Monitoring Air Quality Using Lichens

Methods and Strategy

For the
Columbia River Gorge National Scenic Area
Deschutes National Forest
Gifford Pinchot National Forest
Mt. Hood National Forest
Siuslaw National Forest
Umpqua National Forest
Wallowa-Whitman National Forest
Willamette National Forest
Winema National Forest

of
Oregon and Washington
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1. INTRODUCTION

1.1 LICHEN MONITORING BY THE USFS PACIFIC NORTHWEST REGION AIR RESOURCE MANAGEMENT PROGRAM: HISTORY AND OBJECTIVES

In 1993, managers of the Willamette, Siuslaw, Deschutes and Mt. Hood National Forests pooled resources to develop a unified approach to air quality biomonitoring using non-vascular plants. Other administrative units added to the program were the Gifford Pinchot National Forest and the Columbia River Gorge National Scenic Area in 1994, the Umpqua and Winema National Forests in 1997 and the Wallowa-Whitman National Forest in 1998. The program's primary objective is to help national forest managers obey federal and state laws, and fulfill agency mandates with regard to the detection and quantification of adverse effects from air pollution on forest ecosystems and resources by:

1. Establishing a network of sites on national forest lands on which inventories of lichen communities and chemical analysis of lichen tissue for nitrogen, sulfur and metals are performed on a regular basis.
2. Monitoring lichen community composition to document and map locations where air quality has improved or deteriorated and to document adverse effects to sensitive lichens. (Lichen community composition is considered highly sensitive to sulfur dioxide (SO$_2$), fluorine gas (F), acid rain (including sulfuric and nitric acids), and fertilizing compounds such as ammonia (NH$_3$) and nitrates (NO$_3$). Lichens are less sensitive, but still responsive if levels are sufficiently high, to nitrogen oxides (NO$_x$), ozone (O$_3$) and peroxyacetyl nitrate (PAN).)
3. Using chemical analysis to map areas of concern by documenting enhanced levels of sulfur- and nitrogen-containing pollutants and toxic metals in lichen and moss tissue.
4. Building a publicly accessible, unified lichen database, interfaceable with other Forest, regional and national databases.
5. Providing analysis and interpretation of biomonitoring data, including thresholds for enhancement of sulfur, nitrogen and metals in lichen tissue, sensitivities of lichens to air pollutants, and site scores for air quality based on lichen community composition.

A secondary objective is to provide current and historical information about the diversity, abundance, distribution, and habitat requirements of lichens on national forest lands.

1.2 USFS RESPONSIBILITIES FOR AIR RESOURCE MANAGEMENT

Forest responsibilities for monitoring air quality are founded in law and are described in agency and regional documents and federal legislation. Under these mandates, line officers are responsible to detect, describe and speak up about air pollution affecting the national forests:

1.21 Federally legislated responsibilities

The Clean Air Act (CAA) of 1970 and its amendments in 1977 established 156 Class I areas, 88 of which are managed by the USFS. Stringent air-quality standards protect these clean air regions. Federal land managers are required to participate in the Prevention of Significant Deterioration (PSD) permitting process for new or modified pollution sources. The PSD process requires that managers predict changes that would likely occur to air quality related values (resources that may be affected by a change in air quality) if the permit were granted. Forest Service managers are required to obtain enough information to independently evaluate proposals and effects, rather than merely echo the assessment of either the proponent or the regulatory agency.
Other federal mandates include the:

a. Forest and Rangeland Renewable Resources Planning Act of 1974, recognizing the fundamental need to "protect and, where appropriate, improve the quality of the soil, water and air resources".

b. The Federal Land Management Policy Act of 1976, establishing the policy that the “US national interest will be best realized if the public lands and their resources are periodically and systematically inventoried in a manner that will protect the quality of scientific, scenic, historical, ecological and environmental, air and atmospheric, water resources and archeological values...”

c. State Implementation Plans (SIP), prepared by US states and required by the CAA. SIP is the contract between EPA and each State to attain and maintain acceptable air quality. Air quality standards can be stricter in SIPs than they are in the CAA. The Forest Service can be very influential in the preparation of the State Implementation Plans.

d. National Environmental Policy Act (NEPA) of 1976. Air is an issue common to all NEPA actions and many special use permits.

e. The Wilderness Act, the Farm Bill, the Organic Act of 1897, the Multiple Use-Sustained Yield Act and NFMA all mandate Forest Service roles in the protection of Forest health and ecosystems from impairment, of which air is an intrinsic element.

f. Agenda 21, the document signed by our country in 1992 at the United Nations Conference on Environment and Development in Rio de Janeiro, Brazil, places a high value on understanding ecosystems, including the atmospheric and climatological components.

1.22 USFS Air Resource Management policy

Within the US Forest Service, the Air Resources Management (ARM) program addresses issues of air quality. Although ARM originated as a PSD Class I area protection program, Class I areas comprise only 8% of the National Forest System. The main value of understanding the air resource and the effect of air pollution on other resources is to accomplish quality integrated ecosystem management. The Chief's policy is that air is a fundamental resource and shall be managed as other Forest resources such as soil and water. The ARM program is described in detail by agency documents including the Forest Service Air Resources Handbook (FSH 2509.19 Expired Interim Directive No. 1., and subsequent updates), the Forest Service Manual (2580) and individual Forest Plans.

To underscore the fundamental tenants of the US Forest Service ARM program, an agency framework document was prepared and approved in 1988 (USFS 1988). One of its three basic elements is the protection of Class I and II areas by:

1. Determining locations for high pollutant concentrations and areas of probable adverse effects using existing emission data, air quality monitoring, personal observation, modeling, and professional consultation.
2. Determining the current condition of Forest resources.
3. Establishing monitoring sites throughout forest areas.
4. Determining adverse effects of air-pollution on National Forest resources due to outside generated emissions sources, and obtaining baseline data for modeling potential impacts from proposed new emission sources.
5. Orienting air regulatory personnel to air quality conditions, trends and the significance of findings.
6. Training specialists and managers on operations and management methods.
1.23 Regional guidelines
In May 1992, Region 6 published *Guidelines for Evaluating Air Pollution Impacts on Class I wilderness Areas in the Pacific Northwest* (Peterson et al. 1992). The preface states: "FS ARM managers in the PNW are responsible for protecting Class I Wilderness from air pollution. To do this, they need scientifically defensible information to determine critical concentrations of air pollution having the potential to impact Class I wilderness values". The guide specifically names lichens as an Air Quality Related Value, recommends the use of lichens and bryophytes as biomonitors of air quality, and states that the FS should implement monitoring independent of the PSD review process.

In summary, federal laws and agency guidelines and policies mandate that managers of the national forests monitor air quality and account for adverse effects to forest ecosystems resulting from changes in air quality. Lichen monitoring was added to the current USFS Pacific Northwest Region air program to help satisfy these information needs.

1.3 LICHENS AS BIOMONITORS OF AIR QUALITY

1.31 Monitoring sulfur, nitrogen and metals via chemical analysis of lichens.
Lichens are composite organisms formed by a fungus and a green alga and/or a blue-green bacterium. Lichens lack mechanisms utilized by higher plants for water uptake (e.g. root systems, conducting tissue) and regulation of gas exchange (e.g. waxy cuticles, stomates). Surface area contact with the substrate by many lichen species is relatively low and, when lichens are hydrated, gas exchange occurs over the entire surface. Compared to vascular plants, elemental content of lichens is strongly affected by atmospheric influences: gases, particulate matter and precipitation. Because of their unique biology, lichens accumulate a wide variety of air pollutants, many of which can be predictably correlated with average atmospheric deposition (Herzig et al. 1989, Ross 1990, Saeki et al. 1977, Garty 2001). Sulfur, nitrogen and metal concentrations in lichen tissue reveal pollution concentrations within a region or around a point source, providing a widely accepted method for monitoring air quality (Richardson 1992, Nash and Gries 1991, Stolte et al. 1993, Nash 2002).

Lichens accumulate elements by gas exchange, by entrapment of airborne particulates, by ion-exchange of dissolved metals and other ions to cell walls, and by active transport, particularly of sulfur and phosphorus, across cell membranes (Richardson and Nieboer 1983). Lichens undergo rapid hydration and dehydration. These processes alternately concentrate and leach pollutants, maintaining a dynamic equilibrium with atmospheric and substrate sources of these chemicals. Accumulation or depletion can be rapid as evidenced by the detection of significant changes in elemental status in short term transplant and seasonal studies (Boonpragob and Nash 1990, Gailey and Lloyd 1986, Garty 1988, Puckett 1985).

Two basic approaches to lichen biomonitoring are: 1) element analysis of lichen tissue, and 2) species presence and cover (Wetmore 1988, Will-Wolf 1988). Element analysis can detect gradual changes in tissue levels before conditions become lethal and can be a more sensitive method than species mapping. The presence of specific anthropogenic elements in lichen thalli offers direct evidence of their presence in the air. Lichen tissue concentrations can be compared to background values in the literature, or to baseline studies, to determine whether they are elevated. If tissue data can be calibrated with direct measurements of air pollution at instrumented sites, lichens can be used to estimate annual average ambient levels, or deposition, of S, N and certain metals.

1.32 Sensitivity of lichens to air pollutants.
Lichens have species-specific response patterns to increasing levels of atmospheric pollutants, ranging from relative resistance to high sensitivity. Sensitive species are damaged or killed by annual average levels of sulfur dioxide as low as 8-30 µg/ m$^3$ (Johnson 1979, DeWit 1976,
By short term exposure to nitrogen oxides as low as 564 ug/m$^3$ (Holopainen and Kärenlampi 1985) and by peak ozone concentrations as low as 20-60 ug/m$^3$ (Egger et al 1994, Eversman and Sigal 1987). With regard to ozone, most reports of adverse effects on lichens were in areas where peak ozone concentrations were at least 180-240 µg/ m$^3$ (Scheidegger and Schroeter 1995, Ross and Nash 1983, Sigal and Nash 1983, Zambrano and Nash 2000). Ruoss et al (1995) found no adverse effects on lichens in areas of Switzerland with daily summer peaks of 180-200 µg/ m$^3$. They attributed lack of response to low lichen metabolic activity caused by low humidity at times of the day when ozone was highest; ozone concentrations never rose above 120 µg/ m$^3$ when the relative humidity was over 75%.

In addition to gaseous pollutants, lichens are sensitive to depositional compounds, particularly sulfuric and nitric acids, hydrogen ions, sulfites and bisulfites, and other fertilizing or alkalinizing pollutants such as NH$_3$ and NH$_4^+$+. While sulfites, nitrates, and bisulfites are toxic in themselves, acidic compounds affect lichens in three ways, through toxicity of the H$^+$ ion, fertilization by NO$_3^-$ and, acidification of bark substrates (Farmer et al 1992). For example, in a study of northwest Britain, Lobaria pulmonaria was limited at nearly all sites to trees with bark pH >5 (Farmer et al 1991). In the Netherlands, a number of studies have demonstrated that ammonia-based fertilizers alkalinize and enrich the nutritional composition of lichen substrates which in turn influences lichen community composition and element content (van Herk 1999, van Dobben et al. 2001, van Dobben and ter Braak 1999 and 1998). Finally, it is clear that pollutant mixes can have synergistic protective or adverse effects on lichens and that individual species differ in their sensitivity to these pollutants and their response to pollutant mixes (Hyvärinen et al 1992, Gilbert 1986, Farmer et al 1992).

The ability of lichens to absorb and concentrate sulfur from oxidized sulfur sources is well established, as is their sensitivity to SO$_2$ gas. The first indications of air pollution damage from these sources are inhibition of nitrogen fixation, increased electrolyte leakage, decreased photosynthesis and respiration followed by discoloration and death of the algae (Fields 1988). More resistant species tolerate regions with higher concentrations of these pollutants, but may exhibit changes in internal and/or external morphology (Nash and Gries 1991, Will-Wolf 1980).

A preliminary air quality assessment can be made by studying the lichens present in an area with reference to their sensitivities to sulfur dioxide or other pollutants. If many or all of the more sensitive species are absent from an area where they would be expected to occur, there is a high probability that the air quality has been degraded. If all of the expected sensitive species are present, sulfur dioxide levels can be expected to have little biological impact on other organisms. Denison (1987) cautions: 1) lichen community dynamics are complex and a missing species can also be due to gradual climatological and environmental changes during natural succession, and 2) variation in the skill and meticulousness of the individual researchers who measure and identify the lichens can affect results as much as pollution effects. The most accurate results from this method are achieved where historical records (Wetmore 1988) and a good quality control /quality assurance program to assess and minimize observer error are available (Stolte et al. 1993).

2. METHODS

2.1 SUMMARY OF APPROACH

Methodology and training procedures follow the protocols of the Lichen Indicator section of the Forest Health Monitoring (FHM) program (Tallent-Hassell 1994) [see http://fia.fs.fedu.us/library.htm#Manuals for an electronic copy of the current manual]. FHM was developed under the auspices of the US Environmental Protection Agency to monitor the
condition of the nation’s forests; the lichen indicator assesses the status of and trends in air quality and climate and has been closely scrutinized for repeatability (McCune et al. 1997). FHM is a component of EMAP (Environmental Monitoring and Assessment Program), and is currently administered by the USFS Forest Inventory and Analysis program. All FHM lichen indicator data are archived with the Information Management group for FHM at the EPA office in Las Vegas, Nevada. The methods in this manual produce data for managers of Pacific Northwest regional national forests that is comparable with data produced by the FHM lichen indicator. Plots are located on sampling point #1 of the FIA P2 grid (formerly the regional 5.4 km Current Vegetation Survey grid—see Section 3) and are permanently marked in the field and digitized within each Forest’s geographical information system. Managers on individual Forests select priority areas for monitoring or more intensive sampling. The default monitoring area and intensity is the entire Forest at the P2 scale. (This is an intensification of the same grid by the FIA/FHM. FHM monitors 1/16 of the P2 plots for lichens and provides regional assessments, whereas managers of national forests seek watershed level assessments). Each forest is monitored in a four year round. One-quarter of forested plots are monitored each year. A ten-year monitoring interval is recommended between rounds. For example, if a monitoring round began in 1993, it would be completed in 1996. The next round would then begin in 2003.

