masses generally don’t contain excessive amounts of ozone. One way to lower ozone concentrations is to lower the precursor emissions, such as nitrogen compounds. Successful efforts to lower ozone should result in less acidic rainfall, thereby reducing impacts to structures in historic and cultural parks.

Early Implementation of Ozone Concentration Monitoring within the Cumberland Piedmont Network

During the first two rotations of sampling through CUPN parks, Portable Ozone Monitoring Stations (POMS) were used to measure hourly ozone concentration and meteorological values. These hourly data were then used to estimate SUM060 and W126 indices. The POMS monitors were set up each year at the two parks that were receiving foliar injury monitoring. The POMS monitors gathered data during the growing season (April–October) and foliar injury monitoring occurred July–August.

Beginning in 2017, the use of POMS within the Cumberland Piedmont Network was discontinued. The rationale for discontinuing POMS was based largely on implementation cost and the availability of roughly equivalent information from other sources. Running a POMS is expensive, both in terms of staff time and in terms of money allocated to a contractor for support, data QA/QC, and data preservation. Also, the ozone concentration data collected using a POMS (as we collected them) are not considered to be collected by an EPA-equivalent method, so the data could not be used for

regulatory purposes. The network decided to use interpolated data provided by the NPS-ARD, which is adequate to explain observed foliar injury at CUPN parks. Finally, monitoring for effects to park resources (in this case, damage to plants that is caused by ozone), may be more useful to park managers than ozone indices or concentrations.

Historical Development of Foliar Injury Monitoring within the Cumberland Piedmont Network

Prior to 2006, the network had made no attempt to do any foliar injury assessment. Foliar injury assessments had been completed in Mammoth Cave National Park (MACA) in 1985 and 1986, but these assessments were part of a short-term study and were not part of long-term monitoring activities (McCune et al. 1987).

In early 2006, NPS-ARD worked with Dr. Robert Kohut from Cornell University to initiate some pilot foliar assessments as designed by Dr. Kohut (see Kohut [2005] for specifics on sampling designs and methodologies). The CUPN and MACA staff (along with staff from other parks and networks) participated in conference calls with NPS-ARD personnel and Dr. Kohut to discuss these pilot assessments. It was decided that, with the help of Dr. Kohut, three CUPN parks (Cowpens National Battlefield [COWP], Cumberland Gap National Historical Park [CUGA], and Mammoth Cave NP) would participate in the pilot assessments. Through the collaborative efforts of CUPN staff, park staff, and Dr. Kohut, foliar injury sampling was completed in the summer of 2006. One of Kohut's sampling designs (the one attempted by the network during the pilot) required that branches be removed for examination from the crowns of very large trees. In Figure 6, note the tree climber near the top of a very tall tree. Ultimately, the difficulty and cost of climbing trees to retrieve branches for examination contributed to the network's choosing to use the methods that were documented in version 1.0 of this protocol and are currently in use.

No foliar assessments were completed in 2007. The MACA staff intended to conduct injury surveys, but drought conditions suggested the absence of ozone injury symptoms, and it was informally decided that no surveys would be conducted. It should be noted that, when monitoring as prescribed by this version of the protocol, parks are sampled without regard to the drought index at the time when sampling is to be completed. In 2007, the protocol was being drafted and it allowed for not sampling under drought conditions.

In 2008, the Cumberland Piedmont Network began implementation of the draft version of this protocol. Figure 7 shows CUPN staff during 2009 foliar injury sampling at Fort Donelson National Battlefield (FODO). Specifics of the foliar injury sampling designs and methods are discussed in more detail later in this narrative. The first 12 years of sampling are summarized in Table 2. The only significant deviation during the 2014 to 2019 period was the ending of ozone concentration monitoring after 2016.



Figure 6. Tree climber (circled) during the 2006 Kohut pilot assessment at Mammoth Cave NP.



Figure 7. Sammi Jo Eubank (CUPN staff) examining a sweetgum tree for foliar injury using USFS methods.

Table 2. CUPN sampling schedule of on-park ozone concentration (OC) monitoring and foliar injury (Foln) assessments from 2008 to 2019.

