Identification of Pollutant Sensitive Species and Assessment of the Variability of Air Pollution Injury on Flora in National Park Units

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ABSTRACT

The National Park Service (NPS) has instituted a program of air pollution research and monitoring to identify pollutant-sensitive species and the nature and magnitude of the biological responses to air pollutants. Pollutant-sensitive species become the focus of surveys and long-term monitoring plots to evaluate spatial and temporal trends in injury and determine the ecological ramifications of pollutant injury. Studies in the eastern and western forests in the United States have primarily focused on the effects of chronic ozone pollution and to a lessor degree on the effects of sulfur oxides and toxic metals from point sources.

This paper discusses methods and results of controlled ozone fumigations in Great Smoky Mountains National Park (GRSM) and Sequoia Kings Canyon National Parks (SEKI). In GRSM species were found to be sensitive to ozone below the National Ambient Air Quality Standards (NAAQS). In Sequoia and Kings Canyon NP procedurally similar fumigations have indicated that the seedling age class of giant sequoias (Sequoiadendron giganteum Buchh.) displays visible foliar injury symptoms when exposed to ambient concentrations of ozone. Significant increases in foliar injury, negative physiological effects, and reductions in growth of roots and shoots occurred in treatments of ambient plus 50% ozone. The duration of direct sunlight in the chambers was shown to affect the response. Foliar injury symptoms, macroscopically and microscopically similar to symptoms observed in the chambers, were observed on sequoia seedlings in the field under conditions of reduced durations of direct sunlight and adequate soil moisture.

This paper also discusses field research on the ecophysiology and variability of the response of Western conifers (primarily ponderosa and Jeffrey pines) to chronic ozone injury and the best methods of evaluating the injury. Ecophysiology studies examined chronic ozone effects in situ on the photosynthesis, respiration, and stomatal conductance of Jeffrey pine. Variability studies examined the variability of ozone injury between trees, within stands and the variability of injury within trees. Studies were directed to give information on the number of trees, crowns, and branches that

should be evaluated in surveys and plots. Information obtained in these and other studies were used to construct an additive injury index to summarize pollutant impacts to western conifers.

The NPS has used diverse methods for selecting and locating sites and plots for determining the spatial and temporal variability of sulfur and toxic metal pollution in NPS units. These methods include barbell, grid, and transect sampling, establishment of long-term monitoring plots, and the use of Geographical Information Systems (GIS) and Loran and Geographical Positioning Systems (GPS) land navigation systems.

INTRODUCTION

The Air Quality Division (AQD) of the National Park Service (NPS) (a Bureau of the Department of the Interior of the United States government) is composed of a Research Branch (RB), a Monitoring and Data Analyses Branch (MDAB), and a Policy, Planning and Permit Review Branch (PPPRB). The AQD is mandated under Federal Law (Organic Act, 1916; Clean Air Act Amendments, 1977, 1990) to protect Air Quality Related Values (AQRVs) in areas under NPS jurisdiction from degradation from anthropogenic air pollutants. AQRVs include biological and cultural resources, visibility (day and night), odor, and air that meets Federal health standards for humans. Recognizing that the protection of park resources sensitive to air pollution relies primarily on the actions of state and federal regulatory agencies, the AQD has committed to acquiring information needed to participate in and influence decision making that may affect park air quality. Activities include inventorying park resources that may be sensitive to air pollution, monitoring the spatial and temporal trends of identified pollution effects, determining the significance of the effects, and identifying the sources of the pollutants causing the impact. Data collection and synthesis by the RB and MDAB are based on information needs of the PPPRB.

The RB of the AQD has developed a Biological Effects Program (BEP) that employs diverse methods to assess the impacts of gaseous and particulate pollutants on the biological resources in terrestrial ecosystems under NPS jurisdiction. The BEP approach is to identify species, genotypes, or age classes of species that are sensitive to ambient concentrations of specific air pollutants through controlled exposure studies (fumigations) and field gradient studies. The nature and magnitude of the biological responses to air pollutants of the sensitive species are then determined (microscopic and macroscopic foliar injury, and phenological, physiological, and growth alterations). Pollutant-sensitive species become the focal point for studies assessing the variability in biological responses in the field, for assessing dose-response relationships in the field, and for assessing short-term (in surveys) or long-term (in trend or ecological plots) effects of air pollutants.

Determinations of the nature and extent of air pollution injury initially considers the nature of the pollutant of concern. Ozone and other oxidants leave no readily discernible elemental signature and the pollutant signatures for these toxins are the biological responses of the affected individuals.

These biological responses consist of macroscopic and microscopic foliar injury symptoms that are evaluated by crown assessments and are correlated with diverse secondary pollutant signatures (increased rates of needle and branch senescence and abscission, altered physiological and nutrient cycles, and altered phenological cycles). For other phytotoxic air pollutants such as sulfur oxides, fluorides, or metals the pollutant signature consists of morphological response measured through crown assessments and an elemental response through accumulation of elements unique to the pollutant(s). Evaluation of the effects of particulate pollutants (e.g., sulfur oxides and toxic metals) on coniferous forests have been performed using variability studies (barbell sampling) (Gough and Severson, 1981; Tidball and Ebens, 1976), grid sampling designs (Jackson et al., 1990), geometric radial transects (Crock et al., 1990), and highly stratified random sampling (Duriscoe and Stitt, 1988) using GIS.