In the basic plan, like the FHM lichen indicator, specially trained field crews perform a complete survey of epiphytic macrolichens, including ocular abundance estimates. In addition, and different from the FHM lichen indicator, target lichens and mosses are collected for tissue analysis at all plots. Twenty grams dry weight of two target lichens or mosses are collected at each plot. These samples are used to establish site baselines for toxic elements and to determine sub-regional element profiles. Target lichens are regionally abundant and easy to collect and prepare for analysis.

The methodology described herein emphasizes quality control and minimizes specialized knowledge required by field personnel. The program coordinator spot checks crews once or twice during the summer for quality control, inspects and aids sample preparation for tissue analysis, verifies identities of lichens collected in the field and supervises database entry. Forests share a common database from which statistical analyses are performed. Reports are generated on demand, to summarize trends in, and current status of, air quality and to quantify ecosystem effects of air pollution via its effects on the Air Quality Related Value, lichen communities.

Supplements to the basic plan include expanded tissue analysis for elements of local, but not Forest-wide or regional concern, more intensive sampling in Class I Wilderness or areas of special concern, and the addition of transects to answer specific questions regarding point sources. Photodocumentation, growth studies, fumigations, transplants, or more quantitative measurements of lichen abundance may be added as needed to the basic monitoring strategy. These are not detailed here but can be developed by individual Forests or districts with the assistance of the program coordinator. Crustose and non-epiphytic macrolichens may be collected to inventory and establish habitat requirements of poorly known species.

### 2.2 TISSUE COLLECTION AND ELEMENT ANALYSIS METHODS

Element content refers to the concentration (percent or ppm dry weight) of selected elements. Elements are selected for analysis based on the likelihood of detectable enrichment from anthropogenic sources. ICP analysis provides a suite of elements for a set cost. Typically, this includes aluminum (Al), boron (B), barium (Ba), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), silica (Si), strontium (Sr), titanium (Ti), vanadium (V) and zinc (Zn). Total sulfur (S) and nitrogen (N) are measured by separate methods, and concentrations of these elements provide the best estimate of the exposure of lichens to harmful pollutants. Because of their environmental toxicity, mercury (Hg) and arsenic...
As are important elements to monitor, but they are much more expensive to analyze, and extra care must be taken in the field and laboratory to avoid volatilization leading to low recovery.

Each sample for element analysis must contain only one lichen species and be free of visible dirt, bark and other surface grit. Because lichen species differ in their ability to accumulate elements and because measurements must be comparable across national forest boundaries, only a few regionally common species are used for tissue analysis.

The elemental content of selected lichen species establishes site baselines for toxic elements and determines regional toxic profiles. Lichen elemental content is clearly indicative of key assessment questions, especially those concerning contamination of natural resources. Because a suite of elements is measured, it is sometimes possible to identify the different sources of contamination such as fossil fuels, saltwater aerosols and agricultural dusts (e.g. Sloof 1995, Reis et al. 1996).

Elemental content is determined by various methods. Typically, a 10 g composite sample can adequately represent the mean element content at a plot. Samples are hand-cleaned of debris, oven dried, then ground. A bulk collection of thoroughly dried and ground material if stored in the dark can be used to assess laboratory precision over a period of about ten years. Because lichens hydrate readily, and hydrated material can decompose, storage of the bulk collection in the freezer is preferable to storage at room temperature. Recently, it has become possible to acquire standardized lichen materials (see section 2.38). Lichen SRMs are highly desireable because some important elements, such as S and N, can be considerably lower in lichen samples than in plant SRMs.

### 2.21 Field Procedure

**What to collect:** Collect > 20 grams each of two target lichens, dry weight. Dusty, gritty, discolored, or decaying material should be avoided. Collect replicate samples as described in the quality control section below.

**Where to collect:** The target species should be collected from a minimum of 6 different locations near or within the plot. Lichens attached to tree branches, shrubs or tree boles, in the litter, or on fallen branches, may be used. Exceptions are target species in the genera *Alectoria*, *Bryoria* and *Usnea*. These deteriorate quickly on the forest floor and should not be collected from litter or fallen branches. In non-forested areas, *Xanthoparmelia cumberlandia* may be collected from rocks. Replicates and repeat collections should be made from the same host species and types of substrate locations, in roughly the same proportions.

Collections should be made near or on the plot but not more than 1 km (.65 miles) away from the plot perimeter-- approximately 20% of the distance to the next plot. Lichens should not be collected within 35 m of any road. Off-plot collecting will increase the probability of finding a given target species at each collection site.

**How to collect:** Most samples can and should be collected with the fingers. Non-powdered vinyl gloves are worn to prevent contamination of the samples. While wearing gloves the field crew should not touch anything brought with them onto the plot except the Kapak bag. Unused Kapak bags should be stored in a clean zip-loc plastic bag. New gloves will be used at each plot and replaced if they become torn or contaminated. A clean, stainless steel knife may be used to collect target species, e.g. *Xanthoparmelia*, that are tightly adhered to the substrate. Keep the designated collecting knife in a separate, clean, plastic bag.

The samples should be collected as clean and free from bark and other foreign surface material as is practical and will be cleaned carefully later in the office. Place samples in metalized polyester Kapak bags (Kapak Corp., Minneapolis, MN) and weigh on a 50 g Pesola spring scale. If the lichens are dry, the sample and bag together should weigh ≥ 28 g. If the lichens are wet,
the bag should weigh more than 100 g and adequacy of the sample size should be judged by volume rather than weight. After enough material has been collected, fold the edge of the bag over three times and seal with waterproof, removable, laboratory tape.

**What to record:** The following information should be recorded directly on the Kapak bag, and also on the field data card. Write on the bag with an indelible marker:
- Plot number
- Date
- Substrate(s): Host species name and substrate location in order by the amount of sample in the bag from that substrate. E.g. “*Pinus contorta* branches, *Pinus ponderosa* branches and boles” would indicate that the sample weight collected primarily from *P. contorta* branches and in lesser amounts from *P. ponderosa* branches and boles.
- Target species acronym.
- Collector’s initials
- Moisture status of sample at the time of collection: dry, damp or wet.

**Target Species:** Two species will be collected at each monitoring site from the following list of target species. Whenever possible, one of the target species should be *Platismatia glauca* (*Plagla*). Target species are grouped below by desirability. Target species acronyms are given in parentheses.

**Most Preferred**
- *Platismatia glauca* (*Plagla*), collect whenever possible

**Preferred**
- *Alectoria sarmentosa* (*Alesar*)
- *Evernia prunastri* (*Evepru*)
- *Hypogymnia enteromorpha* (*Hypent*)
- *Hypogymnia imshaugii* (*Hypims*)
- *Hypogymnia inactiva* (*Hypina*)
- *Letharia vulpina* (*Letvul*)

**Good**
- *Bryoria fremontii* (*Bryfre*)
- *Letharia columbiana* (*Letcol*)
- *Lobaria oregana* (*Lobore*)
- *Sphaerophorus globosus* (*Sphglo*)

**Acceptable if no other target species are present.** If a moss is collected, the second target species must be a lichen.
- *Isothecium myosuroides* (*Isomyo*)—moss
- *Lobaria pulmonaria* (*Lobpul*)
- *Neckera douglasii* (*Necdou*)—moss
- *Usnea* (*Usnea*)—shrubby species only

### 2.22 Sample Collection, Preservation, and Storage

Samples should be air dried as quickly as possible, preferably the same day, by spreading onto clean 100% acid free paper laid over a flat surface covered with clean plastic. Lichens and mosses should not be air-dried in areas subject to contamination (e.g. near cooking areas, roads, or in rooms where organic solvents are used, dust levels are high, or smoking is permitted). Place dry material back in Kapak bags and reseal as soon as the lichens are dry. Store in a clean, dry place. **Specimens must be thoroughly air dried to avoid fungal decay.**
2.23 Sample Delivery
After the first two plots are completed, specimens will be mailed or brought to the lichen specialist to allow immediate feedback to the field crews concerning specimen quality and quantity. Thereafter, the bags will be delivered biweekly or monthly to the program coordinator. Bags should be packed closely, but without excessive crushing, in a sturdy cardboard box. Bags from several plots can be mailed in the same box. A packing list should be kept by the field crew specifying the plot number, Forest, species, field replicate number, and mailing date of each sample (see "Forms" section).

2.24 Common Problems and Solutions
- None, or only one, of the target species is present in sufficient quantities for collection, even if the sampling area is expanded to the 1 km maximum sampling radius.
  - In this case, no samples, or only one sample should be collected. Do not substitute non-target species.
- Platismatia glauca is present, but it would be faster to collect other target species.
  - Limit collection time to two hours and collect other target species while looking for P. glauca. Although 20 grams is the desired sample size, if the material is clean, dry, and in good condition, field sample size as low as 12 grams may be useable. Samples that weigh < 8 grams after cleaning in the office are not usually sent to the analytical laboratory.
- More than 1 hour has been spent collecting but sample weight is still very low.
  - Get help from other crew members or switch to a more easily collected target species. Usually it’s a good idea to stop collecting after two hours and process the collection that has been made. The decision to send the sample to the laboratory will be made in the office after the sample is cleaned.
- All the sample material came from one or two trees.
  - This is not acceptable. The material must evenly represent at least six locations. Expand the area of collection up to the maximum size allowed. If material is still too scarce, collect a different species or collect nothing.
- Lichens were collected wet, it is still raining by evening, and the field crew is camping.
  - Drying the lichens is still important to prevent fungal decay. If the distance is reasonable, go to the nearest district office to dry the lichens. Alternatively, store the samples in a cooler with ice up to two days, then air dry lichens in a tent, or in clean mesh bags on a clothes line as soon as conditions improve. Avoid use of heating devices to dry samples.

2.25 Equipment and Supplies
Non-Consumable
- Locking-blade or fixed-blade knife (ca. 4" blade) with belt sheath.
- Pesola spring scale, 50 g.
- Reference samples of target species (provided or approved by the lichen specialist).

Consumable
- Black waterproof markers, such as “Sharpies”, for writing on specimen bags.
- 4 x 7" metalized polyester bags (sold by Kapak Corp., 5305 Parkdale Drive, Minneapolis, MN 55416, 1-800-527-2557 Product #60-4B-IM)). Two bags are needed for most plots, one for each species. Bring extra bags for field replicates.
- Rolls of laboratory tape, ¾ “ or 1” wide. Tape should adhere to wet surfaces and be removable. Masking tape and cellophane tapes are difficult to remove and are not recommended.
- Disposable vinyl gloves, not powdered, one pair of gloves per plot.
- New, gallon size Ziploc bags. Store gloves and Kapak bags in separate Ziploc bags, placed inside a 3rd Ziploc bag with the sharpie, scale and laboratory tape.
2.26 Interferences
This method may be used in any season or weather condition. Normally, the tissue collection season is approximately May 15 – Oct 15, that is, after the winter rains have ceased and before autumn rains have begun. Lichens collected during the rainy season typically have lower concentrations of mobile elements like S, N, K, Na, therefore mid to late summer is the ideal time to capture maximum pollutant loading. The method requires careful discrimination among species in the field and should not be performed in poor light or within an hour of sunset or sunrise.

2.27 Safety
Only minor hazards are associated with the method. Care should be used when removing lichen specimens with a knife. A locking-blade or fixed-blade knife is best. Trees should not be climbed to procure specimens.

2.28 Quality Control and Performance Standards
Only people who have successfully completed lichen training should collect the lichen elemental samples. Data quality will be measured at several times:
1. A post-training evaluation of field methods, based on sampling a test plot.
2. A mid-season evaluation of sample quality by the lichen specialist, and
3. Analysis of accuracy and precision after the specimens have been analyzed by the laboratory.

The following components of data quality will be evaluated:

**Precision:** Precision, or repeat measurement error, is determined in several ways:
1. Revisits by the field crews who re-sample one plot within one month of its initial sampling. Collections are made for the same target species sampled in the previous visit.
2. Field replicates are made at every fifth plot for each species. After collection for the first bag is completed, collection should be made into a second bag. The purpose of the field replicate is to assess variability in elemental content on the site due to the collection method. If each collection contains a representative selection of lichens on the plot, and both samples are in good condition, there should be little variability in element content between the two collections. Field replicates should only be collected at sites where material is plentiful to avoid creating a bias in which the first bag contain samples that are in better condition than the second bag.
3. Other components of precision are determined during lab analysis, by using various kinds of quality control samples (standards, splits, blanks: see section 2.4). The data quality objective (DQO) for precision is a coefficient of variation of 15%.

**Accuracy:** Accuracy is determined in the laboratory by analyzing reference samples with known elemental content. The DQO for precision is a coefficient of variation of 15%.

**Completeness:** Completeness is the proportion of plots that will yield usable data. The DQO for completeness is 90%. The most important aspect of quality control for completeness is ensuring that the lichen elemental samples are adequate, not decomposed, and being received by the program coordinator. The field crew should ensure specimen quality by periodically calling the program coordinator to verify shipments and soliciting comments and suggestions on the quality of the specimens.

2.29 Specialist Procedures
The samples will be processed first by the field crew who air-dry any samples that were damp or wet at the time of collection. The dry samples are then mailed or hand-carried to the program coordinator. The program coordinator processes all samples by 1), checking to see that each sample is thoroughly air dried, 2), verifying the identity of the species contained in the sample, 3), cleaning the sample so that it contains only one species, and 4) assigning a unique sample number to each sample bag. Data from sample bags are entered in a computerized database. The samples are then randomized, and assigned a consecutive laboratory ID number.
coordinator mails the samples to the analytical laboratory where they are processed and analyzed in order by laboratory ID number. Between 1993 and 2001 this laboratory was the Research Analytical Laboratory, Dept. of Soil Science, 135 Crops Research Bldg., University of Minnesota, St. Paul, MN 55108, Attn.: Roger Eliason, (612) 625-9211.

2.3 LABORATORY ANALYSIS

At the laboratory, air-dried samples are first passed through a stainless steel grinder with a 20-mesh sieve and mixed thoroughly. Ground samples are dried at 65°C for 2 hours and cooled in a desiccator before weighing for analysis. The following analyses are made:

2.31 Sulfur

Total sulfur is determined by combusting 200 mg of sample mixed with 500 mg of V$_2$O$_5$ in an oxygen atmosphere at 1370°C in a Leco Corp. SC-132 Sulfur Analyzer. The SO$_2$ evolved from the sample is determined by a nondispersive infrared detector empirically calibrated with LECO plant reference materials (LECO Corporation. 3000 Lakeview Dr. St Joseph, MI 49085). Reference materials used for sulfur analysis in the 1990s were commercially prepared Peach leaves (Alpha Resources, Stevensville, MI), NIST 1575 Pine Needles and NIST 1572 Citrus leaves.