Park	2008/2014	2009/2015	2010/2016	2011/2017	2012/2018	2013/2019
ABLI	OC (2014 only), Foln	Foln (2015 only)	–	–	–	–
CUGA	Foln	–	–	–	–	–
MACA	Foln	Foln	Foln	Foln	Foln	Foln
FODO	–	OC, Foln	–	–	–	–
SHIL	–	OC, Foln	–	–	–	–
COWP	–	Foln (2009 only)	–	–	–	Foln (2019 only)
CHCH	–	–	OC, Foln	–	–	–
STRI	–	–	OC, Foln	–	–	–
LIRI	–	–	–	OC (2011 only), Foln	–	–
RUCA	–	–	–	OC (2011 only), Foln	–	–
CARL	–	–	–	–	OC (2012 only), Foln	–
GUCO	–	–	–	–	OC (2012 only), Foln	–
KIMO	–	–	–	–	–	OC (2013 only), Foln
NISI	–	–	–	–	–	OC (2013 only), Foln

Measurable Objectives

The goal of the CUPN ozone and foliar injury protocol is to document ozone dosing and how it relates to the presence (across species and sites), amount, and severity of foliar injury.

The specific **monitoring questions** addressed by this protocol are:

1. At which network parks are ozone concentrations high enough to cause foliar injury?
2. At select CUPN parks in any given year, what is the presence (across species and sites), amount, and severity of foliar injury?

The specific **monitoring objectives** addressed in this protocol are:

1. Use NPS Air Resources Division interpolated ozone concentration data, Mammoth Cave National Park actual ozone concentration data, and derived indices to identify when ozone concentrations may be high enough to cause foliar injury.

2. In three to four CUPN parks per year, use established biomonitoring methodologies to detect the presence/absence (and, if present, to quantify the amount and severity) of foliar injury.

Addressing these monitoring questions and objectives will help to demonstrate presence/absence of plants with verified injury. Presence of injury would constitute a demonstration of an impact on park resources, regardless of how minor or major that impact might be. Park managers can use this information during state-level review of air quality permits for the prevention of significant deterioration (PSD) of air quality. More PSD info is available at [What is PSD Increment on epa.gov](https://www.epa.gov/nsr/prevention-significant-deterioration-basic-information#:~:text=PSD%20increment%20is%20the%20amount%20of%20pollution%20an,The%20NAAQS%20is%20a%20maximum%20allowable%20concentration%20%22ceiling.%22) (https://www.epa.gov/nsr/prevention-significant-deterioration-basic-information#:~:text=PSD%20increment%20is%20the%20amount%20of%20pollution%20an,The%20NAAQS%20is%20a%20maximum%20allowable%20concentration%20%22ceiling.%22).

Sampling Design

Sampling Design Overview

Foliar injury assessments will be completed for three or four CUPN parks per year as detailed in Table 3. Foliar injury sampling is conducted between late July and mid-August as described by the USFS (2008) in their protocol. For more detail on the timing of foliar injury assessments, please see SOP 4 *Sampling—Version 2.0* (CUPN 2024a).

Table 3. Proposed CUPN sampling schedule of foliar injury (Foln) assessments. Please note Cowpens NB has been moved from Year 2 to Year 6 in the schedule.

Park	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
ABLI	Foln	—	—	—	—	—
CUGA	Foln	—	—	—	—	—
MACA	Foln	Foln	Foln	Foln	Foln	Foln
FODO	—	Foln	—	—	—	—
SHIL	—	Foln	—	—	—	—
CHCH	—	—	Foln	—	—	—
STRI	—	—	Foln	—	—	—
LIRI	—	—	—	Foln	—	—
RUCA	—	—	—	Foln	—	—
CARL	—	—	—	—	Foln	—
GUCO	—	—	—	—	Foln	—
COWP	—	—	—	—	—	Foln
KIMO	—	—	—	—	—	Foln
NISI	—	—	—	—	—	Foln

All sites cannot be sampled each year since the network does not have the resources to conduct foliar assessments at each of its 14 parks every year. However, staff at parks for which CUPN staff is not scheduled to collect foliar injury data may choose to perform injury assessments, provided crew leaders have completed the training exercises presented in this protocol (see SOP 2 *Pre-Sampling—Version 2.0* [CUPN 2024b]). This “episodic monitoring” is discussed in more detail in a subsequent section of this narrative.

