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Lichens as Bioindicators of Air Quality

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Identification of Sensitive Species

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We describe procedures for determining the responses and sensitivities of lichen species to air pollutants. Pollutant chemicals in their primary forms and as derivatives such as ozone or acid rain can cause acute and chronic injury to lichens and other cryptogamic plants. Fumigation studies are designed to reveal plant responses to air pollutants under controlled conditions in enclosed exposure systems, generally through changes in physiological processes. Gradient studies are designed to show responses at predetermined locations along an air pollution gradient. They usually measure visible damage and are done around existing or proposed pollutant sources. Both types of study have limitations, but are useful for detecting air pollution effects and tracking changes in existing or proposed pollution sources, as well as for understanding natural succession and variability.

OVERVIEW

Air pollutants can cause both acute and chronic effects on lichens and other organisms such as liverworts, mosses and cyanobacteria. Atmospheric deposition and concentrations of air pollutants great enough to cause acute injuries to vegetation, such as necrosis, morbidity, and mortality, are usually found only around point sources. Examples of large point sources include fossil-fuel fired steam electric generating plants, gas purification plants, metal smelters, aluminum production plants, cement plants, chemical plants, and pulp mills. Historically, sulfur dioxide (SO_2) and fluorides have received the most study, and acute effects to lichens and other vegetation around point sources of these pollutants are well documented (Shriner et al. 1990; Nash 1988, 1972; Ferry et al. 1973; Gilbert 1973).

Primary pollutants such as SO_2 and fluorides are of biological concern in the same chemical form as they are emitted. Secondary pollutants are created as a result of chemical reactions involving primary pollutants during transport in the atmosphere. Ozone (O_3), peroxyacetyl nitrate (PAN), and acid rain are examples of secondary pollutants. Ozone and PAN are formed in photochemical reactions involving nitrogen oxides (NO_x) and hydrocarbons mostly emitted by vehicles. Acid rain is formed as SO_2 and NO_2 are oxidized to sulfuric acid (H_2SO_4) and nitric acid (HNO_3) during atmospheric transport. Visible injuries such as bleaching and necrotic spots due to acute and chronic ozone exposure occur in the Los Angeles basin (Sigal and Nash 1983), the central Sierra Nevada, and in the eastern United States (Shriner et al. 1990). Acute foliar injury to vegetation from acid rain is rare, with few documented cases (Shriner 1986).

Other types of pollutants, commonly referred to as "air toxics" (e.g., industrial organics, agricultural pesticides, trace metals, and metalloids) are of concern as well (Moser et al. 1992), although there is limited information on the effects of these compounds. Semi-volatile and persistent organics are transported and deposited via atmospheric processes. High concentrations of toxic organics and pesticides have been found in fog and in lichen tissues (Glotfelty 1987; T. Moser, personal communication). Studies have demonstrated that various trace metals affect lichens (Nash 1972, 1975).

A number of other pollutants, known to be present but for which there is little or no information, are of less interest for one or more of the following reasons:

1) pollutants are widely distributed, but seldom cause damage because ambient exposures have mean ratios and mean concentrations that are too low to affect lichens (e.g., ethylene, carbon monoxide);

2) pollutants are not widely distributed, occurring only as accidental releases (e.g., chlorine, ammonia); and/or

3) pollutants are widely distributed, but seldom cause damage to vegetation because the high concentrations necessary to cause damage are rare (e.g., hydrogen sulfide, hydrogen chloride) (Shriner et. al. 1990).

Chronic injury develops after long-term or repeated exposure to sub-acute concentrations of multiple air pollutants associated with urban development, such as small stationary sources and large numbers of mobile sources. Chronic injury is less likely to involve death of tissues, but is likely to involve nonspecific symptoms of plant stress such as chlorosis (loss of chlorophyll, yellowing) and reduced growth. At the community level, sensitive species may disappear from the population. Lists of species known to be sensitive to pollutants under certain conditions have been published (Nash and Greis 1991).

In this chapter we review procedures for determining the responses and sensitivities of lichen species to air pollutants. The most widely used methods are gradient studies and fumigation studies. Historically, gradient studies usually involved observations of visible injury, species richness, or species abundance correlated with pollutant exposure, when exposure data were available. However, these studies did not address proof of cause/effect relationships.

More recently, fumigation studies involving some variation of a closed chamber with air movement through the chamber, or chamberless field fumigations, have been used to develop quantitative relationships between concentrations of air pollutants and various plant responses, thereby establishing cause/effect relationships. Controlled fumigation studies coupled with gradient studies give the best information. For SO_2, scales of sensitivity have been independently established based on controlled laboratory fumigation results and on field observations (Nash 1988). By non-parametric correlation analysis, significant agreement among the various scales is shown and this strongly supports the inference that lichens actually do respond to SO_2 (Nash 1988). For other air pollutants, data are currently insufficient to establish precise sensitivity scales. However, pollutant concentrations measured in the field are high enough to adversely affect species that are demonstrably sensitive to fumigation experiments with air pollutants such as ozone, hydrogen fluoride, and zinc (Nash 1988).