MATERIALS AND METHODS

Identification of Ecological Indicators

The Biological Effects Program of the AQD conducts controlled exposure studies (fumigations) under natural environmental conditions to determine the sensitivity of native plant species to gaseous air pollutants. These controlled studies characterize and quantify the macroscopic, microscopic, physiological, phenological, and growth responses of pollutant-sensitive species. Fumigations are conducted in open-top chambers or branch chambers in selected NPS units using the daily ambient profile of the air pollutant(s) of concern as a base treatment. Manual or computer tracking of ambient air concentrations (ambient treatment) is used to construct non-ambient pollutant profiles (treatments) that are typically sub-ambient (pollutant-filtered control), ambient air plus 50% addition of the pollutant, ambient air plus 100% addition of the pollutant, and an open plot (non-chamber control to evaluate chamber effects). Each treatment is replicated three times.

For example, controlled *in situ* exposures (open-top chamber fumigations) were conducted at the Uplands Research Laboratory in Great Smoky Mountains NP, Tennessee, U.S.A. from approximately June through mid-September in 1988, 1989, and 1990. Twenty-three species of forest plants native to GRSM were selected for fumigation based on their importance in park ecosystems, threatened and endangered status, or because the nature of foliar injury symptoms observed in the field was consistent with symptom expression due to exposure to ozone observed in other air pollution studies, or similar to symptoms observed on species in fumigation chambers. The species were ranked for sensitivity in the following categories: sensitive - visible foliar injury symptoms observed in ambient treatments; intermediate - symptoms observed in 1.5X ambient treatments; and tolerant - symptoms observed only in 2.0X ambient treatments or not observed at all. Detailed foliar injury responses (type and severity of visible injury) were recorded for the species. Similar fumigation studies were conducted in SEKI on the seedling (from 1986-1988) (Miller et al., 1989b) and sapling (Grulke et al., 1989) age classes of giant sequoias. In addition, multiple species fumigations were initiated in Acadia NP in 1990 on a variety of tree, shrub, and herb species (Eckert, 1989).

Since species are screened for sensitivity to ambient pollutant profiles under environmental conditions representative of the species natural habitat, they are considered to be ecological indicators for a Park(s) if they exhibit a biological response that is statistically significant from the control (ambient-minus) treatment. Ecological indicators are species that are the pollutant-sensitive components of the ecosystem and are expected to be the initial indicators of pollution stress in the ecosystem. They are used by the AQD to assess whether existing, or past deposition of air pollutants have caused alterations in the normal biological, physical, or chemical processes of resources in NPS units, and to estimate potential impacts of increased pollutant levels. The AQD also uses pollutant sensitive indicators that have been identified in the air pollution literature or effects data bases and which are found in the vascular (NPFLORA) floras that have been determined for many park units.

Biological response variables that have been measured in fumigation studies include histological (microscopic) (Evans, 1989), morphological (visible; macroscopic) (Miller et al., 1989b; Neufeld, 1990), spectral (Rock et al., 1988), physiological (photosynthesis, respiration, water, carbon dioxide, and nutrient use) (Grulke et al., 1989), and alterations in carbon allocation (root, shoot, foliage growth) (Miller et al., 1989; Neufeld, 1990). The most distinct pollutant responses observed in the ambient treatments are the standards for surveys and plots for pollutant injury determinations in the field (Miller et al., 1989b; Neufeld, 1990; Eckert, 1989). Pollutant responses observed in ambient-plus treatments (particularly ambient plus 50% increase in ozone) may be considered representative of the responses that occur in portions of the park units where environmental factors render the species more susceptible, in other areas of the park where pollutant levels are higher, in other years when pollutant levels may be higher, or in other parks where pollutant levels may be higher.

<u>Variability in the Response of Ecological Indicators</u>

The variability in pollutant response of the sensitive ponderosa and Jeffrey pine species at various levels of biological organization (needles, whorls, branches, crowns, trees, and stands) was determined in variability studies in and near SEKI. The objectives were to improve the methodology for evaluating individual tree injury, correlation of pollution injury with reductions in tree vigor and growth, performance of cruise surveys, and establishment of long-term monitoring plots. Studies were conducted to compare the variability of foliar injury on branches in the lower crowns of these pine species, the difference between the upper and lower crown response variables (visible foliar injury, needle length, number of whorls, percent fascicle retention per whorl, severity of biotic and non-pollution abiotic injury), and optical versus hands-on evaluation of foliar injury (two different types of binoculars and 1 spotting scope) (Muir and Armentano, 1987). Both upper and lower crowns of ten trees were evaluated by hands-on scoring of the foliage.

Injury variability within stands of ponderosa and Jeffrey pine trees was determined by Duriscoe (1989) to estimate the number of trees that needed to be evaluated within stands to differentiate stands as slightly, moderately, or severely injured. He evaluated the crown condition of 50 trees per stand in three stands each having slight, moderate, or severe crown injury due to ozone (based on the incidence and severity of injury within the stand).

The physiological responses of conifers in situ exposed to fluctuating ambient levels of ozone were evaluated to improve injury rating systems for conifers and to better understand the environmental, biological, and physical factors that influence species and individual responses to ozone. Differences in photosynthesis, respiration, water and carbon use efficiency, stomatal conductance, and nutrient status were evaluated for different age classes of needles of Jeffrey pine saplings (Patterson and Rundel, 1989) and giant sequoia seedlings, saplings, and monarchs (Grulke, 1990) using infrared gas analyzers (IRGAs) and standard laboratory elemental analysis techniques.