Site and Species Selection

Foliar injury site selection criteria are adopted from USFS (2008) and Kohut (2007). Non-randomly selected sampling locations are chosen by the project lead in consultation with park management and park natural resources staff based upon accessibility, known sensitive species distributions and

minimal interference with maintenance or visitor activities. Open sites are required (at least 1 acre [43,560 square feet]). Other general rules for choosing sampling locations address topographic elevation and distance from acute sources of ozone precursor emissions (e.g., parking lots). Whenever possible, the sites are chosen to provide spatial coverage of the park as well as cover multiple elevations, soil types, aspects, steepness and soil disturbance. For more specific guidelines on site selection and the list of established sites, see SOP 3 *Establishment and Location of Sampling Sites—Version 2.0* (CUPN 2024c).

In the protocol’s supplemental materials directory, the file “CUPN O3_SensitiveSpeciesbyPark_2020.xlsx” lists sensitive plant species within each of the CUPN parks. Generally, samplers may choose from the full list of sensitive plant species, but the use of species common in CUPN parks is encouraged. An extensive list of ozone sensitive plants is included in the USFS (2008) field guide. The USFS (2008) also gives preference to the following plant species in the southeast (the species in bold and marked with an asterisk are preferred by the network and should be searched for at each park):

- ***Blackberry (Rubus spp.)****
- **Common milkweed (*Asclepias syriaca*)***—CUPN uses common or tall milkweed (*Asclepias spp.*)*
- Black cherry (*Prunus serotina*)
- Yellow poplar or tulip tree (*Liriodendron tulipifera*)
- White ash (*Fraxinus Americana*)—CUPN uses any ash available (*Fraxinus spp.*)
- Sassafras (*Sassafras albidum*)
- Spreading dogbane (*Apocynum androsaemifolium*)—CUPN uses any dogbane (*Apocynum spp.*)
- Sweetgum (*Liquidambar styraciflua*)
- Pin cherry (*Prunus pensylvanica*)

Sample Frequency and Replication

Foliar injury assessments will generally be conducted only at three to four parks per year. In addition to the scheduled CUPN assessments, park-level natural resource managers and park management may opt to conduct additional foliar injury assessments. Foliar injury assessments at each park will take place at multiple (for most parks), non-randomly distributed sites.

General Sampling Schedule

It will take six years to sample each of the 14 parks (Table 3). At the end of the six-year period, the sampling cycle will repeat. Parks are “active” in any given year if they are being sampled. Please note Cowpens National Battlefield was moved from Year 2 (as shown in Table 2) to Year 6. This change was made during Year 1 of the second cycle through the protocol, so the park was not missed during any cycle, although it was done late in the second cycle.

Episodic Monitoring

Since ozone exposure varies from one year to the next, it is possible that during years when ozone exposure is estimated as high (through examination of ozone concentration and weather data from nearby monitors), CUPN foliar injury surveys will not be scheduled at a given park. In such cases, ozone impacts to plants will not be documented if the CUPN sampling schedule is followed.

Allowing episodic monitoring at such a park will help to document ozone effects during off-schedule years. Because ozone exposure can't be effectively predicted, logistical planning for episodic sampling can be difficult. When conditions warrant, we will work with staff at individual parks, who may, at their discretion, conduct episodic monitoring at their park. Under such a scenario, there may be deviations from this protocol (such as fewer than recommended number of species and individuals per species per site). These episodic events will be guided by a written sampling plan that will be developed prior to the sampling event.

Field Methods

Preparations for Sampling

In general, foliar injury sampling events will be scheduled by the project lead in collaboration with the Cumberland Piedmont Network and park staff sometime in July. Sampling occurs in late July or August. Specific sampling schedules may be slightly adjusted to account for inclement weather, or personnel workloads. Sampling order and routes to parks and sites will be finalized at least one or two days prior to maximize efficiency and minimize travel times. If possible, site accessibility is verified by appropriate park personnel.

Lists of pre-trip activities are provided (see SOP 2 *Pre-Sampling—Version 2.0* [CUPN 2024b]). The crew leader must check each item on the lists, especially the supplies list (which includes items such as field forms), at least a few weeks before sampling, in case supplies are needed, and again the day before the trip for final details.

Frequently, the crew leader will be assisted by individuals who have not been trained to identify and estimate foliar injury. Minimal training is provided to these less-experienced crew members prior to the sampling event. This is acceptable since the crew leader will make the final call on all plants that show any form of injury. Less-experienced crew members are trusted to know when a plant has no injury on it and the crew leader will accept that designation without closer inspection. However, any plant that has any injury on it will be inspected by the crew leader. The number of less-experienced crew members participating will be kept to a minimum.