The information collected from gradient and fumigation studies can be used in relation to applications for Prevention of Significant Deterioration (PSD) permits, to show that estimated emissions from a proposed facility may unacceptably affect resources of a Class I area. For areas in which there are no data, the information provides baseline or control data to which subsequent data on effects of new pollution can be compared. For areas where there are historic data, the information may allow comparisons over time that contribute to understanding successional patterns, the natural variability of species, and the effects of existing air pollution. Following permitting, construction, and operation of air pollution sources, continued measurements can show the magnitude and significance of changes in air quality.

FUMIGATION STUDIES

Fumigation studies are designed to show measurable responses to air pollutants, singly and in combination, under controlled conditions in more or less enclosed exposure systems such as continuously stirred tank reactors (CSTRs), open-top chambers, branch chambers, and miniature cuvette chambers. Pollution studies are occasionally done in the field without chambers, using techniques such as Zonal Air Pollutant Study (ZAPS) and simulated acidic rain exposures.

The most commonly measured responses are selected physiological processes. Lichen physiological processes sensitive to fumigation appear to be: nitrogenase activity, K^+ efflux/total, electrolyte leakage, photosynthesis, and respiration pigment status, in order of sensitivity (Fields 1988). There are still problems with fumigation studies because sensitivity depends on such factors as concentration and duration of exposure, environmental conditions, and status of the thallus during exposure (Fields 1988). In addition to the very important influence of thallus hydration level, it

is known that different portions of a thallus vary in their physiological response (Nash et al. 1980, Karenlampi 1970; Moser and Nash 1978; Moser et al. 1983).

Long-term laboratory fumigation studies to determine chronic injury are not currently considered feasible because of problems in maintaining specimen viability in growth chambers. However, short-term fumigation studies (less than a month) are valuable for explaining the mechanisms of plant response and establishing exposure-response relationships. Since lichen material does not generally do well for even short times in artificial chambers, attention must be paid to the treatment of specimens and their physiological condition throughout the study (Link and Nash 1984, Pearson and Benson 1977, Pearson 1970).

Although air pollution is a complex phenomenon involving multiple contaminants, most fumigation studies have used only single pollutants under given sets of conditions. Most laboratories are not equipped to perform the factorial experimental designs necessary to study the responses of plants to pollutant mixtures because of limitations in the number of exposure chambers available. However, additive, antagonistic, and synergistic responses to two or more pollutants are known for vascular plants (Shriner et. al. 1990), and there is no reason to think that lichen responses to multiple pollutants are any less complex.

Exposure Systems

Gaseous and particulate exposure systems range from plastic bags (Anderson 1976) to elaborate chamber systems with automatic control of environmental parameters and pollutant concentrations (Heagle and Philbeck 1984). Over time, systems for studying the effects of single or multiple pollutants have become more sophisticated (Lange et al. 1984). However, there is no perfect all-purpose exposure system. Essential requirements for plant exposure systems, whether they be controlled environment, greenhouse, or field, are listed below in order of their importance (Heagle and Philbeck 1984):

- uniform pollutant concentration throughout chamber and between chambers;
- uniform environment throughout chamber and between chambers;
- non-reactive surfaces;
- precise control of pollutant concentrations; and
- environment resembling ambient conditions.

In addition, the accurate determination of pollutant concentrations and environmental parameters within and between chambers requires acceptable calibration procedures which should be followed before and during an experiment. Properly designed and controlled experimental techniques and comprehensive reporting are important for other researchers working in related areas and for regulatory agencies in developing dose-response curves for the establishment of air quality standards (Drummond and Pearson 1979). The chamber systems described below have the advantage of multiple replicates, but are usually too expensive to build for individual lichen studies. However, it may be possible to conduct experiments at existing facilities. Chamber studies are most useful for determining the effects of pollutants on lichen physiology (e.g., photosynthesis, nitrogenase activity, respiration) and ultrastructure.

Controlled Environment Chambers

Controlled exposure conditions can be attained in three ways:

- by converting commercially available controlled-environment chambers to pollutant exposure capability;
- by putting smaller exposure chambers (plexiglass boxes, bell jars or even plastic bags) within a single controlled-environment chamber; or
- by adding environmental controls to exposure chambers.