2.32 Nitrogen

Between 1993 and 1997, total nitrogen concentration in lichen samples was measured using a semi-micro Kjeldahl digestion method (Horneck and Miller 1998). Since 1997, total nitrogen has been determined by combustion (Matejovic 1995). The combustion method yields slightly higher results because nitrates are determined (Simone et al. 1994 and empirical testing of duplicate lichen material). The standard reference material for nitrogen in the 1990s was NIST 1575 Pine Needles.

Total Kjeldahl nitrogen is determined by converting the various forms of nitrogen to NH$_4^+$, measuring NH$_4^+$ concentration, and subtracting the weight of hydrogen. To accomplish this, 0.150 g of dry, ground plant material is digested in 3.5 ml concentrated H$_2$SO$_4$ with 1.5 K$_2$SO$_4$ and 7.5 mg selenium. This mixture is placed in an electrically heated aluminum block at 400°C and digested for 1 hour. The NH$_4^+$ formed is reacted with salicylate in the presence of hypochlorite and nitro-prusside to form an emerald-green complex. Color intensity is measured spectrophotometrically on a Technicon AutoAnalyzer at 660 nm. The method converts only partial amounts of nitrate, thus samples containing high concentrations of nitrates must be pretreated with salicylic acid to ensure complete conversion. The nitrate reduction step is not necessary for lichen samples. Nitrates comprised less than 0.01% of the total nitrogen in a randomized subset of regional lichen samples.

The combustion method for total nitrogen uses a LECO FP-528 Nitrogen Analyzer. A 150-500 mg sample is weighed into a gel capsule and dropped into an 850°C furnace purged with O$_2$ gas. The combustion products (CO$_2$, H$_2$O and NO$_x$) are filtered, cooled by a thermoelectric cooler to condense most of the water, and collected in a large ballast. A 3 cc aliquot of the ballast combustion product is integrated into a helium carrier stream. The stream first passes through a hot copper column to remove O$_2$ and convert NO$_x$ to N$_2$. A reagent tube then scrubs the remaining CO$_2$ and H$_2$O from the stream. N$_2$ content is measured by a thermal conductivity cell against a helium background and the result displayed as weight percentage of nitrogen.

2.33 Aluminum, boron, cadmium, calcium, chromium, copper, iron, lead, magnesium, manganese, nickel, phosphorus, potassium, sodium, and zinc.

These elements are determined using simultaneous inductively coupled-atomic emission spectrometry (ICP-AES) (Dahlquist and Knoll 1978). Reporting limits during the 1990s are listed in Table 1. For this analysis, one gram of sample is weighed into a 20 ml high form silica crucible, covered, and dry-ashed at 485 C for 10-12 hours in a circulating air muffle furnace (Munter and Grande 1981). After ashing, 5 ml of 20% HCl is added and the mixture is boiled
under reflux for about 3 hours for improved recovery of aluminum, chromium and iron. After cooling, 5 ml of deionized water is added. This digest solution is gently swirled and allowed to settle for 3 hours. The supernatant is decanted and transferred to 15 ml plastic disposable tubes for direct determination. During ICP-AES analysis, measurement of a sample is repeated three times with 10-s gas flow between each measurement.

The procedure is only a partial digestion of the sample that is designed to solubilize the less refractory components of plant material. Silicate matrices that may be present as contaminants of the sample are not as completely solubilized in this procedure as the plant tissue.

**Table 1.** University of Minnesota Research Analytical Laboratory Determination and Reporting Limits for ICP-AES analysis of lichen tissues.

<table>
<thead>
<tr>
<th>Element</th>
<th>Determination Limit</th>
<th>DL Units (dry wt)</th>
<th>Analytical Method</th>
<th>Wavelength</th>
<th>Reporting Limit ug/l (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>0.5 %</td>
<td>Ash</td>
<td></td>
<td></td>
<td>180</td>
</tr>
<tr>
<td>N</td>
<td>0.01 %</td>
<td>LECO</td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>S</td>
<td>0.01 %</td>
<td>LECO</td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Al</td>
<td>3.6 ug/g</td>
<td>ICP-AES</td>
<td>308.215</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>0.78 ug/g</td>
<td>ICP-AES</td>
<td>193.696</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.46 ug/g</td>
<td>ICP-AES</td>
<td>249.773</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>0.12 ug/g</td>
<td>ICP-AES</td>
<td>455.403</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Be</td>
<td>0.04 ug/g</td>
<td>ICP-AES</td>
<td>313.042</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>4.36 ug/g</td>
<td>ICP-AES</td>
<td>317.933</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.12 ug/g</td>
<td>ICP-AES</td>
<td>226.502</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0.24 ug/g</td>
<td>ICP-AES</td>
<td>228.616</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.28 ug/g</td>
<td>ICP-AES</td>
<td>205.552</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.52 ug/g</td>
<td>ICP-AES</td>
<td>324.754</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.96 ug/g</td>
<td>ICP-AES</td>
<td>259.940</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>14 ug/g</td>
<td>ICP-AES</td>
<td>766.491</td>
<td>708</td>
<td></td>
</tr>
<tr>
<td>Li</td>
<td>0.4 ug/g</td>
<td>ICP-AES</td>
<td>670.781</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>3.8 ug/g</td>
<td>ICP-AES</td>
<td>279.079</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.06 ug/g</td>
<td>ICP-AES</td>
<td>257.610</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>0.22 ug/g</td>
<td>ICP-AES</td>
<td>202.030</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>3.6 ug/g</td>
<td>ICP-AES</td>
<td>588.995</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.44 ug/g</td>
<td>ICP-AES</td>
<td>231.604</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.7 ug/g</td>
<td>ICP-AES</td>
<td>214.914</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>1.7 ug/g</td>
<td>ICP-AES</td>
<td>220.353</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Rb</td>
<td>53 ug/g</td>
<td>ICP-AES</td>
<td>780.020</td>
<td>2650</td>
<td></td>
</tr>
<tr>
<td>Si</td>
<td>1 ug/g</td>
<td>ICP-AES</td>
<td>251.611</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>0.06 ug/g</td>
<td>ICP-AES</td>
<td>421.552</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>0.3 ug/g</td>
<td>ICP-AES</td>
<td>334.941</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.36 ug/g</td>
<td>ICP-AES</td>
<td>292.402</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.4 ug/g</td>
<td>ICP-AES</td>
<td>213.856</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Reporting limits are based on the concept of the Lowest Quantitatively Determinable Concentration (LQDC) and are 5 times the instrument detection limit. Precision at the LQDC is approximately ± 10% and analytical results are quantitative. The instrument detection limit is 2 times the standard deviation of eleven replicates of a reagent water sample (blank).
2.34 Barium, beryllium, cobalt, lithium, molybdenum, rubidium, silicon, strontium, titanium, and vanadium

Resources permitting, tissue samples are analyzed for these trace elements by the same ICP procedures described above. During the 1990s, concentrations of Be, Co, Li and Rb in Pacific Northwest lichens from background “clean” areas were below laboratory detection limits.

2.35 Ash

The percentage ash yield is determined by combusting 1 g of the oven-dried sample at 485 °C for 12 hours in a circulating air muffle furnace.

2.36 Fluoride

In this determination, 1 g of dried sample is added to 20 ml of 0.05 M H$_2$SO$_4$ and shaken 15 minutes. Twenty ml of 0.01 M NaOH is then added, followed by another 15 minute shaking period. Finally, the solution is buffered by 5 ml of 3 M NaOAc and 10 ml of 0.5 M sodium citrate to reduce interference from Al, Si, and Fe. Fluoride is measured with a fluoride ion selective electrode under constant stirring and temperature (Jacobson and Heller 1972).

2.37 Mercury

Mercury is analyzed by digesting a 0.50 g sample with 2 ml H$_2$O$_2$ and 0.5 ml HNO$_3$ in a microwave digestion vessel for four minutes at 296 watts and 8 minutes at 565 watts, followed by a 2 hour digestion in a 95°C hot water bath with 0.25 M sulfuric acid, 5% potassium permanganate and 5% potassium persulfate. After reduction with stannous chloride, total mercury is quantified by the cold vapor technique using atomic absorption spectrophotometry on a Monitor Elemental Mercury Detector.

2.38 Reference Materials and Blanks

NIST SRMs

At least 1-2 NIST standard reference materials (Pine Needles, Orchard and/or Peach Leaves) are analyzed with each run. (NIST standards have concentration ranges established by the National Institute of Standards and Technology. They are purchased directly from NIST and submitted as blind samples to the testing laboratory. Analysis results are used to assess laboratory accuracy.)

Lichen SRMs

The use of a standardized lichen material in addition to or in lieu of a NIST SRM is highly desirable because concentrations of some elements are lower in lichens than in plant materials and it is easier for the laboratory to achieve precise, accurate results for the NIST SRM than for the lichen samples. The lichen, *Pseudevernia furfuracea* is available as CRM 482 from IRMM (Institute for Reference Materials and Measurements) Reference Materials Unit: Technical and Sales Information, Retieseweg, 2440 Geel, Belgium, http://www.irmmm.jrc.be. This material is certified for Al, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn (Quevauviller et al. 1996). A second reference material, IAEA-336, *Evernia prunastri* (Heller-Zeisler et al 1999, Stone et al. 1995) is certified for more elements Al, As, Ba, Br, Cd, Ce, Cl, Co, Cr, Cs, Cu, Eu, Fe, Hg, K, La Lu, Mn, Na, Nd, P, Pb, Rb, Sb Sc, Se Sm, Sr, Tb, Th, V, Yb, Zn. It can be ordered from Analytical Quality Control Services Agency’s Laboratories, Seibersdorf A-2444 Seibersdorf, Austria or from the IAEA website at http://www.iaea.org/programmes/aqcs/main_database.htm.

ALSAMH standard

A sample of the lichen check, *Alectoria sarmentosa* from the Mt. Hood National Forest (submitted in large volume to the lab in 1993) is analyzed every 20-30 samples (1-2 for each batch of sulfur, 2 for each batch nitrogen, 4 for each batch of 100 ICP samples). This check is used to compare laboratory precision between batches and years. A new *Alectoria sarmentosa* check was collected in Winter 2002 from Willamette Pass, Willamette National Forest, in the central Oregon Cascades, and submitted to the UMN Research Analytical Laboratory in Spring 2003.
Duplicates
Duplicate analyses of the digests are run every 10 samples for all elements. This determines the laboratory precision within a batch.

Acid blanks
One or two acid blanks are analyzed with each analytical batch (batch size varies between ICP, N and S analyses). These blanks pass through all digestion/analytical procedures for the N, S and ICP analyses and are identical, as far as possible, to the samples. They are used to detect and quantify contamination of the samples from the analytical reagents.

2.39 Receipt and Storage of Analytical Results
Results are received from the laboratory in electronic spreadsheet form and hard copy as %S, %N and ppm (dry weight) of the remaining elements. Individual sample dilution-factors and direct instrument readings for ICP results are also provided. Upon receipt the data is checked for inconsistencies and, if necessary, arrangements are made to rerun samples with anomalous values. Currently the lichens and air quality databases are stored at the Siuslaw National Forest Corvallis Supervisor's Office and at NACSE (Northwest Alliance for Computational Science and Engineering) in the Computer Science department of Oregon State University. Eventually, a copy of the database will be archived by the USFS Natural Resources Information System, updated annually. The lichen module is scheduled for development in 2003-2004. Currently the database can be queried from http://www.fs.fed.us/r6/ag/lichen.

2.4 LICHEN COMMUNITY METHODS
The purpose of the lichen community indicator is to use lichen species and communities as biomonitors of change in air quality, climate change, and/or change in the structure of the forest community. Lichen communities are good indicators of air quality, particularly long-term averages of sulfur dioxide concentrations. Other pollutants that alter natural lichen communities include sulfur and nitrogen-based acid deposition, nitrogen fertilizers, fluorine and, possibly, ozone and other oxidants (see Section 1.32).

The lichen community survey methods employed by our program were developed under the auspices of the USDA-Forest Service Forest Health Monitoring Program and are described in the FIA Field Methods Guide (http://fia.fs.fed.us/library.htm#Manuals). A few differences exist: our abundance rating has more categories than FHM, but can be collapsed to the same rating; substrates are recorded; and it is permissible to collect lichens below 0.5 meter on trunks as long as they are tree and shrub-dwelling epiphytes and not terricolous or rotting wood species.

The objectives of this task are to determine the presence and abundance of macrolichen species on woody plants (using a 34.7 m [114 ft] radius plot) and to collect samples to be mailed to the lichen specialist(s). The method has three parts, performed at the same time:

1. Make a collection of voucher specimens for ID by a specialist, the collection representing the species diversity of macrolichens on the plot as fully as possible. The population to be sampled consists of all macrolichens occurring on woody plants, excluding the 0.5 m basal portions of trees and shrubs (west side of the Cascade crest only). Fallen branches are included in the sampling.
2. Estimate the abundance of each species. Note that the crew member responsible for this task need not be able to accurately assign species names to the lichens (that is done later by a specialist), but must be able to make distinctions among species.
3. Record the substrate from which the lichen was collected. For woody substrates, record the species and location (i.e., branches, bole, limbs, etc.).
2.41 Procedure

1. The area to be sampled (henceforth the "lichen plot") is a circular area with 34.7 m (114 ft) radius. The area of the lichen plot is 3782 m² = 0.378 ha = 0.935 acres.

2. Sampling continues for a maximum of two hours or until 10 minutes elapse with no additional species recorded. At least 30 minutes must be spent searching the plot, even if very few lichens are present.

3. A reconnaissance walk through the lichen plot should be taken to locate lichen epiphytes on woody plants, collect voucher samples and assign abundances. The following method is suggested: Begin at approximately 30 m (100 ft) due north from plot center, measuring with the eye to the limiting boundary of 114 ft. and continue to the right in a sinuous manner 90°. (The plot should be flagged every 90° along the perimeter). The same procedure is followed around the rest of the plot. If time allows, a second circuit of the plot can be made, searching for spots which were not visited in the first pass.