Sampling Methods

Methodologies for foliar injury assessments have been adopted from those used by the USFS in their protocol (USFS 2008). The USFS method involves visually inspecting plant leaves from the target species while walking around the site. The amount and severity of ozone-caused foliar injury for each plant inspected is written on a field form. Except for a voucher specimen for each species when injury is found, no leaves are removed from the sampled plants. Typically, the assessment takes a crew of two or three people between one and two hours to complete. After plants have been surveyed, the center of that site is mapped using a GPS and a site sketch is drawn that shows the position of target plants and patches within that site, as well as significant proximal landmarks (e.g., structures, roads, power lines). Other site-level abiotic data are also collected.

As samplers “sweep” a site, two types of data are collected on each plant sampled. The first datum is referred to as **“injury amount.”** This parameter defines how widespread ozone injury is on a particular plant. The parameter can range from no injury on the plant anywhere up to the entire plant showing some level of foliar injury. The next parameter collected is referred to as **“injury severity.”** This parameter is a measure of how badly, amongst the pool of injured leaves on a plant, a plant is injured on those leaves. Samplers are instructed to select and collect data on a leaf with an injury level that is “typical” of the entire plant. Parameter values can range from no injury on a typical leaf to as much as the entirety of a typical leaf shows injury. These estimates are based only upon parts of the plant that are exposed to sunlight since ozone injury will generally not occur on shaded parts of leaves (see Figure 3).

The USFS protocol (2008) calls for at least two species with 30 plants each. The Cumberland Piedmont Network will adopt the USFS minimum of 30 plants per species over two species, whenever possible, to retain some comparability with USFS data. There is no minimum number of plants per species for any additional species at any given site. Also, the maximum number of individual plants examined for each species is thirty. At some CUPN parks, finding sites with adequate plant counts can be challenging, if not impossible. The network still wants to be able to sample at those parks, so if a site with two species of at least 30 plants each is not available, sampling should still occur and the limited plant quantities be noted in reports.

Quality Assurance Fieldwork

The QA/QC (quality assurance/quality control) fieldwork is completed at one site each year by a second, independent team concurrent with the first team's sampling effort. That first team will be referred to as the standard team and the second team will be the QA/QC team. In addition to keeping costs minimal, this approach adequately addresses the QA/QC goals of the protocol. The purpose of the QA/QC fieldwork is to help ensure the standard team (which, in the past, has been led by the same individual who is responsible for sampling at remaining parks during the year) is, as correctly as possible, finding, identifying, and quantifying ozone-caused foliar injury.

Species diversity at Mammoth Cave National Park's (MACA) Great Onyx Job Corps sites is not adequate to cover all the plants examined across CUPN parks. Consequently, a new site at park's barrens (adjacent to its southern entrance) will be added. This additional site will pick up most of the remaining ozone sensitive species examined at CUPN parks. A few remaining species not at the two MACA sites will be examined at a third QA/QC site—located at Abraham Lincoln Birthplace National Historic Park (ABLI). Some species found in limited quantities will not be targeted for QA/QC. That is, they will not be included in the first 10 QA/QC plants, as discussed in the subsequent paragraph. This is primarily due to the absence of those species at our preselected QA/QC sites. The QA/QC species will be examined by both teams once independent sampling begins if present at the site. The QA/QC details (schedule and species encountered at each QA/QC site) are discussed in SOP 4 *Pre-Sampling—Version 2.0* (CUPN 2024a).

The process begins with each team independently examining the first 10 plants together. These 10 plants are selected using a random starting point as outlined in SOP 4 (CUPN 2024a). Comparison of the results from the two teams will determine whether injury (if present) is identified by both teams and whether each team is assigning similar amount and severity indices to each plant. These comparisons help to gauge the degree to which the standard team is correctly assessing presence or absence and amount or severity of foliar injury.

After the first 10 plants are evaluated, the two teams split up, each starting at opposite ends of a predetermined sampling path and working toward one another, and eventually passing one another. Each team samples plants along the path, but the original 10 plants are not resampled (they are marked with flagging tape by the teams when they were first assessed to prevent resampling). This independent canvassing of the sampling area helps to document the repeatability of the sampling approach and helps to determine whether the standard team is missing significant populations of ozone sensitive plants.