Currently, the most widely accepted system is the continuously stirred tank reactor (CSTR) system, which ensures rapid mixing of pollutants injected into the system and uniformity of conditions within the chambers. The primary concern is how well the results reflect plant response under field conditions, since the dynamic nature of field environmental conditions (e.g., temperature, light, relative humidity) is not reproduced in these systems. (Shriner et al. 1990).

Mini-Cuvettes

An additional form of exposure chamber used successfully for a number of years is a cuvette which encloses a branch or a portion of a branch in a mature tree canopy (Legge et al. 1977, Lange et al. 1984, Amundson et al. 1992). This approach permits the measurement of mechanistic plant tissue

responses, such as photosynthesis, **in situ** in a canopy, eliminating many of the concerns regarding the effects of controlled exposure chambers. In small chambers it is possible to introduce pollutant gases in sub-parts per million as part of the inflow using the flow-through air exchange principle. However, this type of pollution exposure has not yet been used with lichens.

Open-Top Field Chambers

Open-top chambers used in the field duplicate the ambient environment as closely as possible while allowing control of pollutant concentration within the chambers. Appropriate experimental designs for using open-top chamber systems include pollutant-free and ambient chambers as well as non-chambered control plots to estimate any chamber effects. The open-top chambers are the best currently available experimental technique for developing functional relationships useful for predictive purposes (Shriner et al. 1990).

ZAPS Systems

The acronym ZAPS stands for Zonal Air Pollution Systems, referring to means of delivering air pollutants in the field for relatively long-term testing with no control over environmental conditions. The two systems described dispensed only SO_2.

Moser et al. (1980) used a small system at Anaktuvuk Pass in Alaska to fumigate caribou forage lichens *Cladina stellaris*, *C. rangiferina*, and *Cetraria cucullata*. Anhydrous SO_2 was dispensed through paired holes along an aluminum pipe. Sulfate concentrations were measured at intervals upwind and downwind from the pipe.

One large system (ZAPS I) was operated by the US Environmental Protection Agency from 1975-1979 in Custer National Forest in southeast Montana to predict possible effects of emissions from new coal-fired power plants on components of a grassland ecosystem. A duplicate system (ZAPS II) operated from 1976-1979. Both ZAPS I and ZAPS II had four plots, each with different SO_2 concentrations. In 1978, sulfation plates were set at 10, 50, and 100 cm above the soil surface at five locations per plot, adjacent to transplanted lichens. The plates demonstrated slight differences in sulfation levels with elevation above ground level. In general, less SO_2 was recorded at the lowest level, 10 cm, than at the upper two levels, indicating little or no pooling of SO_2 at ground level and vertical variations in SO_2 concentrations within plots (Eversman 1979).

Acid Rain Simulation

Contaminated rainfall has been simulated in the field and laboratory using a variety of approaches, from watering cans to back-pack sprayers and irrigation sprinklers, to very elaborate systems designed to permit variation of all of the major components of dose on a programmable basis (Shriner 1979). Rain simulators have been used in combination with a ZAPS-type fumigation system and are being used with open-top chambers to study the combined effects of acid rain and gaseous pollutants. The more sophisticated systems can reproduce the physical and chemical characteristics of natural precipitation including:

- rainfall rate (intensity controllable between 0.5 and 2.7 cm/hr);
- droplet size range (0.1 - 3.2 mm);
- chemical composition, through metering pumps which supply carefully controlled concentrations of ions to a deionized water stream.

GRADIENT ANALYSIS

The gradient analysis method assumes that measurable attributes of affected plants vary along causative environmental gradients. This assumption is illustrated in figure 1, which shows unimodal relationships among the importance values of three lichens and one moss along a moisture gradient (Flock 1978).

Species are best represented at their environmental optima, and will not be found at limits outside their tolerances. In addition to air pollution, other gradients influencing lichens include climate and substrates, as well as gradients of disturbance factors such as grazing intensity, fire regimes, or trampling.

Gradient analysis can be designed to show measurable responses of lichens at predetermined locations along an air pollution gradient. Studies are usually done around existing or projected sources of contaminants. The sources can be point sources like power plants or diffuse sources like the Los Angeles basin. The most commonly measured response variables are visible injuries such as bleaching and thallus deformation, changes in community structure such as species richness or cover, and physiological