4. Lichen species with the following growth forms will be collected: fruticose and foliose (i.e. macrolichens).

5. Woody plants (all trees and shrubs 0.5 m tall) within the lichen plot will be inspected for lichen species. Fallen and reachable branches will also be inspected.

6. Care should be taken to inspect the full range of substrates and microhabitats available: shaded and exposed, conifers and hardwoods, fallen upper branches and lower branches, large shrubs and trees in particular topographic positions (for example, checking in draws or ravines of an otherwise uniform slope, so long as it occurs within the lichen plot). Rotten logs, stumps, or other semi-permanent features of the forest floor should NOT be sampled.

7. Abundance ratings. Relative abundance within the lichen plot will be recorded. Relative abundance for each species is estimated as follows. Choose the highest rating that is true.

<table>
<thead>
<tr>
<th>Code</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rare (&lt; 3 individuals/colonies in area)</td>
</tr>
<tr>
<td>2</td>
<td>Uncommon (4-10 individuals or colonies in area)</td>
</tr>
<tr>
<td>3</td>
<td>Common (10-40 individuals or colonies in area)</td>
</tr>
<tr>
<td>4</td>
<td>Very Common (&gt;40 individuals or colonies in area but less than half of the boles and branches are covered by the species. Choose one: 4-1 individuals/colonies are few (between 40-80) and widely scattered around the area 4-2 the lichen is restricted to one or two small areas in the area, usually on just a handful of trees or shrubs. The total number of individuals or colonies is ≥40. 4-3 many trees or shrubs have up to 20 individuals or colonies. 4-4 many trees or shrubs have more than 20 individuals or colonies 4-5 more than half the trees or shrubs have up to 20 individuals or colonies. 4-6 more than half the trees or shrubs have more than 20 individuals or colonies.</td>
</tr>
<tr>
<td>5</td>
<td>Abundant (more than half of boles and branches are covered by the subject species).</td>
</tr>
</tbody>
</table>

These codes correspond to the FHM lichen indicator codes as follows: 1 = FHM 1; 2 and 3 = FHM 2; 3 to 4-4 = FHM 3; 4-5 to 5 = FHM 4.

8. A sample of each putative species will be collected and placed in a paper packet labeled with plot number, collector's initials, forest acronym, substrate and relative abundance. The abundance rating can be revised as collection proceeds or given at the end of the collection period. Any relevant comments are recorded on the outside of the packet under "Remarks". For more details, see section 2.52 below. Before leaving the plot all packets should be checked to make sure that, as a minimum, plot number, abundance and substrate has been recorded on
every packet. They should then be alphabetized by genus and species and sequential packet numbers assigned, beginning with “1”.

9. How to handle uncertainties: The field crew will frequently have uncertainties about the classification of an organism. The following rules for the field crew are designed to put the onus of the responsibility for classification on the specialist, not the field crew.

a. When in doubt, assume it is a lichen.
b. When the growth form is in doubt, assume it is a macrolichen.
c. When species distinction are in doubt, assume that two different forms are different species.

The purpose of these rules is to encourage the field crew to make as many distinctions in the field as possible. The specialist can later adjust the data by excluding specimens that are not macrolichens and by combining forms that were considered separately by the field crew but are actually the same species.

2.42 Sample collection, Preservation, and Storage

Optimally, a palm-size (about 5 cm. in diameter) sample of fruticose and foliose growth forms is collected. This includes all species that are three-dimensional or flat and lobed. Even minute fruticose and lobate forms should be included. Squamulose species and *Cladonia* squamules lacking upright stalks should not be included.

In some cases, a small sample should be obtained because of the scarcity of the species. However, if the abundance rating is > 3, the sample should be generous. Large samples containing multiple individuals simplify the identification process and demonstrate that the collector was able to distinguish the species from look-alikes, improving confidence in the assigned abundance code. Collecting large samples also improves the likelihood of picking up inconspicuous species that may not be distinguishable in the field. These can be recorded by the lichen specialist in the office.

Species in the genera *Usnea* and *Bryoria* are most difficult to distinguish in the field and large samples nearly always contain a mix of species within a packet. If species are present in equal amounts, the abundance code may unusable. For these genera, the best strategy is to carefully learn characters that differentiate species and collect smaller samples in multiple packets.

Each specimen will be placed in a separate folded paper packet and labeled with appropriate codes:

- Ocular abundance (can be revised as collection proceeds and the observer becomes more familiar with the plot).
- Occasionally there will be more than one species on a given bark sample. If there is any chance of ambiguity about which species in the packet corresponds with the abundance rating, a descriptive clarifying phrase, such as “the white one” or “the sorediate one”, will be written on the packet.
- Packets will be labeled with an indelible marker. If the packets are damp, a soft pencil (No. 2 or softer) can be used.
- All packets should be labeled with the plot ID code before leaving the plot, or earlier as time allows.
- The lichen worksheet and all of the specimen packets from a given plot will be placed into a paper or Ziploc bag with the plot ID code, collectors initials, and date recorded on the outside of the bag and the top folded closed.
- The bags will be stored in a dry place until delivery to the specialist. Specimens must be thoroughly air dried to avoid fungal decay. If specimens were wet when collected, the individual packets should be spread out and dried inside or in the sun as soon as possible. If temperatures are above room temperature, wet lichens are likely to mold within 2-3 days.
2.43 Sample Delivery
After the first two plots are completed, mail or bring the specimens to the program coordinator. This allows the coordinator to provide immediate feedback to the field crews concerning specimen quality and quantity. Thereafter, deliver the packets biweekly or monthly. Packets should be packed closely, but without excessive crushing, in sturdy cardboard boxes. Packets from several plots can be mailed in the same box. The field crew should save a running packing list (see “Forms” section) specifying the CVS plot numbers, Forest, and date mailed. Send any notes of possible use to the lichen specialist with the packets.

2.44 Equipment and Supplies
Consumable
- Folded labeled paper packets (can be made by recycling one-sided office paper). Carry 40 packets per plot west of the cascade crest, and 25-30 packets for plots east of the Cascades.
- Black waterproof markers for writing plot ID’s and abundance data on paper or plastic bags.
- Larger brown paper bags (16.5 x 9.5 " or similar size), or gallon-sized re-sealable clear plastic bags, one per plot.
- Soft pencils (No. 2 or softer) and indelible pens.
- 6 mailing forms
- 60 field data cards per Forest.

Non-Consumable
- Locking-blade or fixed-blade knife (ca. 4" blade) with belt sheath.
- 14-20x hand lens (Bausch and Lomb Hastings Triplet).
- Guides for lichen identification:
- Hand pruners (useful for collecting small branch segments).
- 1" wide chisel (useful for collecting samples from tough-barked hardwoods, a sheath can be made from a piece of cardboard and strapping tape).
- Clipboard (for field data forms).

2.45 Interferences and Safety
This method may be used in any season or weather condition. Because careful discrimination among species in the field is required the method should not be performed within an hour of sunset or sunrise, or during dark, rainy conditions.

Only minor hazards are associated with the method. Care should be used when removing lichen specimens with a knife or chisel. A locking-blade or fixed-blade knife is best. Trees should not be climbed to procure specimens.

2.46 Quality Control and Performance Standards
Only people who have successfully completed lichen training should collect the lichen community data. Data quality will be measured by (1) a post-training audit, (2), mid-season field audit, and (3) re-sampling at least one plot per field season. A field audit will consist of comparing the results of a lichenologist with the field-crew member. One or more plots will be examined per audit.
The field crew will be audited within 2-4 weeks after the conclusion of training by the lichen specialist. Results of the audit will be included in a summary QA report prepared at the end of the field season. Corrective action by the auditor will be (1) correction of any misunderstandings and (2) provision of additional on-the-spot training so that the crew can better complete its task. Corrective actions cannot include alterations in the basic method.

A second aspect of quality control is ensuring that the voucher specimens are adequate, not decomposed, and being received by the lichen specialist. The field crew should ensure specimen quality by periodically calling the lichen specialist to verify shipments and solicit comments and suggestions on the quality of the specimens.

The performance of the method is assessed by evaluating data quality objectives (DQOs) for detectability, precision, accuracy, and completeness. Each of these is evaluated below, based on the FHM program experience with this method in 1992.

**Detectability:** Detection capabilities of field crews is determined by % of the specialist’s species that was represented in the field crew's data. The DQO for this statistic is 80%.

**Precision:** Precision, or repeat measurement error, is determined by the field crews revisiting and re-sampling one plot sometime during the same field season. The DQO for precision is 85%. Scores are compared as described in the accuracy section, next.

**Accuracy:** Accuracy can be expressed in terms of the percent agreement between species composition of two independent samples of the same lichen plot, one of which is collected by a lichen specialist and is considered the “true” species composition. This agreement is calculated as the concordance in abundance scores. The abundance scores have six possible levels (0,1,2,3,4 and 5). Concordance between two investigators for a single plot will be calculated as the % similarity between the scores for the two investigators, calculated using the sum of the shared species abundance scores divided by the total of all scores from both investigators). The DQO is 80%.

**Completeness:** The DQO of completeness is 90%.

### 2.47 Lichen specialist procedures

**Purpose:** The program coordinator has five roles:
1. Conduct or assist with training of field crews.
2. Verify or supervise verification of lichens collected and identified by the field crew.
3. Conduct or assist with field audits.
4. Supervise database entry.
5. Write or assist with data analysis and annual reports.

**Procedure for processing specimens:**
1. Receive boxes of specimens in the mail or directly from the field crew.
2. Open the boxes immediately and check for damp lichens. If some are damp, thoroughly air-dry them.
3. Identify the contents of each bag by species. In the case of mixed collections or multiple collections of the same species, see the special instructions below.
4. Enter the list of species identifications, along with plot and species numbers, substrates and abundances for each identification in a computerized spreadsheet.
5. Prepare voucher specimens. Select individuals for herbarium specimens such that, ideally, each species is represented by about 3 specimens, for every Forest. These specimens should be stored in standard lichen packets and herbarium labels with as complete label data as possible from the information provided. In all cases, the label data should include the plot ID number. Vouchers are currently stored in the Siuslaw National Forest herbarium.
6. Store packets (with lichens!) for future reference. Packets are currently stored in the Siuslaw National Forest Supervisor’s Office storeroom.
7. Keep a list of comments/suggestions for the field crew. They will call you periodically for feedback.

**Handling multiple collections of the same species:** Because the field crew is instructed to err on the side of making a species distinction when they are unsure whether two organisms belong to the same species, it is expected that in many cases, two or more collections from a given plot will be of the same species. Each collection will be entered separately in the database.

For data analysis purposes, a combined abundance value can be calculated for those species collected more than once on a plot using the following rules for combining abundance values:

<table>
<thead>
<tr>
<th>Recorded Values</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 + 1</td>
<td>2</td>
</tr>
<tr>
<td>1 + 2</td>
<td>2</td>
</tr>
<tr>
<td>1 + 3</td>
<td>3</td>
</tr>
<tr>
<td>2 + 2</td>
<td>2</td>
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<tr>
<td>2 + 3</td>
<td>3</td>
</tr>
<tr>
<td>3 + 3</td>
<td>3</td>
</tr>
<tr>
<td>4 + any others</td>
<td>4</td>
</tr>
<tr>
<td>(Use highest rating recorded)</td>
<td></td>
</tr>
<tr>
<td>5 + any others</td>
<td>5</td>
</tr>
<tr>
<td>1 + 1 + 1</td>
<td>2</td>
</tr>
<tr>
<td>1 + 1 + 2</td>
<td>2</td>
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<tr>
<td>1 + 1 + 3</td>
<td>3</td>
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<tr>
<td>1 + 2 + 3</td>
<td>3</td>
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<td>3</td>
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<td>3</td>
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<tr>
<td>2 + 3 + 3</td>
<td>3</td>
</tr>
<tr>
<td>3 + 3 + 3</td>
<td>4</td>
</tr>
</tbody>
</table>

**Recording species mixed with vouchers but not recognized by the field crew:**
Despite the best efforts of the field crew, the lichen specialist will occasionally encounter species that were not recognized in the field. Although these lichens can easily be recorded by the specialist, they will not have abundance values assigned by the field crew. In these cases, the species should be recorded on the data sheet, and a missing value indicator (0) recorded for abundance and packet number. For data analysis purposes, zeroes are usually converted to the most common abundance rating, 3.

**Quality Assurance Reports to management:** A QA report form will be used during training and audit procedures, and can be made available to the air resource management staff officers and program managers. A summary of QA results from training, audits, re-measurements, and debriefings will be compiled by the program coordinator, along with description of any significant QA problems and recommended solutions.

### 2.48 Data entry and analysis
Field data are recorded on the packets used for collecting specimens. These data are later entered in the office, following determinations of species. Data will be entered onto a computer spreadsheet and backed up on a Forest Service network drive. Currently the lichens and air quality databases are stored at the Siuslaw National Forest Corvallis Supervisor's Office and at NACSE (Northwest Alliance for Computational Science and Engineering) in the Computer Science department of Oregon State University. Data values will be screened against acceptable ranges.
Various community parameters at the plot level can be calculated from lichen species abundance data (also collected at the plot level, but the data are aggregated from individual species to the community). The most commonly used are:

1. Species richness -- the total number of species recorded in the sampling unit (plot).
2. Total abundance-- the sum of the abundance classes across species.
3. Score on compositional gradient-- the score is calculated as a weighted average across species for a given sample unit, the species weights being derived from a measure of sensitivity to air pollution or any other gradient or (ii) scores derived from equations based on ordination of samples varying in the quantity to be indicated (i.e. an "assessment endpoint", e.g. air pollution).

Data analysis will consist of:

1. Analysis of data quality by using data from audited plots. Data will be compared between the field personnel and the auditors (lichen specialists).
2. Derivation of synthetic composite variables representing the major components of variation in lichen communities. This multivariate analysis will be done within bioclimatic regions.
3. Description of regional patterns of lichen community parameters.
4. Establishment of nominal/subnominal boundaries for indications of air quality by comparison of known polluted areas with otherwise similar, but remote areas. Because locally polluted areas occur in essentially all forested ecosystems of North America, the nominal/subnominal boundary can be varied and calibrated throughout the continent.
5. Analysis of the relationship between lichen community parameters and various off-from spatial data (e.g. pollutant emission data), to the extent possible by data availability and funds.