Monitoring of Gaseous Pollutant Impacts on Ecological Indicators

Methods for quantifying the severity of pollution injury on coniferous trees were evaluated in a multiagency workshop (DOI NPS, USDA Forest Service, California Air Resources Board, US Environmental Protection Agency (EPA)-Region 9) in March 1989. Indices to evaluate injury on individual trees that were reviewed included: an additive index that consisted of foliar injury, whorl retention, needle length, and branch mortality (Miller, 1977); an injury class index (Pronos et al., 1978); a non-additive foliar index that consisted of percentage chlorotic mottle severity, fascicle retention per whorl, whorl retention per branch, and needle length (Stolte and Bennett, 1984); and an additive injury index that included class of chlorotic mottle severity, needle length, number of whorls per branch retained, and percent live crown ratio (Duriscoe, 1989). The consensus from the workshop was to develop a comprehensive survey and plot method, named the Western Pine Method (WPM), that includes an additive index (modified from Duriscoe, 1989) to describe the severity of ozone injury on individual trees (Arbaugh et al., 1990) and also includes other parameters to evaluate pollution impacts to conifer stands in the western United States. Additional descriptions for the WPM survey and plot methods are given in Stolte and Miller (1990).

Gaseous Pollutant and Precipitation Monitoring Data

Ozone pollutant monitoring data were recorded hourly in GRSM (1988-1989) and SEKI (1984-1990) by the MDAB of the AQD according to EPA guidelines (NPS, 1990). Ozone data consisted of summarized hourly maxima, daily and monthly means for different averaging times, and cumulative dose exceeding selected concentrations (60, 80, 100, 120 parts per billion). In GRSM, ambient ozone data was not available (not yet analyzed) from the Uplands Lab fumigation site, but ambient ozone data from a nearby pollutant monitoring site in GRSM (Look Rock) was available for evaluating effects on plants in chambers at the lab (ozone at Look Rock is estimated to be about 50-100% higher than at the Uplands site) (Neufeld, 1990). In SEKI the ozone data collected at the Lower

Kaweah monitoring site (elevation approximately 6000 feet) from 1984 - 1990 (June - October for 1984 and 1985 and every month starting in July 1986) was complemented by precipitation monitoring data.

Monitoring of Toxic Element Impacts on Ecological Indicators

Grid sampling techniques were used to determine the toxic element concentrations in plants and soils. Sample sites were selected within defined geographical areas by overlaying the sample population with grids of various sizes (based on known or estimated variability of the element(s) of interest and available financial resources). Grid sampling insures adequate spatial resolution of elemental concentrations and allows construction of elemental contour maps (Jackson et al., 1990). The most efficient grid size for each element can be determined by evaluating the spatial variability of each element of concern using a barbell sampling technique (Tidball and Ebens, 1976; Gough and Severson, 1981). Barbells are composed of randomly oriented axes of varying linear distances. Analyses of barbell samples involves the determination of the highest degree of variability at each different spatial scale using a nested analysis of variance test of the elemental concentrations (Severson et al., 1990; Jackson et al., 1990; Armentano, pers. comm., 1990). Adequate grid sizes can be determined by identifying spatial scales that have the highest degree of variability. In Everglades NP (EVER) grid size was based on the number of sites that could be sampled with limited resources. In these studies spatial element variability is evaluated post priori.

Another method of determining toxic element impacts on resources is by using radial transects around point sources. Radial transects can consist of sites located at geometric (e.g. 1, 2, 4, 8, 16, etc. km) or arithmetic distances from a point source (Severson et al., 1990; Crock et al., 1990) . The geometric sampling design is based on the log linear accumulation of elements typically found in biological receptors around point sources (Severson et al., 1990).

An initial step in elemental surveys and plots is to determine the geographical distribution of pollutant sensitive species (target ecological indicator species) throughout a park unit of concern from vegetation maps or GIS. For example, in North Cascades NP (NOCA) a GIS was used to produce a stratified random sampling design that initially identified the target populations then further stratified by drainage basin, elevation, slope, aspect, and canopy closure (Duriscoe and Stitt, 1988). This method is particularly useful when financial resources and/or very rough terrain (slopes frequently in excess of 60%) limit the number of plots that can be established. In other studies, sample sites were located systematically using a grid or transect sample design based on known or estimated population variability (Gough and Severson, 1981; Tidball and Ebens, 1976; Gough et al., 1990; Severson et al., 1990). Latitude and longitude or Universal Transverse Mercator (UTM) coordinates were obtained and mapped on 1:250,000 or 1:64,000 US Geological Survey topographic maps. In areas of relatively flat terrain or in remote forest locations, land navigation systems (Loran and Geographical Positioning Systems) were used to locate sample points and record plot coordinates after the plot had been established.

RESULTS

Identification of Ecological Indicators

Fumigation studies performed *in situ* in National Parks have established the relative gaseous pollutant sensitivity of forest tree, shrub, and herb species. Controlled ambient-based exposures conducted in GRSM from 1988 - 1990 showed that of the 23 species evaluated for sensitivity to ozone (based on visible foliar injury), 12 were considered sensitive (visible foliar ozone injury in ambient treatments), 7 intermediate (visible foliar injury in ambient plus 50% treatments), and 4 resistant (visible foliar injury only at ambient plus 100% or no visible foliar injury) (**Table 1.a.**). The most common foliar injury symptom was a dark stipple of the upper leaf surface. Symptoms observed in the ambient treatment chambers were macroscopically similar in appearance to symptoms observed on sensitive genotypes of species in the field. Sweetgum and winged-sumac had significant increases in foliar injury at the elevated ozone treatment levels but no effects on biomass were observed. Ambient pollutant monitoring data from the nearby Look Rock monitoring site indicates that ambient ozone in GRSM in 1988 and 1989 was relatively moderate (**Table 1.b.**), with few hourly averages exceeding 80 ppb ozone in 1988 and 1989 (42 and 56 hours exceeding 80 ppb, respectively). Hourly averages exceeding 100 ppb ozone in 1988 and 1989 were 9 and 0, respectively. The highest daily one-hour maximum of ozone was 112 ppb in 1988 and 98 ppb in 1989 (NPS, 1990).