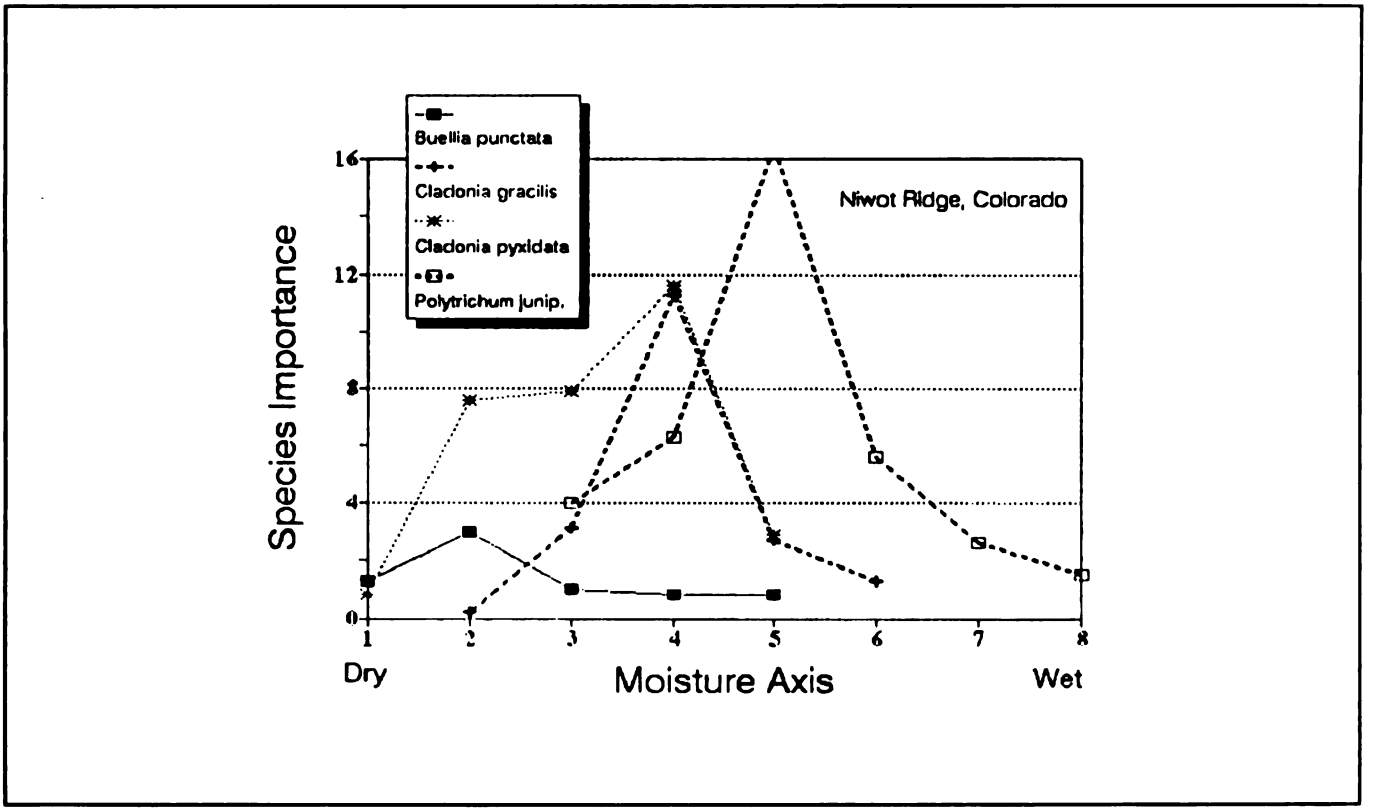

Figure 1.—Importance of 4 cryptogams along an indexed moisture gradient from dry to wet (from Flock 1978).

processes such as photosynthesis, nitrogenase activity, element uptake, membrane integrity (electrolyte leakage), pigment quantity, degradation, and fluorescence.

Gradient analysis is appropriate for studies of the effects of air pollutants on lichens, since pollutant loadings often vary with distance from point sources. It is not necessary for other environmental factors to vary continuously in the real world in order to use gradient analysis. There are often sharp discontinuities in spatial or temporal dimensions. Gradient analysis is also useful for analysis of regional effects derived from non-point sources. In this case, the regional airshed is assumed to be large enough that there is wide latitude in pollutant exposure levels.

The use of lichens in gradient studies has some limitations of which the researcher should be aware (Johnson 1976). These include:

- difficulties associated with identification of species;
- determination of the best indicator species for the particular study; and
- demonstration that the observed distribution patterns reflect pollution stress and not other biotic and/or abiotic factors.

This last problem can be especially vexing, since pollution impacts on lichens may be minor relative to more direct effects of microclimate, substrates, trampling, or extremes of weather variability.

The theory of gradient analysis is well developed (Ter Braak 1987). Measurements are taken across a variety of environments, and differences among the measures are assumed to be controlled by environmental differences between the sites. Data can be analyzed in several ways, which are summarized here from the treatise by Ter Braak and Prentice (1988). Readers are referred to Ludwig and Reynolds (1988) for mathematical details.