### 3. STRATEGY

#### 3.1 GRID SYSTEM AND SELECTION OF PLOTS

Plot locations were selected from the USFS Pacific Northwest Region Current Vegetation Survey (http://www.fs.fed.us/r6/survey/) 5.4 km grid, now the Phase 2 (P2) grid of the Forest Inventory and Analysis program (http://fia.fs.fed.us/). One quarter of monitored plots are measured each year over a four-year period to complete one round of sampling. The Current Vegetation Survey plot locations are field-marked and entered in the USFS regional geographic information system in UTM. There are three grid intensities between points: 5.4 km (3.4 miles), 2.7 km (1.7 miles), and 1.3 km (0.8 miles). The grid is laid over the Forests without regard to where the plots fall; some plot centers fall in streams, lakes or alpine areas and were not installed.

The EMAP-FHM program was initiated on a separate hexagonal grid. Recently FIA has taken over administration of both programs and the CVS and FHM plots are now integrated with the FIA grid into a single plot network. FHM lichen indicator plots are approximately 28 km apart.

The default monitoring plan was to monitor all the CVS 5.44 km grid plots at the Forest level. Forest-wide monitoring was preferred to place Class I areas in a regional context. Also, because good air quality is a prerequisite to ecosystem health, managers need to understand the status and trends in air quality on all Forest lands. However, the choice and number of plots for each Forest depended on local pollution sources, Forest priorities, and availability of funding.

### 4. NATIONAL FORESTS

#### 4.1 COLUMBIA RIVER GORGE NATIONAL SCENIC AREA

4.11 Local Sources of Pollution

The Columbia River/Portland area is the site of several major point sources (Reynolds Aluminum, Boise Cascade Papers, Longview Fiber as well as Weyerhaeuser, Vanalco, James River II, Simpson Timber Chemical Division and the Portland International Airport). There are many
additional smaller industrial pollution sources and multiple smaller home heating wood stove and incineration sources. The Scenic Area is a major transportation corridor; high traffic density from the Portland area Interstate Highway 84 follows the Oregon side of the river and State Highway 14 follows the Washington side. The Columbia Gorge is a major rail conduit, with frequent rail traffic on both sides of the river and active barge and recreational boating activity in the river itself. Diurnal winds carry pollutants up and down the river valley from Portland, neighboring industries and agriculture.

4.12 Monitoring Priorities
Monitoring priorities include sites with high visitor use and federally owned lands within the Scenic Area. There are no Class I areas in the Columbia River Gorge National Scenic Area (CRGNSA).

4.13 Sampling Strategy
There are FIA/FHM but no CVS plots in the CRGNSA. Lichen surveys have been made in north-south river valleys and along an east-west transect on the valley floor (See Maps A and B). Lichens were sampled for tissue analysis approximately every 4.8 km (3 miles) on alternating sides of the Columbia River. In treeless areas, the epiphytic lichen, *Xanthoparmelia cumberlandia*, was collected for tissue analysis. No lichen community surveys were made in treeless parts of the scenic area. Coordinates and relocation directions have been recorded for the initial monitoring round, but no permanent markers were installed. Approximately 10 FIA plots were surveyed for lichens.

4.14 Potential Interfaces
There are two IMPROVE sites in the CRGNSA, one in the west at Mt. Zion and the other in the east, near Wishram. Visibility and chemistry of fine particulates are monitored on a weekly basis. A passive monitoring station at Horsethief State Park was used to estimate ambient levels of SO2, H2S, NH3, NO, NO2, and NOx (biweekly means in summer, monthly means in winter) from July 2002-June 2003. IMPROVE and passive monitoring data is available from the USFS PNW Regional Air Resource Management Program website at http://www.fs.fed.us/r6/aq/. The nearest National Acid Deposition Program (NADP) monitor is in the Bull Run Watershed of Mt. Hood National Forest. NADP monitors wet deposition chemistry, including concentrations of hydrogen, sulfate, nitrate, ammonium, calcium, magnesium, and sodium ions in precipitation, rain volume and total seasonal and annual deposition. Data from this program is accessible via the NADP website at http://nadp.sws.utuc.edu/.

4.2 DESCHUTES NATIONAL FOREST

4.21 Local Sources of Pollution
Other than forest fire, the Deschutes National Forest has been remote from large emission sources. However, human population in Bend and Deschutes counties is growing rapidly. From 1960 to 2000, Deschutes County has grown from 20,000 to nearly 120,000 people, and nearly half that growth has occurred within the last 10 years. Baseline monitoring in the 1990s should provide a good benchmark against which future measurements of air quality can be compared.

4.22 Monitoring Priorities
There are two Class I areas on the Deschutes National Forest: Three Sisters Wilderness and Mt. Washington Wilderness. The Newberry National Volcanic Monument is a high priority area because of the potential for geothermal development. Other areas of special concern are the Mt. Jefferson Wilderness and the Research Natural Areas.
4.23 Sampling Strategy
Baseline monitoring has been completed for the entire Forest. At the 5.44 km (3.4 mile) grid level, there are 211 plots (See Map C). A special baseline study for the Newberry National Volcanic Monument included an additional 12 plots.

4.24 Potential Interfaces
There is an IMPROVE site for the Three Sister’s Wilderness located on the west side of the Cascades near the crest at Carmen. IMPROVE monitors visibility and chemistry of fine particulates on a weekly basis. The nearest NADP monitor is behind the Silver Lake Ranger District office of the Fremont National Forest in Silver Lake. Data from these sites can be accessed via the USFS PNW air resource management website: http://www.fs.fed.us/r6/aq.

4.3 GIFFORD PINCHOT NATIONAL FOREST

4.31 Local Sources of Pollution (excerpted from Horner and Peterson, 1993)
Pollution sources that have the potential to impact the Gifford Pinchot National Forest lie primarily in western Washington and Oregon between Anacortes and Portland. In 1991 the counties which border the Puget Sound, including the cities of Seattle and Tacoma, reported the following point source emissions (in tons/yr.): volatile organic compounds (39300), nitrogen oxides (10000), carbon monoxide (6500), air toxics (6500), sulfur oxides (800), PM$_{10}$ (800), suspended particulate matter (1300). The largest point source of criteria pollutants in western Washington is the Centralia coal-fired power plant in Lewis county. Its emissions include 53, 030 metric tons of sulfur oxides and 21, 650 metric tons of nitrogen oxides per year. It is the single largest source of sulfur oxides in the Pacific Northwest and lies west of the Gifford Pinchot National Forest.

4.32 Monitoring Priorities
The two Class I areas on the Forest are Goat Rocks Wilderness and Mt. Adams Wilderness. Other priority areas include Indian Heaven, Trapper Creek, William O Douglas, and Tatoosh Wildernesses and the Mt. St. Helens National Volcanic Monument. An air quality monitoring plan for the Goat Rocks Wilderness was prepared in 1993 (Horner and Peterson).

4.33 Sampling Strategy
Baseline monitoring has been completed. At the 5.44 km (3.4 mile) grid level, there are 183 plots Forest-wide (See Maps D and E). The Northern Skill Area of the Forest (formerly Packwood and Randle Ranger Districts) was first surveyed from 1995-1996, and the Southern Skill Area (formerly Mt. St. Helens and Mt Adams Ranger Districts) was surveyed in 1997-1998, completing the first round of monitoring.

4.34 Potential Interfaces
Two NADP monitors are located near the Gifford Pinchot National Forest, one in University of Washington’s Pack Experimental Forest near Eatonville, and another at the Marblemount Ranger Station of Cascades National Park, in Marblemount, WA. These monitors are used to measure precipitation and wet deposition of sulfates, nitrates, ammonia and other ionic components of acidic precipitation. Six lichen plots were installed within 4 km (2.5 miles) of each of these monitors in 1998, and an additional 36 plots were installed within 7.2 km (4.5 miles) of each monitor between 1999 and 2000. Lichens were collected for chemical analysis at all 42 plots, lichen communities were surveyed in 1998 only.

25
4.4 MT. HOOD NATIONAL FOREST

4.41 Local Sources of Pollution
The Mt. Hood National Forest has the highest visitor use and is the closest to high-density centers of industry and population of the national forests in this program. The Columbia River/Portland area is the site of several major point sources (Reynolds Aluminum, Boise Cascade Papers, Longview Fibre as well as Weyerhaeuser, Vanalco, James River II, Simpson Timber Chemical Division and the Portland International Airport). There are many additional smaller industrial pollution sources, high traffic density from the Portland area and also within the Forest, and multiple smaller home heating wood stove and incineration sources.

4.42 Monitoring Priorities
The Mt. Hood Wilderness is the only Class I area on the Mt. Hood National Forest and has the highest priority for monitoring. Other areas of special concern are the Columbia Wilderness, and the Bull Run watershed (source of drinking water for the Portland area); both located in the Columbia River Gorge, the Salmon-Huckleberry, Badger Creek and Bull of the Woods Wildernesses, the Resource Natural Areas, and the northern Mt. Hood National Forest in the Hood River, Barlow and Estacada Ranger Districts closest to regional pollution sources.

4.43 Sampling Strategy
Baseline monitoring was completed from 1994-1997. At the 5.4 km grid level, there are 152 plots (See Maps F and G).

4.44 Potential Interfaces
a. Air quality monitors. There is an IMPROVE site at Mt. Zion in the Columbia River Gorge NSA and an NADP site near the west end of Bull Run Reservoir #2. These sites offer opportunities for calibrating instrument and biological data using transplants or by setting up lichen monitoring plots near these monitors. In 1993, one monitoring plot was established near the IMPROVE station. Six lichen plots were installed within 4 km (2.5 miles) of the NADP monitor in 1998, and an additional 36 plots were installed within 7.2 km (4.5 miles) of the NADP monitor between 1999 and 2000. Lichen tissue data were collected at all plots, lichen communities were surveyed in 1998 and 1993.

b. Water quality monitoring. There is a water-quality monitoring program for Bull Run Watershed.

4.5 SIUSLAW NATIONAL FOREST

4.51 Local Sources of Pollution
The largest permitted stationary point sources in the vicinity of the Siuslaw National Forest are in Toledo (Georgia Pacific), Gardiner (International Paper) and North Bend/Coos Bay (Weyerheuser and Sun Plywood). Other important emission sources are smaller industries, motorized vehicles, agriculture, and forestry activities. The Tillamook vicinity is an important local source of ammonia emissions from dairy farming.

4.52 Monitoring Priorities
There are no Class I areas on the Siuslaw National Forest. Areas of special concern are the Oregon Dunes National Recreation Area, Sutton Creek Recreation Area, the Research Natural Areas and the Drift Creek, Cummins Creek and Rock Creek wildernesses. The immediate coastline is habitat for a rich and unique lichen flora, not found elsewhere in the region.
4.53 Sampling Strategy
The Siuslaw has no Class I areas and therefore the default plan, a Forest wide monitoring strategy using the 3.4 mile grid, has been implemented. There are 78 plots on this grid (See Map H). 10 additional off-frame plots were installed in the Oregon Dunes National Recreation Area. Baseline monitoring occurred from 1994 through 1997.

4.54 Potential Interfaces
An NADP monitor is located on the Siuslaw National Forest, near the town of Alsea. The monitor is used to measure concentrations and deposition of sulfates, nitrates, ammonia and other ionic components of acidic precipitation. Six lichen plots were installed within 4 km (2.5 miles) of this monitor in 1998, and an additional 36 plots were installed within 7.2 km (4.5 miles) of the monitor between 1999 and 2000. Lichen tissue data were collected at all plots, lichen communities were surveyed in 1998 only.

4.6 UMPQUA NATIONAL FOREST

4.61 Local Sources of Pollution
Emission sources are primarily located to the west of the Umpqua National Forest. They include stationary and mobile sources in the Eugene/Springfield metropolitan areas, the southern end of the Willamette valley and the I-5 corridor including Roseburg, Grants Pass, and Medford. Total SO$_2$ and NO$_x$ emissions from counties bordering the Umpqua National Forest (Lane, Douglas, Jackson and Josephine) were: 14,150 and 56,725 tons/year, respectively, during the mid-1990s (OR DEQ). State Route 138 is the main traffic route through the Forest. It leads to the northern entrance of Crater Lake National Park.

4.62 Monitoring Priorities
Three Wildernesses: Mt. Thielsen, Rogue-Umpqua Divide and Boulder Creek Wildernesses have boundaries within the Umpqua national forest. None are Class I areas. Wildernesses and Botanical Special Interest Areas are the highest priorities for air quality monitoring.

4.63 Sampling Strategy
Initial Forest-wide monitoring was completed from 1997 to 2000. There are 135 plots on the 5.44 km (3.4 mile) grid (See Maps I and J), about 34 per year on a four-year rotation.

4.7 WALLOWA-WHITMAN NATIONAL FOREST

4.71 Local Sources of Pollution
Local sources of air pollution are primarily related to agriculture. The nearest notable stationary source of sulfur dioxide is a power plant approximately 144 km (90 miles) northwest of Eagle Cap Wilderness at Boardman in Morrow County. About 23,000 tons of sulfur dioxide are released from this source annually. Total SO$_2$ and NO$_x$ emissions from counties bordering the Wallowa-Whitman National Forest (Wallowa, Union, and Baker) were 1,320 and 9,119 tons/year, respectively, during the mid-1990s (OR DEQ). Additional emissions from bordering Idaho counties could affect the Forest. No major highways traverse the Wallowa-Whitman National Forest.

4.72 Monitoring Priorities
There are two Class I areas on the Wallowa-Whitman: Eagle Cap Wilderness and Hells Canyon Wilderness. Part of the North Fork John Day and Monument Rock Wildernesses are also in the Wallowa-Whitman N.F. The Class I Wildernesses have the highest priority for air quality monitoring.
4.73 Sampling Strategy
Monitoring to date has been limited to the Eagle Cap Wilderness, Hell's Canyon National Recreation Area and Hells Canyon Wilderness. There are 43 plots in the 5.44 km (3.4 mile) CVS grid within the boundaries of Eagle Cap Wilderness (See Map K), about 11 plots per year in a four-year rotation. The initial round of monitoring was completed from 1998 through 2001. In 1999, lichen monitoring sites were established along the Snake and Imnaha Rivers and five tributaries of these rivers all inside Hell's Canyon National Recreation Area and Wilderness. At the HCNRA plots, *Xanthoparmelia cumberlandia* was collected for tissue analysis and lichen communities were surveyed in *Celtis occidentalis* plant communities only.