Miller et al. (1989b) fumigated emergent giant sequoia seedlings in Sequoia National Park from 1986-1988 and found that emergent seedlings were a relatively sensitive age class of this species to ozone. Ozone concentrations in 1988 at Highlands (site of the fumigation) typically ranged between 50 to 100 ppb, with 120 ppb hourly averages observed six times during the 1988 fumigation period. The authors observed distinct visible foliar injury symptoms (chlorotic mottle/banding, necrosis, and purple-red discoloration) in ambient and ambient-plus ozone treatments (**Figure 1.a.**). In treatments of ambient plus 50% ozone, Grulke et al. (1989) found significant alteration of photosynthetic processes of seedlings and rooted sequoia saplings, and Miller et al. (1989b) found significant reductions in growth of roots and shoots of seedlings. Low soil moisture and durations of direct sunlight exceeding two hours were shown to reduce injury. The foliar injury symptoms observed on post-fire emergent seedlings in sequoia groves in 1988 were macroscopically similar to symptoms observed on fumigated seedlings and were found in locations with similar conditions of short durations of direct sunlight (**Figure 1.b.**) and high soil moisture (**Figure 1.c.**). Evans (1989) found that the injury patterns on seedlings from the chamber fumigations and the field were histologically (microscopically) similar. The mesophyll cells of the needle-like leaves of the sequoia seedlings were the most sensitive to ozone, with cellular symptoms of plasmolysis, amorphous staining, and necrosis that generally increased with increasing ozone dose.

Research and Application of Ecological Indicators

Muir and Armentano (1987) found that the number of branches that needed to be evaluated for different crown response variables on ponderosa pines to detect a 10% difference between tree means were: other abiotic and biotic injury (7 branches), chlorotic mottle (8 branches), needle length to 3 cm (11 branches), and needle retention within whorls (42 branches). In comparisons of injury between the upper and lower crowns of 10 trees, they found percent chlorotic mottle, fascicle retention per whorl, and number of whorls were not significantly different between the upper and lower crowns, but needle length, percent other injury, and percent necrosis were different. Remote observations (optical instruments) of the lower and upper crowns were not always an accurate representation of the amount of pollution injury (based on comparisons with foliage evaluated in-hand). The coefficient of determination was $r^2 \leq .31$ for injury in the upper tree crowns and $r^2 \leq .66$ for injury in the lower crowns. Optical instruments tended to overestimate chlorosis and whorl retention when injury was low and underestimate these two variables when injury levels were high. The hands-on injury evaluations of two experienced observers (over 20 years experience each) were not different for severity of chlorotic mottle, needle necrosis, other injury (biotic and abiotic), and percent needle retention per whorl. Needle length determinations were significantly different. These studies provided information that allowed the AQD to evaluate the effectiveness of crown assessments, and suggested efficient and more precise improvements for quantifying individual tree injury in surveys and plots.

Duriscoe (1989) found that the number of trees required to differentiate three classes of stand injury (slight, moderate, and severe classifications based on the severity and incidence of injury in each stand) and most accurately assess the injury level in any one stand varied according to the severity of injury in the stand (**Figure 2**). Sample sizes of at least 30 trees were determined to be a minimum necessary to differentiate injury ($p \leq .05$) between stands or within any stand over time (Duriscoe, 1989). Variability in plot injury was highest in stands where the severity of injury to individual trees was the highest and the incidence of injury was high. In general, when the incidence of injury at any level of biological organization is 50%, e.g., stands of trees with 50% of the individuals injured, a larger sample is needed to detect differences between stands. The number of trees that need to be sampled depends on the desired level of precision (**Figure 3**) (Duriscoe, 1989; Muir and Armentano, 1987).

Field studies on the physiological responses of Jeffrey pine trees in SEKI indicated that all whorls of needles evaluated on resistant (asymptomatic) and sensitive (symptomatic) genotypes were found to be significant contributors of fixed carbon (Patterson and Rundel, 1989) (**Figure 4.a. and b.**). Chlorotic mottle symptoms on remaining needles of ozone-sensitive genotypes of Jeffrey pine were shown to decrease the amount of carbon fixed (**Figure 4.c.**), increase respiration, and alter water and carbon dioxide use efficiency (Patterson, 1990). This information was used to improve the rating of air pollution stress on pines by reflecting the decreases in photosynthesis that accompanied needle loss and symptom expression on remaining needles. These studies also indicated that photosynthesis of Jeffrey pines was reduced in August due to seasonal summer droughts (**Figure 4.a. and b.**), when ozone concentrations are the highest, and the trees are afforded a photosynthetic recovery period during the relatively mild winter days in the Sierra Nevada when ozone is lowest. For example, in SEKI from 1984-1990 there were an average of 7.1 hours each day in August when hourly ozone concentrations were $\geq$ 80 ppb, as contrasted to the October to May period (1986-1990) when the average maximum number of hours of hourly ozone averages $\geq$ 80 ppb was 1.1 (NPS, 1990).