Direct gradient analysis is used where lichen abundances, probabilities of occurrence, or morphological or physiological symptoms are described as a function of measured environmental variables. Each sample can be associated with its elevation, soil pH, on-site air chemistry measurements, or long-term exposures to pollutants as given, for example, by air quality maps. Measured attributes of lichens or other cryptogamic plants are plotted along an elevation, soil pH, or pollution loading axis. The relationship between the lichen variable and the environmental quantity can be determined by regression.

Gradient analysis is also applied by using community composition as a measure of environmental values when the regressions are known. Ter Braak and Prentice refer to this as a calibration problem. If environmental variables are not measured, one can use information from the species assemblage to compute abstract axes that account for much of the variation in species composition. This is indirect gradient analysis, an ordination problem according to Ter Braak and Prentice. Ludwig and Reynolds (1988) define ordination as "a set of techniques in which sample units are arranged in relation to one or more coordinate axes such that their relative positions to the axes and to each other provide maximum information about their ecological similarities". Indirect gradient analysis is most useful when lichens of known sensitivities to air pollutants are present. The method may be less effective when subtle pollution effects are likely to be overwhelmed by effects of other environmental variables.

Gradient analysis is widely employed by ecologists. A variety of multivariate software packages are available, such as ORDIFLEX, PC_ORD, DECORANA, and CANOCO (Gauch 1977, Hill 1979, Ter Braak 1988). Which of the various methods to use on a particular data set, and interpretations derived from the various multivariate analyses, are discussed by Ter Braak and Prentice (1988) and Ludwig and Reynolds (1988).

ANALYTICAL TECHNIQUES

Many analytical techniques are available for examining the effects of air pollutants on lichens. Which techniques are appropriate for a given study will depend on the type and levels of pollutants involved, the lichen species used, the nature of the research (gradient studies as opposed to fumigation work, for example) and the resources available.

The analytical techniques used most often are listed below. These are generally applicable to both field and laboratory work, unless otherwise indicated. Using any of these techniques will require additional literature research to determine their suitability for the proposed work, instrumentation needed, specific procedural protocols and other recommendations, including quality assurance, quality control, levels of injury and levels of exposure.

Characteristics measured in air pollution studies include individual morphological and physiological characteristics and population characteristics. Specific limitations and/or advantages of each technique are noted here.

Morphology

Morphological analyses detect change to the form or structure of an organism. A disadvantage to morphological analyses is that not all pollution injury will produce a response that can be distinguished visibly. Morphological changes can be analyzed macroscopically, without optical aids, or microscopically.

Macroscopic

This is the unaided visual assessment of lichen characteristics such as coloration, size, and appearance of the species chosen. An advantage to macroscopic analyses is that they can be done with limited instrumentation and funds. These types of analyses can also be done with still or video cameras. Comparisons can be made before and after treatments, throughout time at the same sites, and/or along a distance gradient from a known pollution source.

Microscopic

Microscopic analyses allow for observation of cellular injuries that may or may not be otherwise visible. Two types of microscopes are used in analysis of injury assessment in lichens: light microscopes and transmission electron microscopes. Light microscopy has been useful in estimating the effects of pollutant stress, especially of SO_2 on algal cells. Eversman (1978) and Will-Wolf (1980) estimated the percentage of plasmolyzed algal cells in fresh mounts of lichens exposed to SO_2, showing that the percentage of plasmolysis increased significantly with increasing exposure time. Observations of fresh mounts use color and shape of chloroplasts, which comprise most of the algal cell, to estimate the algae's health. Healthy cells are green and round; plasmolyzed cells are yellowed and shrunken. Kauppi (1980) demonstrated the usefulness of fluorescence microscopy in determining the status of algal cells.

An alternative method to determine dead and plasmolyzed algal cells uses fixed and embedded sections of lichens stained with toluidine blue (Holopainen and Kauppi 1989). Empty cells are

interpreted as dead; cells with shrunken contents are considered plasmolyzed. Again, increased plasmolysis was observed with SO_2 fumigation.

Some cells are too small to accurately observe with light microscopy. Transmission electron microscopy is a helpful tool to visualize or verify cellular effects when physiological effects have been observed. Many characteristics of chloroplasts and mitochondria change in intermediate stages of injury; severe injury results in near collapse of all recognizable cell structure (Eversman and Sigal, 1984, 1987; Holopainen and Kauppi 1989). Some specific changes recorded are: deformation of thylakoids and pyrenoglobuli of the chloroplast, swelling followed by degeneration of mitochondria, unusual accumulation of starch granules and lipid bodies, and disappearance of recognizable organelles and membrane structures.