Voucher Specimens

In situations where ozone-caused foliar injury is suspected, leaves showing injury are collected from each species at each site showing injury. These voucher specimens are placed into a plant press at the site (see SOP 4 [CUPN 2024a]). After the specimens have dried, they are sent to an expert who can identify and differentiate ozone injury from other, similar looking forms of injury. This inspection helps to assure that ozone injury is not being incorrectly identified in the field. After the regional expert has finalized inspection of the specimens, they are returned to the CUPN office where they are photographed and prepared for storage at their final park-designated repository. Details on this process are described in SOP 5 *Post-Sampling—Version 2.0* (CUPN 2024d).

Reasons for Choosing this Method

The USFS program examined sites across the U.S. from 1994 to 2010 using established and accepted methods. Their approach is easy to execute provided the sampler is properly trained. The protocol by Kohut, while demonstrated to be effective in detecting ozone injury, is difficult to execute since leaf samples are obtained by tree climbing teams from the tops of tall, mature trees. For the USFS approach, tree climbing is not necessary and a crew with only a few members can sample a park. Sampling a site also takes little time, usually a couple of hours. Though the USFS has now suspended their foliar injury field efforts, the Cumberland Piedmont Network still uses several USFS resources, such as training materials, reference materials and field forms.

The only significant difficulty associated with the approach is in some park units it can be hard to find an ideal site meeting all criteria (see SOP 3 *Establishment and Location of Sampling Sites—Version 2.0* [CUPN 2024c]). Populations of target species suitable for monitoring can be rare, and suitable open sites, meadows, or early successional stands of plants may also be uncommon.

Data Management

Database Design

Foliar injury data are entered into a relational Microsoft Access database utilizing the Natural Resource Database Template (NRDT), which includes an established set of core tables and fields developed by the NPS Inventory and Monitoring Program (NPS 2007). Custom tables for foliar injury data were incorporated into the database and are based upon field forms used by the USFS Forest Inventory Analysis program (USFS 2008).

Data Processing

Foliar injury data are entered from hardcopy field forms into the electronic database by the project lead. Immediately following data entry, a verification step is conducted to check for hardcopy-to-electronic transcribing errors. During data entry, some validation checks (those incorporated in database design) occur, such as limiting values to allowable ranges. In addition, a final validation overview is performed by the project lead to capture any further inconsistencies not resolved during data entry.

Data Stewardship

Good data stewardship practices play a critical role in long-term data security and help prevent irretrievable loss or corruption of data. This includes using established directory structures, maintaining backups, following naming conventions, and preparing metadata. Final data that have been verified, validated, and accepted are documented with metadata by the data manager. The metadata document accompanies all archived annual data sets on the network server, and it is also distributed with data requests and uploads to national databases.

For more information on data management procedures, see SOP 6 *Data Management and Quality Assurance / Quality Control—Version 2.0* (CUPN 2024e).

Analysis and Reporting

Data Analysis

The monitoring questions and subsequent analysis of the supporting data address whether ozone concentration is high enough to cause ozone foliar injury and whether that injury is occurring. Estimates of ozone exposure indices are retrieved from NPS Air Resources Division's (NPS-ARD) website, as discussed subsequently in *SOP 7 Data Analysis and Reporting—Version 2.0* (CUPN 2024f). Ozone concentration data from Mammoth Cave National Park are downloaded from NPS-ARD's monitoring support contractor's website, as discussed in SOP 7.

Foliar injury data consist of injury amount (how much of the total plant shows any level of ozone injury) and injury severity (on average, how much of the plant's leaf surfaces show ozone injury). These data are recorded as indices from zero to five, with zero meaning there is no injury, up to five, meaning nearly all the plant or leaf surface shows injury. In annual reports, these data will be presented in various tables without statistical analysis.

The amount and severity of ozone-caused foliar injury will be addressed using a simple presentation of the data collected, specifically by listing the plants examined, whether injury was suspected and subsequently confirmed, and the amount and severity indices for plant species that were confirmed to have injury.

Relationships between ozone exposure indices (as indicated by NPS Air Resources Division interpolated data), Palmer Drought Severity Indices, and foliar injury data will be explored in annual reports through a discussion in the text. For example, for a site and year when ozone concentrations are low, there is no drought, and very little foliar injury is discovered, it will be explained this is the expected finding and the reasons why can be discussed.