<u>Western Pine Method</u>

The studies referenced above on the sensitivity and variability in response of forest species to air pollution in the western United States served as a foundation for the development of an interagency pine-ozone monitoring system (NPS, USDA Forest Service, EPA - Region 9, and the California Air Resources Board). The Western Pine Method (WPM) is designed for use in the western United States to evaluate air pollution stress on Western conifers (Stolte and Miller, 1990). The method consists of using a stratified random sampling design to locate plots or survey points, the selection of 50 trees of a species of known-pollutant sensitivity as the focus of the plot or survey point, and the hands-on evaluation of the foliage from 5 branches of the lower crown of each tree. Foliage is evaluated for severity of ozone foliar discoloration, retention of needle fascicles and whorls, modal needle length, and severity of biotic and abiotic injury. The boles of the trees are evaluated for diameter at breast height (dbh), injuries (pitch tubes, fire scars, lightning scars), and percent live crown. Severity of foliar injury (chlorotic mottle), needle retention, needle length, and percent live crown are summed into an additive injury index (Eridanus Injury Index - EII) (Duriscoe, 1989) that improves quantification (0 - 100 range) of the degree of pollution stress to the tree (Stolte and Miller, 1990). The major features of the EII modified for use in the WPM are:

$$\text{(a)} \quad EII\ (CM, WR, NL, LCR) = [(\text{mean } CM / 3)(20)] + [(5 - WR)(15)] + [10.5 - (NL/2)] + [11 - (LCR/10)] \qquad (1)$$

$$\text{(b)} \quad EII_{max} = 20 + 60 + 10 + 10 = 100$$

where

- CM = chlorotic mottle injury class (0 - 3)
- WR = number of annual whorls of needles (1 - 5)
- NL = average modal length of needles ($\leq$ 21 cm)
- LCR = live crown ratio (10 - 100% in 10% increments)

Whorl level values for chlorotic mottle severity, needle retention, and needle length, and tree level values for live crown ratio, were standardized to ratio values by dividing them by values expected from an uninjured tree (1.a.). These ratios were weighted and summed to give a tree-level injury index value (0 - 100) (1.b.). An uninjured ponderosa or Jeffrey pine could be expected to have no CM (injury class 0), 5 or more annual whorls of needles (depends on the species), needles 21 centimeters long or more, and a live crown ratio of 100%. Asymptomatic (no visible foliar chlorotic mottle injury observed) trees were automatically given an injury index value of 0, based on the premise that if no chlorotic mottle symptoms were observed on remaining foliage then any reductions in whorl retention, needle lengths, or live crown ratio could not be attributed to air pollution. The maximum injury rating of 100 would indicate a severely injured tree with only the current year's growth of foliage remaining (WR = 1), more than 30% of the foliar area injured (CM = 3), average modal needle length $\leq$ 1 cm (NL = 1), and a live crown ratio of 10% or less (LCR = 10) (Stolte and Miller, 1990).

Monitoring of Ecological Indicators for Effects from Toxic Elements

In the Kenai National Wildlife Refuge in Alaska, a barbell design for spatial variability and three radial geometric transects were established to evaluate any impacts from the industrial complex in that area (**Figure 5.a.**) (Severson et al., 1990). The authors found a log-linear response in concentration of many elements with decreasing distance from the plant. Similar radial geometric transect plots or survey points have been established near planned or existing point sources in Wrangells-Saint Elias NP and Denali National Park and Preserve in Alaska. Samples of white and/or black spruce, feather moss, and ground lichens are being analyzed to establish baseline conditions in these parks and evaluate the pollutant-sensitivity of selected biological resources.

In Everglades NP (EVER) the elemental spatial variability of slash pine (Pinus elliottii var. densa) is being evaluated using a barbell sampling technique (**Figure 5.b.**). Grid sampling is also being employed to establish long-term elemental plots and construct element contour maps (**Figure 5.c.**). A GIS was used to stratify the slash pine type from the surrounding sawgrass wetlands, abandoned farms, and inclusions of hardwood hammocks. The GIS then analyzed various grid sizes that would insure extensive spatial resolution and provide a number of sample points that were within financial and logistical constraints. The GIS then provided UTM coordinates of the sample points. A Loran-C land navigation system was used to locate the sample points and take coordinates of each final plot location (Jackson et al., 1990). Similar long-term elemental monitoring plots were established in seven major water basins in North Cascades NP, using the target species subalpine fir (Abies lasiocarpa) (Duriscoe and Stitt, 1988).

DISCUSSION

Identification of Ecological Indicators

Controlled pollutant exposures (fumigations) performed in situ in NPS units under natural conditions of ambient pollutant concentrations and profiles, ambient environmental conditions of temperature, light, and relative humidity, and using species and genotypes native to the area appear to realistically assess the relative pollutant sensitivity of species. In addition, the nature and severity of foliar symptoms are defined and potential impacts on physiological processes and biomass accumulation that may be occurring to the same species in the forests are determined. Foliar injury symptoms observed on species in controlled fumigation exposures are macroscopically and microscopically similar in nature and severity to symptoms observed on the same species in the field under environmental conditions similar to those in the fumigation chambers.

By identifying sensitive species, determining the nature and magnitude of the species response to an air pollutant, and understanding how some of the most important environmental factors (e.g., soil moisture) affect the response to air pollutants, the extent and severity of air pollution injury under field conditions can be determined in long-term monitoring plots or surveys. Ranking species according to sensitivity to ozone under natural, fluctuating ozone concentrations agrees fairly well with rankings performed in the field or in fumigation chambers under non-ambient exposure profiles (Davis and Wilhour 1976). Although it may be more difficult to define exact air pollution threshold concentrations that cause injury (alterations of biological processes) for the purposes of standard setting, linking results obtained from chamber fumigation studies to possible or probable effects in the field is much more realistic. In situ controlled gradient studies could be performed for many types of stress (e.g. drought, UV-B, temperature) to identify sensitive ecological indicators specific for one or more stresses.

Research and Application of Ecological Indicators

To accurately assess impacts, it is important to understand the variability in response of ecological indicators to stress that occurs under field conditions. Variability studies have resulted in refinement of survey and monitoring methods to assess pollutant effects on sensitive biological species. The high degree of variability in response to pollutants that occurs in the field due to differences in genetics (Scholz et al., 1989), environmental conditions (Heck, 1968), and physiological status (Patterson and Rundel, 1989) results in the need for relatively large numbers of individuals of pollutant-sensitive species to be evaluated to delineate differences between stands and differences within stands over time (Duriscoe, 1989).