Since cellular changes due to pollutant stress can mimic ordinary seasonal stress or normal senescence, it is important to have fresh material and to control conditions under which samples are stored before fixation so that moisture and light conditions of the control and treated samples have been identical. Unfortunately, all pollutants seem to cause the same ultrastructural changes in algal cells so one cannot distinguish among pollutants. It does seem, however, that the oxidant pollutants such as O_3 and PAN cause cellular damage at lower concentrations and in less time than SO_2 and acid treatments (Eversman and Sigal 1987, unpublished data).

Physiology

Physiological analyses detect alteration to the normal functioning of an organism or any of its parts. There are many techniques available to determine physiological changes resulting from air pollution; the more commonly used ones are listed here. All require some level of instrumentation. Most are sensitive to seasonal or geographical variation, which should be taken into account when sampling. It is important to recognize that lack of homogeneity within a lichen thallus has been documented for various characteristics (e.g., photosynthesis, nitrogenase activity, chlorophyll concentrations), and care must be taken if experimental material does not include the entire thallus (Karenlampi 1970, Nash et al. 1980, Moser and Nash 1978, Moser et al. 1983).

Pigments

Chlorophyll and its degradation products are the pigments most often used to assess pollution injury. Studies have shown chlorophyll levels to be significantly affected by pollutants (Beekley and Hoffman 1981, Belnap and Harper 1990, Garty et. al. 1985, Henriksson and Pearson 1981, Kardish et. al. 1987, LeBlanc and Rao 1973, Nash 1973, Nash 1976, Ronen and Galun 1984, Eversman 1980, Moser et al. 1980). Researchers have traditionally reported effects on chlorophyll as total chlorophyll concentrations, percent reduction in chlorophyll concentrations, chlorophyll a:b ratios, or the ratio of chlorophyll to its degradation products.

There are several means of quantifying chlorophyll and degradation products. The more commonly used method is extraction of these pigments in dimethyl sulfoxide (DMSO). Extracts are then spectrophotometrically scanned to determine levels of chlorophyll at optical density 435 nm and degradation products at optical density 415 nm (Ronen and Galun 1984). It is important to reduce chlorophyll degradation during sample analysis; techniques to do so include the use of red light during sample preparation, cold storage, and standardization of time between extractions and analysis. For further details on these techniques, see Brown (1980), Brown and Hooker (1977) and Vernon (1960).

Nitrogenase Activity

Lichens with cyanobacterial phycobionts are capable of "fixing" atmospheric nitrogen, converting it into a form of nitrogen usable by vascular plants. These lichens may be important contributors of nitrogen to the ecosystems in which they occur (West and Skujins 1978, Evans and Ehlringer unpublished data, Denison 1973). Atmospheric pollutants have been shown to affect nitrogenase activity levels (Belnap 1990, Denison et. al. 1977, Hallbom and Bergman 1979, Hallgren and Huss 1975, Henriksson and Pearson 1981, Kallio and Varheenman 1974, Sheridan 1979, Sigal and Johnston 1986).

Measurement of nitrogen fixation is difficult and costly. Measuring nitrogenase activity, however, is fairly simple and cheap. In the presence of the nitrogenase enzyme, acetylene is converted to ethylene. Consequently, nitrogenase activity levels are reflected in the amount of ethylene that is produced. Levels of ethylene and acetylene can be measured on a gas chromatograph. Incubation in an acetylene atmosphere can be done in the field or the laboratory, using gas-tight containers with an

atmosphere of approximately 10% acetylene under standard conditions of light and temperature in the lab, or ambient conditions in the field (see above references). In the field, portable chambers can be placed over the experimental material, and vacutainers can be used to transport samples (Rychert and Skujins 1974).

Respiration (Gas Exchange)

A significant decrease in respiration rates of lichens exposed to increasing pollutant levels has been demonstrated repeatedly in the literature (Baddeley et al. 1971, Eversman 1978, 1979, 1980; Fields and St. Clair 1984a, 1984b). The most common methods of measuring respiration rates employ an oxygen electrode to measure O_2 absorption or an infrared gas analyzer to measure CO_2 evolution in the absence of light.

Photosynthesis

Photosynthetic rates have been determined using chambers and gaseous $^{14}CO_2$ (Fields and St. Clair 1984a, Hallgren and Huss 1975, Ross and Nash 1983, Sigal and Johnston 1986, Lechowicz 1982), immersion in liquid $^{14}CO_2$ (Fields and St. Clair 1984a) or measured as the rate of CO_2 absorption or O_2 evolution (Beekley and Hoffman 1981). Determining net gas exchange in either flow-through or closed chamber systems depends on the availability of an infrared gas analyzer (IRGA), while estimating gross photosynthesis with $^{14}CO_2$ labeling techniques requires a liquid scintillation counter.