4.74 Potential Interfaces
The following data can be accessed from the USFS PNW Regional Air Resource Management website at [http://www.fs.fed.us/r6/aq](http://www.fs.fed.us/r6/aq).

An NADP monitor is located in Starkey Experimental Forest and Rangeland. The monitor is used to measure precipitation and wet deposition of sulfates, nitrates, ammonia and other ionic components of precipitation. Six lichen plots were installed within 4 km (2.5 miles) of this monitor in 1998 and an additional 36 plots were installed within 7.2 km (4.5 miles) of the monitor between 1999 and 2000. Tissue data were collected at all sites, lichen communities were surveyed in 1998 only.

There are two IMPROVE sites, one near the south end of Hell's Canyon NRA in Oxbow, OR and the other at Wallowa Lake, near the NE boundary of Eagle Cap Wilderness. Hourly ozone means were recorded using a 2Btechnologies field monitor from July to October 2002 at the Oxbow site.

At Hell's Canyon NRA, passive sampling stations were maintained for one year at Cache Creek ranch, Pittsburg Ck Ranch, Dug Bar, Kirkwood Cr ranch, and Hell's Canyon Dam Visitor's Center. Mean ambient concentrations of NO\textsubscript{x}, NO\textsubscript{2}, NO, NH\textsubscript{3}, H\textsubscript{2}S and SO\textsubscript{2} were collected on a biweekly (summer) and monthly (winter) basis from July 2002 through June 2003.

4.8 WILLAMETTE NATIONAL FOREST

4.81 Local Sources of Pollution
Emissions with potential to affect the Willamette National Forest originate primarily in the Willamette Valley. The largest industrial point sources are Weyerhaeuser in Springfield and Cottage Grove, Georgia Pacific Chemical Division and Western Kraft in Albany. In addition there are multiple smaller stationary sources concentrated along the I-5 corridor between Albany and Cottage Grove. The Willamette NF is adjacent to prime agricultural areas of the Willamette Valley and could be affected by volatilized fertilizers, pesticides and manures. Other important emissions are from mobile sources, forest fires and field burning.

4.82 Monitoring Priorities
The Willamette has two Class I areas, the Three Sisters Wilderness and the Mt. Washington Wilderness. Under federal law, these areas have more stringent protection requirements and information needs are therefore greatest. There are five Class II Wilderness: the Mt. Jefferson, Middle Santiam, Menagerie, Waldo Lake, and Diamond Peak Wildernesses. Other special interest areas are the HJ Andrews Experimental Forest, and the Research Natural Areas.

4.83 Sampling Strategy
Baseline monitoring was completed for the entire Forest from 1995 through 1997. There are 237 5.44 km (3.4 mile) grid plots on the Willamette National Forest (See Maps L and M).

4.84 Potential Interfaces
a. **Instrumental monitors.** There is an IMPROVE site (monitors visibility and deposition chemistry) close to the Three Sisters Wilderness and a National Acid Deposition Program monitor at the HJ Andrews Experimental Forest headquarters (deposition chemistry). These sites offer opportunities for calibrating instrument and biological data using transplants or by setting up lichen monitoring plots near these monitors. In 1993, one monitoring plot was established near the IMPROVE station. Six lichen plots were installed within 4 km (2.5 miles) of the NADP monitor in 1998, and an additional 36 plots were installed within 4.8 km (3 miles) of the NADP monitor between 1999 and 2000. Lichen tissue data were collected at all sites; lichen communities were surveyed at the 1993 and 1998 plots only.

b. **Air Toxics Study.** Samples of lichens and mosses were collected from the Three Sisters and Mt. Washington Wildernesses as part of an international air toxics study in 1993. These samples were analyzed for persistent organochlorines and metals. An international standard was included with the metals analysis. Metal and organochlorines concentrations in lichens were compared to sites in southeast and south central Alaska, the Russian Far East south to the Primorsky Region, arctic Alaska, Siberia (Taimyr Peninsula) and Scandinavia/Northern Europe.

c. **Water quality monitoring.** The Blue River Ranger District has begun a water quality monitoring program.

d. **USFS Forest Sciences Laboratory, Corvallis, Research.** Potential exists for collaborating with research scientists at HJ Andrews Experimental Forest, a LTER (Long Term Ecological Research) site.

### 4.9 WINEMA NATIONAL FOREST

4.91 **Local sources of pollution**
Local emissions are primarily from stationary point sources in Klamath Falls and Medford, mobile sources and agriculture. Total SO\(_2\) and NO\(_x\) emissions from counties bordering the Winema N.F. (Klamath and Jackson) were 4,546 and 21,680 tons/year, respectively, in the mid-1990s (OR DEQ). State Route 97 passes through the center of the Winema NF. It is the main transportation artery along the east side of the Cascade Mountains between Bend and Klamath Falls, and connects to State Routes 138 and 62 to Crater Lake National Park.

4.92 **Monitoring priorities**
Mountain Lakes Wilderness is the only Class I area in the Winema National Forest. There are two other Wildernesses: Sky Lakes and Mt. Thielsen. Monitoring the Class I area and other Wildernesses is the highest priority for this national forest.

4.93 **Sampling strategy**
There are approximately 145 5.44 km (3.4 mile) CVS plots on the Winema National Forest (See Maps N and O). The first round of forest-wide monitoring occurred from 1997 through 2000.

4.94 **Potential Interfaces**
An NADP monitor is located on the adjacent forest to the east of the Winema, in the Fremont National Forest. The monitor is used to measure concentrations and wet deposition of sulfates, nitrates, ammonia, hydrogen ions and other ionic components of precipitation. Six lichen plots were installed within 4 km (2.5 miles) of this monitor in 1998. Lichen community and tissue data were collected at these sites.
5. REFERENCES CITED


6. FORMS

6.1 FIELD EQUIPMENT LIST

Community survey:
Collecting packets
Pens
Pencils
Knife
Hand lens
Clippers
Rubber bands
Zip-loc bags
Watch
Large paper or plastic bags

Tissue Analysis
Kapak bags
Tape
Sharpie marker
Gloves
Spring scale

Plot Documentation
Field data sheet
Clipboard with codes, acronyms
Plant indicator species guide
Plant association book
Off-frame plot card
Methods summaries

Finding and measuring plot
Aerial photos
Plot description (1st page of CVS data

Topographic map (with resource orthoquad overlay)
Compass
Clinometer
Altimeter
Calculator
Paper
Hip chain
District map
Field vest/backpack
dbh tape
120’ tape measure
flagging

Safety/Personal
First aid kit
Extra clothes
Water
Lunch
Rain gear
Radio (maintain twice daily communications)
Extra radio batteries
Matches in waterproof case
Whistle
Hard hat
Insect repellent
Sunscreen
Garbage sack (bright orange)
Leave notice where you went and when you expect to be back.
6.2 FIELD DATA SHEET FOR LICHEN MONITORING
USFS PACIFIC NORTHWEST REGION AIR RESOURCE MANAGEMENT

PLOT # ___________________________ Date
Lichen surveyor ______________________ Other observers:
Lichen Tissue collector

1. Plot type (circle one): Standard, Rep, Audit, Training, Off-Grid

2. Stand location
National Forest County
District State
Location in words

3. Stand Age (check one)
   _____ Shrub/forb (very early seral & pioneer vegetation, 1-30 yrs)
   _____ Seedling/sapling (early seral, 30-100 years)
   _____ Pole timber (mid-seral 100-200 years)
   _____ Mature (late-seral, 200 years until seral species are gone from the overstory)
   _____ Old-growth (climax vegetation, late seral species absent in the overstory)

4. Stand Structure (circle one)
   1. Open sampling-pole, < ll” dbh, open canopy
   2. Closed sapling-pole, < 11” dbh, closed canopy
   3. Small sawtimber (11-20.9” ave dbh)
   4. Large sawtimber (> 21” ave dbh)
   5. Old growth

5. Vegetation cover by species (write spp. acronyms in appropriate box)

<table>
<thead>
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<th>26-50%</th>
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<tr>
<td>forbs and grasses</td>
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<tr>
<td>mosses lichens</td>
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</table>
7. Lichen chemistry

<table>
<thead>
<tr>
<th>Lichen species collected</th>
<th>Substrate(s): Record plant acronym and location (e.g. bole, branch, litter, d/d)</th>
<th># Grams (- bag)</th>
<th>Moisture status (dry, damp, wet)</th>
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Describe spatial extent of sampling area for lichen chemistry

8. Notes. Briefly describe any deviations from standard operating procedures, describe the habitat (stand age and structure, surrounding landscape, plant community and ecozone, e.g. riparian area, beach) and outstanding features of the plot (clear-cut, two different stand ages, stream lake or road through plot, etc.) and the lichens (overall abundance, dominant and unusual species).

________________________________________________________________________
________________________________________________________________________
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________________________________________________________________________

9. Plot Audit (Complete before leaving the plot).

Was the lichen community surveyed?
   How much time was spent on the lichen community survey? __________
   Are community packets completely labeled? CHECK
   Are community packets alphabetized? __________

Were lichens collected for chemical analysis?
   Are the Kapak bags correctly labeled (plot #, target species, substrate acronyms and locations in order by sample volume, collectors initials, collection date, forest acronym, moisture status)? __________
   Do the field data card and Kapak bags have the same spp/substrates? __________

Is the field data card complete?
   __________

If off-grid, was the plot location marked on a map?
   __________

Signature of Auditor _________________________ Date __________
### 1. Location
- a. Latitude ___________________ N
- b. Longitude ___________________ W
- c. Township/Range/1/4 section ____________________________
- d. Map name and size _____________________________________
- e. Aerial photo number (optional) ___________________________

### 2. Physiography (use topographic map)
- a. Topographic position (Circle one):
  1. Flat or rounded ridgetop or peak > 120’ wide
  2. Narrow ridge top or peak (<120’ wide
  3. Sidehill, upper 1/3
  4. Sidehill, middle 1/3
  5. Sidehill, lower 1/3
  6. Canyon bottom <660’ wide
  7. Bench or terrace
  8. Broad flat 660’ or more wide
  9. Other, describe ____________________________

- b. Slope ___________ %
- c. Aspect _________ degrees
- d. Elevation _________ feet

### 3. Trees/shrubs
- a. Plant association ____________________________
- b. Largest size class with 8 or more trees (Circle one):
  - pole 5-9” dbh
  - small 9.1-20.9” dbh
  - medium 21-31.9” dbh
  - large 32-47.9” dbh
  - giant 48” or greater dbh
4. DBH measurements

Center subplots on plot center when practical. Size should be large enough to be representative of the entire plot. Minimum subplot radius varies with size class (see chart below), maximum radius for any class is 185.1’. Measure subplot radius and record under “r1” if a circle. If an ellipse, record minimum and maximum radii as r1 and r2. Tally number of trees in each size class, recording an H for a hardwood, C for a conifer. Circle C’s or H’s that represent dominant or codominant trees.

<table>
<thead>
<tr>
<th>Size Class</th>
<th>Minimum radius (ft)</th>
<th>r1</th>
<th>r2</th>
<th>Tally of Trees</th>
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<tbody>
<tr>
<td>0-4.9”</td>
<td>11.8</td>
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<tr>
<td>5-9.9”</td>
<td>26.3</td>
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<tr>
<td>10-14.9”</td>
<td>51.1</td>
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<tr>
<td>15-19.9”</td>
<td>51.1</td>
<td></td>
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<tr>
<td>20-29.9”</td>
<td>51.1</td>
<td></td>
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<tr>
<td>30-39.9”</td>
<td>51.1</td>
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<tr>
<td>40-47.9”</td>
<td>51.1</td>
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<tr>
<td>&gt;48”</td>
<td>185.1</td>
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</tbody>
</table>

5. Age of oldest tree cohort. Collect tree core samples from 2-3 of the oldest trees.
6.4 LICHEN SPECIMEN MAILING FORM--Lichen Surveys

Please enclose a copy of this form whenever specimens for the community analysis section are mailed. Keep a copy for your records.

FIELD CREW TO LICHEN SPECIALIST:

Sent by: _______________________   to:  _________________________   Date:  ______

Sender's comments:  __________________________________________
________________________________________________________________________

Received by:  _______________________   Date:  _____

Receiver's comments:  __________________________________________
________________________________________________________________________

CONTENTS

<table>
<thead>
<tr>
<th>Plot (CVS) number</th>
<th>Forest</th>
<th>District</th>
<th>Notes</th>
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<tbody>
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</table>
6.5 LICHEN SPECIMEN MAILING FORM-- Element Analysis

Enclose this form whenever specimens for tissue analysis are mailed. Keep a copy for your records.

FIELD CREW TO LICHEN SPECIALIST:
Sent by: _______________________ to: _______________________ Date: ______
Sender's comments: ______________________________________________________
Received by: _______________________ Date: _____
Receiver's comments: ______________________________________________________

<table>
<thead>
<tr>
<th>CONTENTS</th>
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<tbody>
<tr>
<td><strong>FIELD USE</strong></td>
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<td>Plot (CVS) Number</td>
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</table>

* Number collections sequentially WITHIN the plot (start with #1 for first bulk sample in each plot).
6.6 DATA QUALITY EVALUATION: LICHEN COMMUNITIES

Name ________________________________       National Forest ______________
Date ______________
Plot ______________
 Trainer/auditor ________________________

Evaluation

_____ Percent of species detected
_____ Percent of agreement in species composition

Comments


6.7 FEEDBACK FORM

To the field crews: To help us improve the lichen procedures, logistics, and training, please send comments to the lichen specialist. Addressed, stamped envelopes are inserted into the back pocket of this notebook. After the training or at any time during or after the field season, please write down your comments and send them in.

Date _________________ Your trainer ____________________

Your Name ___________________________

TRAINING

Did you receive sufficient training to effectively collect the lichen data?

What areas were covered well in the training?

What areas need improvement in the training?

Other comments on training

FIELD WORK

Suggestion box:
7.0 FIELD TRAINING

7.1 TRAINING OBJECTIVES

Training usually takes place over five days and consists of the following:
1. Study of the field methods manual and lichen community survey protocols.
2. Study of characteristics used to differentiate lichen species.
4. Tutorial on collection of lichens for chemical analysis.
5. Field practice in locating CVS plots, orientation and use of aerial photos.
6. Field practice in lichen community survey, tissue collection and completing field data cards.
7. Discussion of equipment, logistics, organization, sample handling and processing.
8. Recognition of Survey and Manage lichens.
10. Certification

Training will be followed within 2-5 weeks by a field visit and audit by the program coordinator.