Physiological studies (Patterson, 1990) have indicated the relative loss of fixed carbon from each abscised whorl of needles and the effects that ozone-induced visible foliar injury has on reducing the amount of fixed carbon on remaining needles. Research on the variability in injury between upper and lower crowns of ozone-sensitive conifers has indicated that the lower crown of trees can serve as a surrogate for injury in the upper crown, at least for ponderosa pines that have two or more whorls of needles remaining (Muir and Armentano, 1987). In trees with very extreme ozone injury, such as those sometimes observed in the mountains of southern California, where lower crown needle loss and branch mortality might be very high, lower crown injury may be more severe than upper crown injury (Miller, 1977).

Hands-on evaluation of branches is preferred over remote (optical instrument) evaluation of upper or lower tree crowns. The precision of optical evaluations of the severity of chlorotic mottle and retention of needle whorls was variable, with overestimation when injury was low and underestimation when injury was high. Since these two variables are considered to be of primary importance when quantifying pollution injury on Western conifers (based on reductions in net photosynthesis), the use of optical instruments in evaluating pollution injury on forest trees is not recommended except for cursory evaluations of crown condition. In addition, the differentiation of chlorosis due to ozone injury (chlorotic mottle) from chlorosis due to natural senescence, scale injury, or other foliar agents is best done by examining foliage in-hand (Stolte and Miller, 1990). Since there is no size class of most species (with the possible exception of emergent seedlings of some species) that is most susceptible to injury from gaseous pollutants, evaluation of smaller size classes of trees (with pruneable lower crowns) probably estimates the injury to all age classes in the stand with reasonable precision. In the forests of the western United States (in the more southern latitudes), tree stands are often open, and even very large trees (with dbh > 100 cm) have lower crown branches that are occasionally obtainable with modern pole-pruners.

Monitoring of Ecological Indicators

The AQD began in 1984 to standardize procedures for assessing pollution impacts on conifers in NPS units, principally ponderosa and Jeffrey pines in the West and white pine and slash pine in the East (Stolte and Bennett, 1984). The procedures were based on methods developed by the US Forest Service in southern California (Miller et al., 1963) and the USFS in northern California (Pronos et al., 1978). Methods utilized by the AQD consisted of hands-on evaluation of the foliage of each whorl of needles from branches collected from the lower crowns of conifers for symptoms of pollution (ozone, sulfur dioxide, hydrogen fluoride), biotic (scale, fungus, insects), and abiotic (weather fleck) injury on each whorl of needles. Information was also collected on the density of both the upper and lower crown of each tree, as well as injury to the bole (lightning, fire, mistletoe, beetles, conchs). Site characteristics such as slope, elevation, aspect, and associated vascular species were also recorded. Each long-term monitoring plot contained 15 trees (> 10 cm dbh) that were tagged and mapped. The AQD established similar plots of hardwood and shrub species, sometimes co-located with the pine plots.

Synthesis of information from early plot work and the variability studies referenced earlier has resulted in methods for design and implementation of plots and surveys that are based on results of field studies and not solely on statistical estimates. The WPM assesses pollutant-injury on 50 individuals of a pollutant-sensitive species by performing hands-on evaluation of branches from the lower crown. Injury assessments consist of foliage retention, severity of chlorotic mottle on the remaining foliage, needle length, and live crown ratio that are summed in an additive injury index. Additional information on bole injury, crown class, and site factors is also obtained (Stolte and Miller, 1990).

Determination of the nutrient and toxic element content of conifer foliage, bole, roots and associated soils is considered by the NPS to be essential baseline information. The nutrient and toxic element contents are an important link between ambient pollution concentrations, visible crown conditions, and physiological, growth, and survivorship effects. The interaction between elements and biological systems is determined by the inherent properties of the element, particularly the chemical reactivity, solubility, and interactions with other inorganic and organic molecules (Lepp, 1981a and b). Determining the nature and concentration of the element is usually, but not always, relatively simple since each element of the periodic table can be detected in biological and abiotic samples with varying degrees of precision using different analytical techniques. Differentiating anthropogenic accumulation in tissues from natural concentrations of the same elements and determining levels of thresholds for injury are more difficult due to the natural variability of toxic elements in ecosystems. Sampling designs that assess the variability of elements of concern are important in establishing long-term monitoring plots. Sufficient replication of individuals at a site and sites within a study area are essential for determining gradients of toxic element accumulation from point or regional sources.

Description of Research Needs and Future Directions

Continued identification of sensitive ecological indicators through *in situ* fumigation studies is needed. Identification of sensitive species through field studies around point sources is also lacking. The nature and magnitude of responses of sensitive species should be well defined. Increased understanding of modifications in pollutant response by environmental factors (soil moisture, radiation, temperature) and biological factors (phenology, age class, biotic stresses) is needed, such as the possible effects of cold winters on needle morphology and subsequent increased resistance to ozone. Additional field and laboratory research on the interactions between air pollutant stress and biotic (insects, fungi) and abiotic (drought, fire) stresses is essential in ascertaining the true impacts of air pollution on our forests.

Continued and expanded field variability studies of pollution injury are needed. Evaluation of variability in pollution injury for more species, ranges of injury, and more parameters is lacking. More field research on the variability of toxic elements that leave pollutant signatures (sulfur, fluorides, metals) is essential.

Increased establishment of long-term monitoring plots (many decades) in forests to improve the understanding of *in situ* dose-response in stands of trees under diverse environmental conditions is needed. Greater emphasis on pollutant and environmental instrument monitoring in forests co-located with trend and ecological plots is necessary to begin to model the response of forests to air pollutants. Regional and landscape models of susceptibility to pollutants based on pollutant and environmental monitoring data should be developed for use in source permit reviews.