Chlorophyll fluorescence is a set of response variables associated with the light reactions of photosynthesis, particularly those associated with photosystem II. It is a convenient, sensitive, rapid, and non-invasive way to assess photosynthetic efficiency. The fluorescent behavior of photosynthetic systems most commonly analyzed is the kinetics of the fluorescence rise to maximum levels at saturating light. This induction curve is markedly altered after exposure to air pollutants. Fluorescence is measured using a chlorophyll fluorometer, or a modified gas exchange system (T. Nash, personal communication).

All methods which measure photosynthesis in lichens have found decreased photosynthetic rates with exposure to pollutants. Determining which methods to use, (instrumentation required, replication and portability for field work) is discussed in Link et al. (1984).

Membrane Integrity

Exposure to sulfur dioxide and trace metals has been shown to compromise membrane integrity in lichens. This has been demonstrated using measurements of potassium efflux, total electrolyte leakage, and scanning electron microscope work (Beckerson and Hofstra 1980, Hart et al. 1988, Pearson 1985, Pearson and Rodgers 1982, Belnap and Harper 1990, Fields and St. Clair 1984b, Moser et al. 1983). Membrane leakage can be estimated by measuring the conductivity of de-ionized water before and after immersion of the lichen. Instrumentation is limited to a conductivity meter, which is both cheap and portable.

Elemental Analysis

This is covered in Chapter 7.

Population Characteristics

These are species characteristics which can be measured in the field and require little or no instrumentation. Measurements are made using quadrats as samples. Variables such as reproductive characteristics, growth rates, mortality, presence/absence, cover, and biomass can be measured within quadrats. These measurements can be done by eye (Daubenmire 1959; Armstrong 1991; Karenlampi 1970, 1971) or by photography. Photography, whether done with a traditional camera or with video, can be computer-analyzed. Details are provided in Chapter 4. Table 1 provides an example of studies done with different pollutants and the effects of those pollutants on chosen lichen species.

EXPERIMENTAL DESIGN

The experimental design of studies addressing the sensitivity of lichen species to air pollutants should be similar to any research design. The choice of method should be determined by the nature of the problem, the questions to be answered, the anticipated use of the data, site characteristics and available resources. First, decide upon a set of broad initial hypotheses or working questions. Then do a literature search to determine the lichen species in the targeted area, the sensitivity of these species to the air pollutants in question, and other related work. Related research might include work with the same species in the same locality or with the same pollutants, and/or using the same analytical techniques.

Table 1. — Selected authors and papers describing effects of various pollutants on lichens. Source: Eversman and Sigal (1985).

Pollutant	Effect(s) observed
Sulfur dioxide	
Baddeley et al., 1973	Respiration decreased
Puckett et al., 1973, 1977	Membrane permeability increased
Tomassini et al. 1977	Permeability increased (ions lost)
Richardson & Nieboer, 1983	Review: physiology, ecology
Fields & St. Clair, 1984	Permeability increased, photosynthesis decreased
Holopainen & Karenlampi, 1984	Ultrastructural changes
Ozone	
Brown & Smimoff, 1978	No effect on photosynthesis
Rosentreter & Ahmadjian, 1977	No effect on chlorophyll content
Nash & Sigal, 1979	Photosynthesis decreased
Ross & Nash, 1983	Photosynthesis decreased
Sigal & Nash, 1983	Distribution limited, morphological changes
Eversman & Sigal, unpub. ms.	Ultrastructural changes, photosynthesis decreased
PAN (peroxyacetyl nitrate)	
Sigal & Taylor, 1979	Photosynthesis decreased
Eversman & Sigal, 1984	Ultrastructural changes
NOx	
Nash, 1976	Loss of chlorophyll pigment
Fluoride	
Nash, 1971	Loss of chlorophyll pigment
LeBlanc, Comeau, Rao, 1971	Plasmolysis, color change, loss of chlorophyll pigment
LeBlanc, Rao,Comeau, 1972	Species die, disappear
Roberts & Thompson, 1980	Discoloration, morphological changes; species disappear
Takala, Kauranen, Olkkonen, 1978	Morphological changes; species disappear
Acid precipitation	
Denison et al., 1977	Nitrogen fixation decreased
Robitaille, LeBlanc, Rao, 1977	Loss of species
Lechowicz, 1982	Photosynthesis decreased
Lechowicz, 1984	Growth decreased
Sigal & Johnston, 1986	Photosynthesis decrease
Sigal & Johnston, 1986	Photosynthesis, nitrogen fixation decrease
Eversman & Sigal, unpub. ms.	Ultrastructural changes

Available resources should be identified. Resources include people, dollars, time, equipment, transportation, access to study areas, collaboration possibilities, and any other factor that may influence your capability to carry out the intended research.