7.2 SAMPLE TRAINING AGENDA

Day 1

8:00-12:00 AM
Introduction to lichen biology—work stations to learn to differentiate lichens based on color, size, morphology and reproductive structures,
Learn to recognize common lichens (lists provided in Section 4)
Slide show of common lichens.

1:00-5:00 PM
Drivers safety training
Short field trip to practice finding lichens
Collecting lichens for tissue analysis
Practice recognizing genera
Slide show quiz of common genera.

Day 2

8:00-12:00
Introductions, Field Schedule
Review of procedures, datacards, forms.
Recognizing rare species, slide show & handouts
Study rare lichens, review forest lichens

1:00-5:00
Slide show on air resource management program: issues, monitoring results
Review of Forest lichens
Written exam
Review exam

Day 3
Day 4

8:00-12:00, 12:30-6:30
Two field sites. Work in pairs.

Day 5

8:00-12:00
Certification Plot-- Local

Bring suitable clothing for work in the woods each day, including field vest, boots, rain-gear, hard hats, long pants and long-sleeved shirts and a day-pack. For lichen work, bring a hand lens, knife and field guides. Bring water and a bag lunch.

Lichen community certification is achieved when the trainee meets the data quality objectives for the training. This is documented in writing by recording the trainee's score on the Data Quality Evaluation sheet. The trainer(s) should clearly indicate that the evaluation was done for the certification process.

<table>
<thead>
<tr>
<th>Percent of Species and Abundance Ratings</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 65</td>
<td>Not passing</td>
</tr>
<tr>
<td>65-79</td>
<td>Good effort- need more field practice</td>
</tr>
<tr>
<td>≥ 80</td>
<td>Certified</td>
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</tbody>
</table>

Consideration is also given to the kinds of species missed and whether collection sizes were adequate. Individual arrangements can be made for non-certified personnel to begin fieldwork with certified field crew and to re-take the certification test within 1-2 weeks.
7.3 TRAINING DOCUMENTS

7.31 What is a lichen?

Lichens are made of symbiotic relationships between fungi and a photobiont. The photobiont can be either a green alga or cyanobacteria ("blue-green algae"). The fungus is almost always an Ascomycete (sac or cup fungus).

Lichens can be confused with algae, fungi and mosses. You can usually tell them apart by the following characteristics:

- Terrestrial algae tend to have little differentiation into macroscopic structures and they never produce disc-like reproductive structures. The most common algae that might be confused with lichens are: powdery green algae on tree trunks (Protococcus like algae), orange felty growth on tree trunks (often Trentepohlia), and blackish, amorphous growths on soil or rock (often Nostoc, a cyanobacterium).

- Non-lichenized fungi do not have a photosynthetic partner. In most cases, if it looks like a fungus and you can't find the green of blue-green pigmented partner, it is a fungus.

- Mosses and leafy liverworts have a distinct stem and leaf structure and are usually bright green (occasionally purplish or brownish). Thalloid liverworts are strap-shaped, bright green to purplish or brownish green. Break these open and they are greenish inside. Break a lichen open and it will either be partly whitish inside or completely black inside (use a hand lens).

Protocol: When in doubt, assume it is a lichen.

GROWTH FORMS

The basic growth forms for the thallus (the body of the lichen) are...

Fruticose: 3-D forms. The most common forms are shrubby, pendulous-stringy, or upright stalks.

Foliose: flat to ascending thallus with definite lobes.

Squamulose: Clusters of small dabs of lichens. These may be ear-shaped, convex, or concave.

Crustose: Tightly attached crusts, often appearing like they were painted on the substrate.

Macrolichens include foliose and fruticose forms.

Protocol: When in doubt, assume it is a macrolichen.

SPECIES DISTINCTIONS

1. Color is important. However, most species show color variation depending on light levels. In general individuals of a given species will be more deeply colored, often with a brownish tinge, when growing in strong light. Shade forms tend to be paler and often more greenish than the sun forms.

2. Reproductive structures are important.

Asexual structures (have both the algal and fungal components):

- Soredia have the consistency of flour and have a dull appearance. Soredia usually occur in patches called soralia.

- Isidia are shiny, slender bumps that occur scattered or in clusters. They are shiny because they are covered by a cortex, like the rest of the upper surface of the lichen.
Sexual structures are produced by the fungus:

a. **apothecia** are disk-like fruiting bodies of the fungi (Ascomycetes).
b. **perithecia** are flask-shaped fruiting bodies of the fungi, but these are usually immersed in the thallus, so that all that can be seen with a hand lens is a black dot where the tip of the flask reaches the surface.

*Note that two lichens that look alike, except that one has apothecia and the other soredia, are different species. Likewise, isidia vs. soredia on otherwise similar lichens will indicate different species.*

3. **Position of reproductive structures** is important. For example, do the soredia occur on the lobe tips, the margins, or on the lobe surface?

4. **Habit** is important. Some species are drooping, others erect, others tightly appressed.

5. The **lower surface** is important. Look at the color. Look for rhizines, feltiness, white spots and veins.

6. **Chemistry** is important, but is not used very often in the field. Lichenologists routinely use several specific reagents in the lab to make “spot tests”. A spot test is made by applying a small drop of a reagent to the lichen and looking for a color reaction.

*Protocol: When in doubt, assume it is a different species.*
7.32 Look-alike lichens, overlooked lichens and difficult genera.

**Alectoria**

*A. vancouverensis* is morphologically indistinguishable from *A. sarmentosa*—they can be distinguished in the office by the C+ medulla.

Problem: Because everyone collects only a small voucher of the more abundant species, *A. sarmentosa, A. vancouverensis* is rarely detected. The best habitat for *A. vancouverensis* is the transition between valley forests and mountain forests (below 2100’, 700 m) usually dropping below the elevation of highest dominance by *Alectoria*; it may be most frequent on the immediate coast.

Remedy: When in the range of *A. vancouverensis*, collect many individuals of *Alectoria* per packet.

*A. lata*—This lichen looks like *A. imshaugii* without isidia. Typically, it has ascocarps but so far all the collections made in our Region have been sterile. It is rare in the PNW and easily overlooked. Study examples in the Siuslaw herbarium to develop a search image for this species.

**Bryoria**

Problem: *Bryoria* species often grow intermixed, and collection packets often contain species mixes, making it difficult to apportion abundance ratings.

Remedy: Try to collect single species. Examine material with a hand lens before placing in a packet. *Bryoria* can be differentiated by color, branching pattern and angles, filament diameter, thallus habit (erect or pendent) and presence of soralia, apothecia, or pseudocyphellae.

Also, there are some rare Bryorioid lichens. Please learn these so you can spot them in the field:

- *Bryoria tortuosa*
- *Bryoria subcana*
- *Bryoria pseudocapillaris*
- *Bryoria spiralifera*
- *Sulcaria badia*

**Candelaria concolor and Chrysothrix chorina/candelaris**

These are rather small but brightly colored lichens that appear to be good indicators of nitrogen deposition; collect them if they occur on the plot. Examples can be found in the Siuslaw herbarium. *C. chlorina* and *C. candelaris* are distinguished by the diameter of the soredia (0.1 and 0.01 mm respectively). *Chrysothrix* species may be hard to distinguish in the field, but are easily separated in the office.

**Cetrelia/Esslingeriana**

Problem: These may be under-collected because they look like *Platismatia glauca*. *P. glauca* is often found at the same sites, usually in greater abundance.

Remedy: Learn to recognize these species by looking at examples in the herbarium.

*Cetrelia cetariodes* can be confused with *Parmotrema* (but lacks cilia) or a very broad lobed *Parmelia* (but lacks abundant rhizines) or with *Platismatia glauca*. It is distinguished by its pseudocyphellae on the upper surface and the elongate marginal soredia, often following the edge of a lobe for 5-10 mm or more. It is most common on the Siuslaw NF (i.e. in the Coast range) and is not known east of the Cascade crest. It occurs in moist riparian and valley bottom forests, especially on older *Alnus rubra*.

*Esslingeriana idahoensis* differs from small individuals of *P. glauca* by its lack of soredia or isidia and a pored black lower surface. It is most common east of the Cascade crest but is occasionally found in low to mid elevation forests on the west side.

**Cladonia**

Problem: These tend to be under-collected and, when they are collected, packets frequently contain a mixture of species.

Remedy: Make a point to look for Cladonia species (at heights > 1/2 meter) on tree boles. Continue to collect only colonies with at least 10 podetia. Learn to differentiate species. Cladonia are
differentiated by color (yellow green vs gray green), presence or absence of cups, color of apothecia and pycnidia, presence ad distribution of soredia, and size, shape and distribution of squamules.

**Hypogymnia**
Problems: Although we are finding a lot of species diversity,

1) it is easy to mix different species mixes in the same packet—making it difficult to know which species the abundance rating recorded on the packet should be applied and

2) many times packets with high abundance ratings are incorrectly labeled. If only one individual is in the packet, it is not possible to know if the field person incorrectly identified the lichen, or happened to collect an example of a different species even though most of what s/he saw was the labeled species.

Remedy: Learn to differentiate species better. Collect more material. Put more *Hypogymnia* in the packets and/or collect more packets if you are not sure it is the same.

Species that are easy to confuse with one another:

**Hypogymnia enteromorpha and appinata**: This may be hopeless. Separated definitively by a P test though supposedly *appinata* lacks the small side buds. Study examples in the herbarium and in the original *Byrologist* report by Goward and McCune.

**H. occidentalis, enteromorpha and metaphysodes**: *H. occidentalis* is narrower lobed and more appressed than *H. enteromorpha*. The upper surface of *H. occidentalis* usually has a dark, continuous margin and older parts are more rugose than *H. enteromorpha*. *H. occidentalis* and *enteromorpha* must be separated in the office with a P test. *Metaphysodes* forms circular colonies like *H. physodes* but without soredia. Small individuals of *occidentalis* can look like *metaphysodes*. But the lobes of *H. occidentalis* always have a black ceiling inside, whereas those of *H. metaphysodes* have a white ceiling.

**H. inactiva and H. imshaugii**: These species are easily confused. Check insides of lobes in several places. *H. imshaugii* is white inside throughout, *inactiva* has a black floor.

**H. occidentalis and H. rugosa**: *H. rugosa* is rare. Most rugose *Hypogymnia* will be *H. occidentalis*. *H. rugosa* differs from *H. occidentalis* in having regularly dichotomous branching, no bud-like side lobes, and a papery (rather than cartilaginous) texture. *H. rugosa* typically occurs at high elevations at passes in the Cascade crest and in a narrow band on the east side of the crest.

**Leptogium**
This genus is often overlooked because it is darkly colored and is associated with dark, moist microsites. *Leptogium* species often grow mixed with mosses on trunks of deciduous trees and on shrub stems.

**Melanelia**
Problems: Packets often contain more than one species and some species are under-collected. The most frequently collected *Melanelia* are *M. exasperatula* and *M. subaurifera*. The dominance and high abundance of a few species seems to camouflage the more rare but similar-looking species.

Remedy: Examine species with a hand lens before collecting to note distinguishing features. Collect more material. Learn some of the less common species by examining herbarium specimens under a dissecting microscope.

Under-collected but common epiphytic *Melanelia*:

- *M. fuliginosa*
- *M. elegantula*
- *M. subelegantula*
- *M. multispora*
- *M. subolivacea*

Under-collected and rare epiphytic *Melanelia*:

- *M. subargentifera*
- *M. disjuncta*
- *M. sorediata*
- *M. glabra*
**Pannaria/Fuscopannaria/Psoroma**
Problem: All the species in these genera are easy to overlook and have been under-collected. These lichens are difficult to spot because of their small size and dark color.
Remedy: Study collections in the herbarium to learn to recognize the species. Study lichen guide to learn their preferred habitats and substrate. Make a point to look for them on the plots.

Epiphytic species to learn:
- **Psoroma hypnorum**: separates from *Pannaria* because the photobiont is a green alga.
- **Pannaria rubiginosa**: forms neat little rosettes with elongated lobes. The upper surface is light blue-gray to brownish. Apothecia are common, reddish brown, and have an even, persistent thalline rim. Margins of the apothecia are not white felted tomentose.
- **Fuscopannaria saubinetii**: from a distance, looks like a dark or greyish crust. Minutely incised lobes can be observed with a hand lens. The apothecia are pale to orangish-brown and lack a thalline margin.
- **Fuscopannaria leucostictoides**: has bluish-gray tinged lobes. The apothecia have thick, thalline, white felted-tomentose margins.

**Parmelia**
Problem: Mixed species in packets, several species under-collected. (Read *Melanelia* section of this handout). *Parmelia sulcata* is often much more abundant than other Parmelia species.
Remedy: Learn to differentiate species better. Collect more material in packets and/or collect more packets.

Under-represented species to learn:
- **P. squarrosa**: shiny isidia, squarrose rhizines
- **P. hygrophila**: dull isidia, forked rhizines
- **P. pseudosulcata**: shiny isidia, simple rhizines
- **P. saxatilis**: occurs on trees but the most common substrate is rock. *P. saxatilis* looks like *P. pseudosulcata* but has a K+y medulla. In the absence of a K test, the best field guess for Parmelia with shiny isidia is *P. pseudosulcata*.

**Physcia/Physconia**
Problem: Some people have trouble recognizing or separating these genera.
Remedy: Study examples in the herbarium. Goward and McCune (Lichens of BC) has a good key. Study species distinctions in the key.

**Ramalina/Niebla**
Problem: A few species appear to be under-collected:
Remedy: Learn to differentiate under-collected species from more common ones.