Development of injury indices based on *in situ* tree responses to pollutants will allow for more realistic correlations between pollutant stress and tree growth, pathogen resistance, and environmental resistance. Refinement of existing indexes to include other variables affecting tree response to pollutants such as available water capacity, dbh of trees, and site factors will improve our understanding of the biological and environmental variables that are most important in evaluating pollution stress. For example, in the western U.S. we know that some ozone-sensitive ponderosa and Jeffrey pine genotypes have less than expected radial ring growth. These trees are in the larger size classes, have poor whorl retention, and chlorotic mottle on the remaining whorls. Are there other variables that could be included in an additive index, such as site factors, tree dbh, and degree of biotic and abiotic stresses, to predict when trees will suffer growth declines from air pollution injury?

Improved instrumentation for evaluation of tree crowns is needed. Field portable spectral and/or fluorescence instruments are needed to evaluate needle condition on the trees, or at a minimum, serve as quality assurance/quality control for human evaluations. Improved methods are needed for obtaining branches from trees in wilderness areas where limited perturbations are allowed.

Acknowledgements

The authors are especially grateful to the highly qualified researchers Paul Miller, Dan Duriscoe, Dave Peterson, Nancy Grulke, Mark Patterson, Diane Ewell, Pat Muir, Tom Armentano, Lance Evans, Larry Gough, Larry Jackson, Jim Crock, and Ernie Gladney who have contributed their research findings to this paper. We would also like to thank AQD staff members Miguel Flores and Dave Joseph (MDAB) for providing ozone data summaries from NPS units, Dale Breitenfeld (RB) and Shirley Vaninger (MDAB) for assisting in the preparation of this manuscript, and Mark Scruggs (RB) for providing critical review comments for this document.

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A

YEAR	SPECIES	COMMON NAME	LIFE FORM	[illegible] OF SENSITIVITY
1988	Acer rubrum	Red maple	Tree	S
	Cercis canadensis	Eastern redbud	Tree	S
	Cornus florida	Flowering dogwood	Tree	S
	Pinus pungens	Table-mountain pine	Tree	S
1989	Asclepias exaltata	Tall milkweed	Herb	S
	Platanus occidentalis	American sycamore	Tree	S
	Prunus serotina	Black cherry	Tree	S
	Rhus copallina	Winged sumac	Shrub	S
	Rudbeckia hirta	Black-eyed susan	Herb	S
	Verbesina occidentalis	Crown-beard	Herb	S
	Liquidambar styraciflua	Sweetgum	Tree	I
	Rudbeckia laciniata	Cutleaf coneflower	Herb	I
	Robinia pseudoacacia	Black locust	Tree	T
	Tsuga canadensis	Eastern hemlock	Tree	T
1990	Sassafras albidum	Sassafras	Tree	S
	Rubus canadensis	Thornless blackberry	Shrub	S
	Aster divaricatus	White-wood aster	Herb	I
	Aesculus octandra	Yellow buckeye	Tree	I
	Pinus virginiana	Virginia pine	Tree	I
	Krigia montana	Dwarf dandelion	Herb	I
	Magnolia tripetela	Umbrella magnolia	Tree	I
	Rubus idaeus	Red raspberry	Shrub	T
	Pinus rigida	Pitch pine	Tree	T

B

YEAR	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL
1988							0.91/25*	0.48/93	1.23/73	0/64	0/68	0/102	0.38/69
							85/1	89/3	112/1	72/1	58/1	65	112/1
1989	0/94	0/67	0.15/66	0.53/95	0.48/95	0.60/94	0.41/95	0/92	0/83	0.03/96	0/94	0/53	0.21/80
	55	61	84/2	87/1	89/1	98/1	93/1	76	68	80/1	63	47	98/2
TOTAL	0/94	0/67	0.15/66	0.53/95	0.48/95	0.60/94	0.51/60	0.24/93	0.58/78	0.02/80	0/81	0/73	0.26/77
	55	61	84/2	87/1	89/1	98/1	98/1	89/3	112/1	80/1	63	65	112/1

*0.91(A)/25(B) A = AVERAGE NUMBER OF HOURS/DAY OF OZONE CONCENTRATIONS GREATER THAN OR EQUAL TO 80 PPB
85(C)/1(D) B = % OF TOTAL POSSIBLE HOURS MONITORED
C = MONTHLY HOURLY MAXIMUM OZONE CONCENTRATION IN PPB GREATER THAN OR EQUAL TO 80 PPB
D = NUMBER OF OCCURRENCES OF MAXIMUM OZONE CONCENTRATION

Table 1. A. Tree, shrub and herb species fumigated with ambient, sub-ambient, ambient plus 50% ozone, and ambient plus 100% ozone in Great Smoky Mountains National Park in Tennessee, U.S.A. Species were ranked on the basis of foliar injury expression at the lowest level of ozone exposure (sensitive-ambient; intermediate-ambient + 50%; tolerant-ambient + 100% or no symptoms). B. Monthly maximum hourly ozone concentrations and monthly averages of number of hours/days of ozone concentrations greater than or equal to 80 parts per billion (PPB) at Great Smoky Mountains National Park, Look Rock monitoring site from 1988-1989.