From the collected information, develop a set of refined hypotheses. These hypotheses are a distillation of the initial broad hypotheses into questions that are feasibly answerable given available resources.

Sample design is the next stage. Sample design will vary greatly depending on geography, accessibility, species used, analytical techniques chosen, and resource availability. Elements common to most study designs include:

Species Selection

The species selected for study will depend on the questions being asked, the types of pollutants and species present, and available resources. Make a list of likely candidates from an inventory of available species and species known to be sensitive to the pollutants in question. Select species from the list based on the questions being asked and available resources. Another option is to run preliminary analyses on species that are selected for reasons other than known sensitivity, such as abundance or ease of collection, to ascertain whether they are suitably sensitive to the pollutants present.

Gradient Sampling

Gradient sampling can vary from survey data to more elaborate vegetation sampling techniques (Chapter 4). Lichens respond to natural gradients of moisture, temperature, light, nutrients, and biotic competition as well as to anthropogenic influences. The response of lichens to more direct natural or anthropogenic influences may overwhelm the subtle effects of pollution (Jackson and Gough 1990). Any secondary effect of pollution upon lichens is likely to be confounded with the influence of other variables. To minimize effects of confounding factors, we recommend a sample design which incorporates known pollution levels.

If the pollution source is local and fixed and the pollution "shadow" is known, sampling should be spatially explicit with respect to the source. Considerable environmental variation will still remain, which again may overwhelm possible secondary influences of the pollutant. Therefore, use a sample design that limits other sources of variation as much as possible. This can be done by narrowing the range of soil, topographic, grazing, or other variables across the widest range of pollutant loadings.

Direct measurements of pollution loadings should be obtained at as many of the sample sites as feasible. If only a few direct measurements can be obtained, they should be concentrated at places where the highest and lowest pollutant levels occur, with a few near the middle (to test for linearity). If no direct measurements of pollutant loadings are possible, then an attempt to measure pollution indirectly can be made by using known sensitive or accumulator species. This is the calibration problem referred to above. Samples of the accumulator species can be harvested for chemical analyses (Chapter 7). These samples should also be spread across the gradient, or if this is not practical, then near the high, low, and middle portions of the gradient.

If the pollution source is regional or non-local, we suggest using gradsect sampling (Austin and Heyligers 1989). This is a method of efficient sampling across the most important environmental features that control species distributions in a region. The concern is not so much with total floristic variation across a region as with floristic variation as affected by pollution. Therefore, gradsect sampling can be limited to one or several regionwide communities with known sensitive or accumulator species. If sensitive or accumulator species are unknown, sampling sites can be established across a wide range of cryptobiotic communities in different airsheds as discussed in Chapter 4. Particular attention should be given to the substrate and microclimate of the sample communities, since these may have a large effect on cryptobiotic species (Northrup Environmental Sciences 1987, Hawksworth and Rose 1976, McCune et al. 1987).

Sample Size

Sample size includes both the number of samples per site and the number of sites. Sample size depends on the variability of the characteristics being measured and the degree of confidence with which comparisons between samples can be made. Data from a pilot study are useful in determining variability and then computing adequate sample sizes to detect possible changes (testing null hypotheses).

Repeatability

Repeatability influences the timing of sampling and site selection. Factors to consider are the phenology of the organism, variability in pollutant levels, physiological condition of the organism, slope, aspect and substrate of sites, distance from influencing factors such as large bodies of water, roads, etc., and any other factors that may affect study results. Confounding variables that cannot be eliminated need to be identified.

Plot Protocol

Plot protocol determines the way in which current or future samples are taken. Permanent plots in which no destructive sampling takes place must be distinguished from temporary plots from which material is collected. Designation of sampling areas within temporary plots may be desirable so that the location of disturbance is known. Certain types of activities may need to be restricted at sites to prevent unwanted influences on materials. For example, indirect trampling may affect soil lichens. Methods for marking and mapping plots must be identified. Current options for establishing coordinates for study sites include topographic maps and global positioning systems.

Collection Techniques and Material Treatment

Collection techniques and handling, transportation, and storage of collected material depend on the end use of the material. In addition, requirements for different analytical procedures must be kept in mind. If fumigation work is to be done, pre-treatment protocol and the physiological condition of the material before and after experimental procedures must be considered.

Comparison with Past, Current, and Future Techniques

It is desirable to design studies so that results can be compared with past and current studies. To facilitate valid comparisons with future studies, field and analytical techniques should be kept as simple as possible. If new techniques are employed, find ways to compare data using statistical calibration and design studies with this in mind. Consider desired future studies and whether resources required for the current study will be available in the future. Create a monitoring manual with instructions for future monitoring personnel. Monitoring systems commonly fail because sites are lost or different analytical techniques are used that cannot be calibrated to previous techniques.

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