In analyses of multiple years of data, the long-term data and relationships between variables will be explored. This includes trend analysis of presence or absence data of ozone injury on individual plants at Mammoth Cave National Park along with the relationship between ozone injury and abiotic variables. It also includes similar explorations of foliar injury data from the remaining CUPN parks, but without trend analysis because data at those parks are only collected one out of every six years. We will look for relationships between ozone indices, environmental conditions, and ozone injury. If foliar injury amount and severity are large enough, it may become possible to also explore trends in those indices. Further, how widespread the injury is across species and parks will be explored. The exact topics and approach to be discussed in synthesis reports are provided in SOP 7 (CUPN 2024f).

Reporting

Annual resource briefs are completed within one year of data collection, although delays may be necessary if the voucher specimen evaluations from the regional expert are delayed. A trend and synthesis report for Mammoth Cave National Park and synthesis reports for the remaining parks are written at the end of each six-year cycle through the parks. The data collected are also available to the public and could be used by other entities for their analyses and publications. For more information on data analysis and reporting, see SOP 7 (CUPN 2024f).

Personnel Requirements and Training

Roles and Responsibilities

Implementation of this protocol requires a team of staff members and a foliar injury expert who independently evaluates specimens with suspected injury. The overall project lead oversees and directs project operations. The project lead is the principal scientist for implementing this protocol and is supervised by the Mammoth Cave National Park (MACA) Science and Resource Management Division Chief with oversight by the CUPN program manager. The project lead obtains park research permits, leads foliar injury training, coordinates and conducts foliar injury surveys, filing trip reports, entering data, verifying, and validating data, summarizing data, and analyzing and reporting on data.

The CUPN program manager ensures staff members receive appropriate training for foliar injury identification and data management. A combination of trained CUPN and park staff are responsible for data collection, data recording, post-sampling equipment inspection and storage, and shipping or receiving of sampling materials. Data entry is the responsibility of the project lead with assistance from the CUPN data manager and other CUPN staff. Data management responsibilities are shared among the project lead, CUPN data manager and park staff. The CUPN data manager ensures that adequate QA/QC procedures are built into the database management system. The data manager and project lead ensure data handling procedures are followed.

Voucher specimens resulting from foliar injury surveys will be handled by the CUPN biologist/curatorial specialist in conjunction with the protocol lead. The protocol lead communicates the details about vouchers to the regional foliar injury expert and coordinates the expert's review. The biologist/curatorial specialist photographs and ships specimens to the regional expert. When specimens are returned, the biologist/curatorial specialist catalogs specimens and photographic vouchers in the Museum Collections Management System (MCMS) according to park instructions. The protocol lead helps with these tasks as requested.

Qualifications and Training

To ensure data quality, staff must be knowledgeable on the tasks for which they are responsible. New crew leaders need to be familiar with SOP 4 *Sampling—Version 2.0* (CUPN 2024a) and given hands-on training by the project lead, or a designee. Further, foliar injury crew leaders and QA/QC persons must complete the training outlined in SOP 1 *Training of Field Personnel—Version 2.0* (CUPN 2024g) to collect foliar injury data. Pre-sampling “training and handling preparation” meetings are held to review sampling procedures for the assessment of foliar injury symptoms, especially to provide some level of training for less-experienced crew members. Prior to any fieldwork each year, all members of the field crew must read and sign the protocol's Job Hazard Analysis. See SOP 1 (CUPN 2024g) for further training guidelines. In addition to internal staff qualifications, this protocol requires the assistance of an external expert in the identification of foliar injury symptoms. This person must have the appropriate knowledge and experience to conduct these assessments. Previously, the Cumberland Piedmont Network used the expert evaluator that was employed by the USFS ozone monitoring program. Since that program was discontinued, the network has used a university professor with the appropriate background to evaluate and confirm ozone injury.

Operational Requirements

Foliar Injury Startup and Annual Costs

Startup costs of foliar injury were reduced because the Cumberland Piedmont Network was able to use extant monitoring methods developed by the USFS (2008). The bulk of the development costs were related to staff time for writing the CUPN protocol and a day of training with USFS personnel at Mammoth Cave National Park. A rough estimate of equipment costs and time required (with resultant cost) are shown in Table 4.