- **Ramalina thrausta**: occurs sporadically on the east side of the Cascades in low elevation moist forest, especially riparian spruce or fir. Occurs in low-elevation old-growth PSME west of the Cascades. It is most common in conifer forests of the immediate coast. *R. thrausta* is most easily confused with *Alectoria sarmentosa*. It is separated from that species by an absence raised pseudocyphellae, a slightly flattened thallus and hooked tips, sometimes with minute terminal soredia.
- **Niebla cephalota**: looks like *Ramalina farinacea* in poor condition, and may be overlooked for this reason. The thallus has black spots throughout, and has slightly larger and rounder (vs. elliptical) soralia than *R. farinacea*. So far, it is known only from the immediate coast. Collect sparingly as this is a rare lichen.
**Usnea**

Problem: mixed species in packets, some species probably under-collected.
Remedy: learn to differentiate species better

Tips: Characters separating species:
--color of axis or cortex
--pendant vs. shrubby
--with or without papillae
--with or without colored cortex or axis
--foveolate vs. smooth branches
--isidia and soredia: Do they occur together? Are soredia concave or convex? Do soredia erode the cortex so much that the central cylinder shows? How large are they relative to the branch diameter. Look for isidio-soredia and soredio-isidia.
--branching patterns and stiffness/softness of thallus
--blackening or not of holdfast (always collect the holdfast when you collect Usnea).
--ratio of medulla to central axis (make cross section). Is the medulla dense or cobwebby? -- presence or absence of articulations (annular rings).

**DISTINGUISHING SPECIES OF ** **USNEA IN THE FIELD**

(References: 15 Mar 2000 key by B. McCune and distribution data in Air Quality database)

**Boldfaced species** are most common in the area of our monitoring study

**Pendant Usnea ( > 11 cm long).**

*Usnea cavernosa*-- main branches strongly pitted and ridged, no papillae isidia or soredia, fibrils lacking or sparse (Alectoria-like). Willamette Valley, Umpqua NF.

*Usnea chaetophora*-- base slightly to distinctly blackened, papillae usually present but may be sparse and low, soralia absent to scattered, usually minute, borne on small tubercles, isidia occasionally present, Alectoria-like. Wide distribution: coast range, west and east side Cascades.

*Usnea filipendula* -- similar to U. longissima but with more main branches. Papillae tall, cylindrical and abundant; tuberculate isidio-soralia, often arising from scars of detached fibrils. Base blackened or not. Widely distributed from coast range to east-side Cascades.

*Usnea hesperina*-- main branches smooth, no papillae, annular cracks common, sometimes Alectoria-like, sometimes fibrillose. Soralia absent to abundant. Isdia absent to sparse, soon abraded. Immediate coast.

*Usnea longissima* -- very long, rarely dividing main branches with many short branches perpendicular to the cylindrical main axis. No papillae or isidia. Soralia occasionally present. Coast range and low elevation, west-side Cascades.

*Usnea madeirensis (= silesiaca*)—base blackened, annular cracks esp. at base, soralia > ½ branch diameter, isidiate at least when young. Mainly immediate coast, Coast Range and Puget Trough.

*Usnea scabra*—main branches wrinkled and ridged, abundant isidia, papillae may be weakly developed, few annular cracks. Widely distributed from coast range to east-side Cascades.

**Shrubby Usnea (< 11 cm long)**

1. With Cigar-shaped branches (pinched at the nodes and slightly to distinctly expanded in the internodes):
Usnea wirthii—pale lemon yellow central axis, cortex often with red spots. Isidia absent; base pale; branch apices recurved; branches usually with annular cracks; soralia plane to slightly concave, sometimes confluent; papillae distinct and numerous; fibrils usually abundant. Widely distributed.

Usnea cornuta—white central axis, thallus large (5-15 cm), papillae limited to main branches or absent. Isidia usually small, <1/2 branch diameter, sometimes coalescing into larger patches. Base pale or blackened. Widely distributed, especially common in Coast Range.

Usnea glabratat—white central axis, thallus small (<5 cm). Papillae sparse or absent, no isidia but soralia may become large and wrap around the branches. Base pale or slightly blackened. Branch apices straight to recurved. Soralia mostly near the apices, usually large and tuberculate, often confluent and wrapping around the branches when mature. Isidia lacking but spinules may be present around the soralia. Papillae limited to main branches or absent. Widely distributed.

Usnea fragiliscens var. mollis—Similar to U. cornuta but soralia are usually >½ branch diameter. OR Base distinctly blackened; thallus subpendent to 20 cm long. Thallus sparsely branched; isidia present; soralia present arising from slow tubercles; papillae low and numerous sometimes indistinct; fibrils sparse to abundant. Coast range.

Usnea esperantiana—Central axis white, isidia absent. Thallus to 8 cm long. Base pale; branch apices recurved, soralia plane to slightly concave, sometimes confluent; papillae distinct and numerous. Fibrils usually abundant. Limited to coast range?

2. With Cylindrical Branch Segments

Usnea diploptypus—soralia raised, not exposing central axis, becoming isidiate. Short to cylindrical papillae; terminal branches more or less similar in diameter, tapering only toward tips. Widely distributed, most common east of the Cascade crest.

Usnea glabrescens (=fulvoreagens)—Base is blackened, has concave soredia, no isidia. Soralia similar to U. lapponica; branches cylindrical, branching isometric dichotomous; base conspicuously blackened. Fibrils and lateral branches divergent (as opposed to the rare species U. wasmuthii, with narrow, ascending fibrils and black base splitting into right angled segments). Widely distributed.

Usnea lapponica—branches often deformed with foveoles or irregularly swollen; base pale or blackened; soralia becoming strongly concave, exposing the central axis, the edges of the ruptured cortex flexed outward, eventually the soralia wrapping around the branches. Most common east of the Cascade crest.

Usnea nidulans—cortex translucent; fibrils often in fascicles of two to four; soralia tuberculate when present. Papillae low. Rare. Immediate coast and coast range.

U. subfloridana—annular cracks few to scattered; soralia tuberculate to slightly excavate, mature soralia often rounded; papillae warty to cylindrical; fibrils often abundant near the base. Widely distributed.

Usnea substerilis—soralia initially raised, become concave, not exposing central axis. Usually isidiate; base pale or blackened; papillae low to cylindrical, usually numerous. Willamette Valley and east Cascades, Columbia River Gorge.

Usnea pacificana—papillae warty, terminal branches tapering; soralia punctiform and tuberculate; thallus initially erect, becoming pendent to 20 cm; base slightly to distinctly blackened; annular cracks common near bases with whit everted medullary rings common on main branches; soredia usually sparse; isidia short to long, occurring on young soralia but easily abraded. Occurring west of the Cascade crest.
Colored Axis or Cortex (red, yellow, brown or black)

Usnea rubicunda—thallus reddish brown, immediate coast.

Usnea wirthii—see entry under Cigar-shaped shrubby Usnea.

Usnea ceratina—Central axis reddish, pinkish brown or rose; raised tubercles commonly bearing isidia and coarse soredia. Annular cracks often abundant and conspicuous; tubercles sometimes coalescing into ridges. Cortex thick and gossy, base pale to rarely blackened. Pendent to 30 cm. Rare. Coastal.

Usnea sphacelata—cortex blackening toward branch tips; thallus tufts to 2 cm; branches often black spotted or banded. Rare. On rock in subalpine to alpine.
7.33 Summary of Lichen Survey Procedures

Above all:

**WRITE NEATLY!!!!**
**PUT PLENTY OF MATERIAL IN LICHEN SURVEY PACKETS**

**Sampling area.**
The area to be sampled is a 114’ r circular plot centered on sampling point #1 of the CVS plot.

**Sampling time.**
Sampling continues for a maximum of two hours or until 10 minutes elapse with no additional species recorded and all sectors of the plot have been covered. At least 30 minutes must be spent searching the plot, even if very few lichens are present.

**Reconnaissance walk**
Walk through the entire lichen plot to locate lichen epiphytes on woody plants, collect voucher samples and assign abundances.

**Lichens to collect**
Collect epiphytic fruticose and foliose lichens.

**Substrates for collections**
Woody plants (must be >0.5 m tall west of the Cascade crest to avoid ground lichens creeping up over moss on bases of trees and shrubs) within the lichen plot will be inspected for lichens species. Fallen and reachable branches will also be inspected. Rotten logs, stumps, and branches overgrown with ground mosses on the forest floor should not be sampled.

**Where to look**
Carefully inspect the full range of substrates and microhabitats available: shaded and exposed, conifers and hardwoods, fallen upper branches and lower branches, large shrubs and trees in particular topographic positions (e.g. checking in draws or ravines of an otherwise uniform slope).

**Abundance ratings.**
Record abundance within the lichen plot on each packet. Use the highest rating that is true, estimated as follows:

<table>
<thead>
<tr>
<th>Code</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rare (≤3 individuals on the plot)</td>
</tr>
<tr>
<td>2</td>
<td>Uncommon (4-10 individuals on the plot)</td>
</tr>
<tr>
<td>3</td>
<td>Common (10-40 individuals on the plot)</td>
</tr>
<tr>
<td>4</td>
<td>Very Common (&gt;40 individuals observed but covering less than half the available substrate. Choose one:</td>
</tr>
<tr>
<td></td>
<td>4-1 individuals are few (close to 40) and widely scattered around the plot</td>
</tr>
<tr>
<td></td>
<td>4-2 most of the individuals are restricted to one or two small areas on the plot</td>
</tr>
<tr>
<td></td>
<td>4-3 many trees have ≤20 individuals</td>
</tr>
<tr>
<td></td>
<td>4-4 many trees have &gt;20 individuals</td>
</tr>
<tr>
<td></td>
<td>4-5 &gt;50% of the trees have ≤20 individuals</td>
</tr>
<tr>
<td></td>
<td>4-6 &gt;50% of the trees have &gt;20 individuals</td>
</tr>
<tr>
<td>5</td>
<td>Abundant (the lichen physically covers more than half of the available substrate)</td>
</tr>
</tbody>
</table>

**Recording substrate**
Record substrate species (4 letter acronym OK). If species cannot be determined use the most specific term that is certain, e.g. conifer branch, hardwood snag. Also record location on the substrate, e.g.
branches, boles, fine branches, fallen, over moss on branch, base of boles over moss. If the collection is on wood rather than bark, write “on wood”. If the lichen was collected from the litter, specify “fallen branch” or “litterfall”. If collecting non-epiphytic species, describe the substrate as specifically as possible-- on limestone rock, on duff and decayed organic mater, in mineral soil, etc.

**Packet labeling**
A sample of each putative species will be collected and placed in a paper packet labeled with the CVS plot number. If it is not a CVS plot, assign a unique number or letter code, e.g. Eugene 1- 5.30.97. Number packets sequentially (we use these numbers to track individual collections in the database), and record abundance, collector’s initials, forest acronym, and collection date.

**How to handle uncertainties**
Field observers will frequently have uncertainties about the classification of an organism. The following rules are designed to put the onus of the responsibility for classification on the specialist, not the field crew:
--When in doubt, assume it is a lichen.
--When the growth form is in doubt, assume it is a macrolichen.
--When the species distinction is in doubt, assume two different forms are two different species.

**Sample collection**
Optimally, a palm-size sample of fruticose and foliose growth forms is collected. Even minute fruticose and lobate forms should be included. Cladonia sp. squamules lacking upright stalks should not be included. Collecting large samples improves the likelihood of picking up inconspicuous species that may not have been noticed in the field. These additional species can be recorded in the office.

**Packaging samples, preservation and storage.**
Each specimen will be placed in a separate folded and labeled paper packet. Often there will be more than one species on a bark sample. If there is any ambiguity about which species in the packet corresponds with which abundance rating, a clarifying phrase, such as “the white one” or the “sorediate one” should be written on the packet. *Air-dry samples thoroughly to avoid decay.* Packets should be stored in a dry place until delivery to the program coordinator.

**Quality control.**
Only those who have successfully completed lichen training should collect the lichen community data.

**Mailing packets.** Periodically send or deliver packets to the program coordinator. Bind packets from a single plot with rubber bands and place in a separate paper or Ziploc bag with the corresponding field data card.
7.34 Summary of Tissue Collection Procedures

What to collect: Collect > 20 grams each of two target lichens, dry weight. Avoid dusty, gritty, discolored, or decaying material.

Target species:
MOST PREFERRED
- Platismatia glauca (collect whenever possible)

PREFERRED
- Alectoria sarmentosa (Alesar)
- Evernia prunastri (Evepru)
- Hypogymnia enteromorpha (Hypent)
- Hypogymnia imshaugii (Hypims)
- Hypogymnia inactiva (Hypina)
- Letharia vulpina (Letvul)

GOOD
- Bryoria fremontii (Bryfre)

ACCEPTABLE if no other target species are present.
- Letharia columbiana (Letcol)
- Lobaria oregana (Lobore)
- Sphaerophorus globosus (Sphglo)
- Xanthoparmelia cumberlandia (Xancum)

If a moss is collected, collect a lichen for the second target species.
- Isothecium myosuroides (Isomyo)--moss
- Lobaria pulmonaria (Lobpul)
- Neckera douglasii (Necdou)--moss
- Usnea (Usnea)--shrubby species only

Replicates
For each species, make one replicate collection for every five collections. To save time and improve repeatability, collect replicates at sites where the species is plentiful rather than waiting until the fifth plot or until later in the field season.

Repeat Visits
Revisit one plot per Forest during the field season and sample the same species again.

Where to collect
Make collections within 1 km (.65 miles) of plot center. Collect > 35 m away from roads. Collect from > 6 locations per sample. Lichens on tree branches, shrubs or tree boles, in the litter, or on fallen branches, may be used. Collect Alectoria, Bryoria and Usnea spp only from live or standing substrates. Collect replicates and repeats from the same host species and types of substrate locations.

How to collect
While collecting wear unpowdered vinyl gloves and avoid crew touching anything brought onto the plot. Store unused Kapak bags in clean zip-loc plastic bags. Wear new gloves at each plot and replace if they become torn or contaminated. Place clean samples in Kapak bags and weigh on a 50 g. Pesola scale. If the lichens are dry, the sample and bag together should weigh > 28 grams. If the lichens are wet, the bag should weigh more than 100 g and adequacy of the sample size should be judged by volume rather than weight. Fold the edge of the bag over three times and seal with waterproof, removable, laboratory tape.

What to record
Write on the bag with an indelible marker: plot number, date, substrate(s), target species acronym, collector’s initials, and moisture status of sample: dry, damp or wet. List host species name and substrate location in order by the amount of sample in the bag from that substrate. E.g. “Pinus contorta branches, Pinus ponderosa branches and boles” would indicate that the sample weight collected from P. contorta branches> P. ponderosa branches> P. ponderosa boles.

Drying Samples
Dry any damp or wet samples within two days of collection. If samples are kept wet more than one day, store them on ice in a cooler. Dry lichens in mesh bags attached to a clothesline, or on clean 100 cotton herbarium sheets on a clean desk or other flat surface, preferably covered by glass or plastic.