Table 4. 2008 Start-up costs for version 1.0 of this protocol.

Category	Description	Quantity (hours for personnel, count for items)	Unit Cost (\$)	Total Cost (\$)
GS-11 Physical Scientist	Training	8	45	360
	Protocol/SOP Development	500	45	22,500
GS-12 Biologist	Training	8	50	400
	Protocol/SOP Development	200	50	10,000
GS-12 MACA Air Quality Specialist	Training	8	50	400
	Protocol/SOP Development	200	50	10,000
GS-09 Biologist	Training	8	40	320
Equipment	Camera	1	200	200
	Plant press	1	80	80
	GPS Unit	1	350	350
	Rangefinder or Tape Measure	1	200	200
	Compass	1	20	20
Equipment Total	–	–	–	850
Grand Total	–	–	–	44,830

Annual costs for foliar injury assessments are comprised primarily of staff time. Foliar injury assessments are generally handled by centralized CUPN staff with help from park personnel. For fieldwork at Mammoth Cave National Park, there are two teams, usually with two people each: the standard team and the QA/QC team. At other parks, personnel involved varies because some sites are more difficult to access safely. Annual costs for each year (based upon what has happened in past years) of the cycle are shown in Table 5. The average annual cost across those six years is \$7,664. These estimated costs do not include time contributed by the foliar injury estimate, and costs associated with occasional synthesis reports.

Table 5. Recurring annual costs of executing this protocol.

Category	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Personnel GS-11 Physical Scientist (110 hours per year)	4,950	4,950	4,950	4,950	4,950	4,950
Personnel GS-9 Curatorial Specialist (20 hours per year)	800	800	800	800	800	800
Personnel GS-11 GIS Specialist (4 hours per year)	180	180	180	180	180	180
Personnel GS-09 Field Assistant (16 hours per year)	640	640	640	640	640	640
Personnel – CUGA (16 hours contributed)	640	0	0	0	0	0
Personnel – SHIL (16 hours contributed)	0	640	0	0	0	0
Personnel – LIRI/RUCA (16 hours contributed)	0	0	0	640	0	0
Personnel – CARL (8 hours contributed)	0	0	0	0	320	0
Personnel – NISI (8 hours contributed)	0	0	0	0	0	320
Personnel – KIMO (8 hours contributed)	0	0	0	0	0	320
Equipment replacement	100	100	100	100	100	100
Fuel	110	190	110	110	190	190
Travel	330	360	210	110	360	510
Postage	50	50	50	50	50	50
Totals	7,800	7,910	7,040	7,580	7,590	8,060

Foliar Injury Annual Workload

Table 6 details the tasks typically carried out each year (along with general timeframes) and staff that have generally been involved with each task in the past.

Table 6. Annual protocol tasks carried out by various staff and timeframes for their completion.

Task	Timeframe for Completion	Staff Involved
Complete NPS Investigator's Annual Reports for previous field sampling year.	January–February	Project lead
Write sampling proposal and submit research permit at each park for current year's sampling efforts.	January–February	Project lead
Produce GIS maps for resource briefs that cover previous year's sampling efforts.	February	GIS specialist
Write resource briefs for previous year's sampling efforts and publish.	March–June	Project lead
In preparation for upcoming season's sampling efforts, communicate with active parks about upcoming sampling efforts about a month prior to sampling.	June–July	Project lead

Table 6 (continued). Annual protocol tasks carried out by various staff and timeframes for their completion.

Task	Timeframe for Completion	Staff Involved
Complete training exercises.	July	Crew leader and QA/QC person
Complete travel authorizations, make reservations and arrange other travel details for going to active parks.	July–August	Crew leader and others as necessary
Assemble supplies and send reminders to active parks about upcoming visits.	July–August	Crew leader
Complete fieldwork.	July–August	Crew leader, QA/QC person, and others as necessary
Complete travel vouchers.	July–August	Crew leader and others as necessary
Put away supplies and equipment. Assess for damage and need for repair or replacement.	August–September	Crew leader and project lead
Finalize field forms. Note: the final section of the voucher specimen field form must be completed after receiving assessment from regional expert.	July–August	Crew leader, QA/QC person and project lead
Send trip reports to each park where sampling occurred.	July–September	Crew leader and project lead
Offload, rename, and store digital site photographs.	August–September	Crew leader and project lead
Communicate with regional expert about voucher specimens that will be sent.	August–September	Project lead
Prepare voucher specimens for shipping to regional expert.	August–September	Curatorial specialist
Send vouchers to regional expert.	August–September	Curatorial specialist
Scan, rename, and store site sketches.	September	Project lead
Conduct operational review of current year's field sampling. Operational review and its follow-up will be initiated and coordinated by project lead.	October	Project lead, crew leader, QA/QC person, and any other available field staff
Upon return of voucher specimens from regional expert, take, rename, and store final images of specimens. Laminate vouchers and log vouchers into ICMS. Place vouchers into final storage.	Within two months of receipt from regional expert.	Curatorial Specialist
Complete voucher specimen field form with assessment results provided by the regional expert.	Within one month of receipt from regional expert.	Project lead

Table 6 (continued). Annual protocol tasks carried out by various staff and timeframes for their completion.

Task	Timeframe for Completion	Staff Involved
Enter data into CUPN ozone foliar injury database. This includes 100% review by data entry person at time of data entry and 10% independent review by other staff.	Within one month of completion of voucher specimen field form.	Project lead and project lead designee
Scan finalized field forms. Rename and store scans of field forms. Store hardcopy of finalized field forms.	Within one month of receipt of regional expert assessments.	Project lead and project lead designee
Complete annual data review.	Within one month of completion of data entry into CUPN ozone foliar injury database.	Project lead and data manager
Archive digital scans and hardcopy of field forms.	Within one month of completion of field forms being scanned.	Curatorial specialist
Update protocol tracker tabs, including completion of Application Inventory Registry compliance, on NPS Inventory and Monitoring protocol tracker website.	December	Project lead and data manager

Facility and Equipment Needs

Ozone foliar injury sampling requires normal office space and equipment storage. Additionally, storage for voucher specimens is needed. There are two types of vouchers: specimen vouchers and photographic vouchers. Specimen vouchers must be stored in specialized storage cabinets and in accordance with guidelines as set forth in the NPS museum handbook. Photographs are digital and are archived on the CUPN server, also in accordance with guidelines set forth in the [NPS Museum Handbook](http://www.nps.gov/history/museum/publications/handbook.html) (<http://www.nps.gov/history/museum/publications/handbook.html>). SOP 2 *Pre-Sampling—Version 2.0* (CUPN 2024b) contains lists of field equipment needs.

Procedure for Revising the Protocol and Archiving Previous Protocol Versions

No protocol is expected to remain unchanged. We anticipate the foundation, the logic and rationale of program design, to remain as written over the term of the program. However, there will come a time when program logistics, such as sample locations, cost estimates, methods, and reporting criteria may require updating. Careful documentation of changes to the protocol are essential when people, in the future, examine data trends, for example, to know exactly under what conditions the data were gathered. All ozone monitoring data will be entered into a database, which contains a field that denotes the protocol version under which the data were collected. Each protocol version will be properly archived to ensure future retrieval.

The rationale for dividing a sampling protocol into a Protocol Narrative with supporting SOPs is based on the following:

- The Protocol Narrative is a general overview of the protocol that gives the history and justification for performing the work as well as a summary of the sampling methods. It does not contain all methodological details which are found in the accompanying SOPs. The Protocol Narrative is rarely changed.
- The SOPs, in contrast, are specific detailed instructions for performing given tasks and are expected to be revised more frequently than the Protocol Narrative.
- When an SOP is revised, in most cases, it will not require modification to the Protocol Narrative, unless it affects the Narrative direction or rationale.
- All electronic versions of the Protocol Narrative and SOPs are archived on the network server in the CUPN protocol folder under the Vital Signs project folder for Ozone. Revised versions of the protocol are posted to the Integrated Resource Management Application (IRMA) site with links added to the NPS I&M protocol database and CUPN websites.

Steps for changing the Protocol Narrative or SOPs are detailed in SOP 8 *Revising the Protocol and Documentation of Deviations and Major Changes—Version 2.0* (CUPN 2024h). The Protocol Narrative and each SOP also contains a Revision History Log (Table 7) that is filled out each time the Protocol Narrative or an SOP is changed.

Table 7. Example of Protocol Narrative Revision History Log.

Revision Date	Author	Changes Made	Reason for Change	New Version #
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—

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