YEAR	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	ANN. MEAN	[illegible]	[illegible]
[illegible]						[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			[illegible]	[illegible]	[illegible]
1985						13.5/20 130/1	8.3/43 120/1	12.5/91 120/4	2.2/49 110/2	1.5/31 90/3			8.2/47 130/1	41.5	249.0
1986							7.7/14 [illegible]	14.3/78 [illegible]	2.3/74 [illegible]	0/64	0/69	0/76	3.8/63 [illegible]	76.0	220.0
1987	0/99.6	C/78	0/99	0.1/60 81/1	0.1/85 81/1	2.5/98 118/1	2.3/95 108/1	2.7/95 109/1	1.0/95 93/1	2.3/94 106/1	0/95	0/93	1.0/91 118/1	27.7	111.0
1988	0/94	0/95	0.1/94 130/1	0.6/95 95/1	1.9/95 97/1	2.6/95 97/1	6.5/96 117/1	4.4/94 108/2	4.6/93 102/1	1.4/95 106/3	0/94	0/71	1.9/93 130/1	30.3	145.0
1989	0/87	0/88	0/95	0/88	0.2/92 86/1	2.0/94 108/1	6.2/93 112/1	4.1/88 100/2	2.1/94 101/1	.03/95 90/1	0/93	0/94	1.3/92 112/1	31.9	231.5
1990	0/92	0/94	0/95	0.2/94 91/1	1.2/93 94/3	4.4/94 112/1	4.0/93 110/2	3.5/94 131/1					1.7/94 131/1		
MEAN*** HIGH****	0/93	C/89	0.03/91 130/1	0.2/84 95/1	0.9/91 97/1	3.1/81 130/1	4.7/76 120/1	7.1/90 131/1	2.6/84 110/4	1.1/72 106/4	0/88	0/84	2.1/85 131/1	47.0*****	206.3*****

B. YOSEMITE

YEAR	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	ANN. MEAN
1987				0.2/94* 84/1	0.6/90 112/1	3.0/86 128/1	2.1/91 133/1	2.8/95 112/1	1.9/95 116/1	0.8/95 91/1	0/95	0/94	1.2/93 133/1
1988	0/76	0/94	0/95	0.4/95 90/1	0.3/95 82/1	0.5/95 97/1	3.0/94 106/2	2.7/94 111/1	3.4/95 119/1	0.9/94 98/1	0/91	0/71	1.0/91 119/1
1989	0/98	0/88	0/95	0/95	0.3/93 93/1	0.3/94 95/1	1.4/95 94/1	1.3/95 111/1	0.2/95 87/1	0/94	0/85	0/87	0.0**/93 111/1
MEAN*** HIGH****	0/87	C/91	0/95	0.2/95 90/1	0.4/92 93/1	1.2/92 128/1	2.2/93 133/1	2.2/95 112/1	0.1/95 119/1	0.6/95 98/1	0/90	0/84	0.8/92 133/1

C. SAGUARO

YEAR	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	ANN. MEAN
1982	0/0						0.3/99* 91/1	0.2/75 85/1	0.1/96 100/1	0/76	0/99	0/98	0.0/91 100/1
1983	0/100	0/100	0.1/45 98/1	0/100	0.5/96 87/2	0.4/92 88/4	0.1/99 88/1	0.5/75 107/1		0/57	0/96	0/96	0.1/86 107/1
1984	0/99	0/70	0/98	0.1/99 84/1	0.1/97 81/2	0.0/92 82/1	0.2/88 86/1	0.3/95 108/1	0.2/90 91/1	0/84	0/94	0/92	0.1/92 1/08/1
1985	0/75	0/89	0/14	0.8/100 105/1	0.3/100 90/2	0.5/100 98/1	NO DATA	NO DATA	NO DATA	NO DATA	NO DATA	NO DATA	0.3/79 105/1
1987					0/93	0/89	0/74	0/95	0.1/95 82/1	0/94	0/94	0/94	0.0/91** 82/1
1988	0/94	0/95	0/86	0/94	0.0/92 82/1	0.1/94 85/1	0.6/93 100/2	0.2/92 89/1	0.2/93 89/1	0/95	0/93	0/81	0.1/92 100/2
1989	0/94	0/88	0.0/94 83/1	0.0/93 81/1	0.0/92 81/1	0.1/89 87/1	0.4/90 93/1	0.2/91 99/1	0/95	0/90	0/95	0/90	0.1/92 99/1
MEAN*** HIGH****	0/92	0/89	0.0/67 98/1	0.2/97 105/1	0.2/95 98/2	0.2/93 98/1	0.2/91 100/2	0.2/87 108/1	0.1/94 108/1	0/83	0/95	0/92	0.1/89 108/1

*0.3(A)/99(B) A = AVERAGE NUMBER OF HOURS/DAY OF OZONE CONCENTRATIONS GREATER THAN OR EQUAL TO 80 PPB
91(C)/1(D) B = % OF TOTAL POSSIBLE HOURS MONITORED
C = MONTHLY HOURLY MAXIMUM OZONE CONCENTRATION IN PPB
D = NUMBER OF OCCURRENCES OF MAXIMUM OZONE CONCENTRATION

** INDICATES A NUMBER GREATER THAN 0 BUT LESS THAN OR EQUAL TO 0.05

*** MEAN = MEAN OF PERIOD OF RECORD

**** HIGH = HIGHEST OZONE CONCENTRATION DURING PERIOD OF RECORD AND NUMBER OF OCCURRENCES

***** AVERAGE FOR 58 YEARS (1971 - 1989)

Table 2. Monthly maximum hourly ozone concentrations and monthly averages of number of hours/days of ozone concentrations greater than or equal to 80 parts per billion (PPB) at: A. The lower Kaweah Monitoring site (elevation 6000 feet) in Sequoia National Park from 1984-1990. Precipitation (includes snowfall and rainfall amounts) and snowfall annual (from July 1-June 30) amounts (inches) taken at Lodgepole ranger station near lower Kaweah. B. The Wawona monitoring site at Yosemite National Park from 1987-1989. C. Saguaro National Monument.

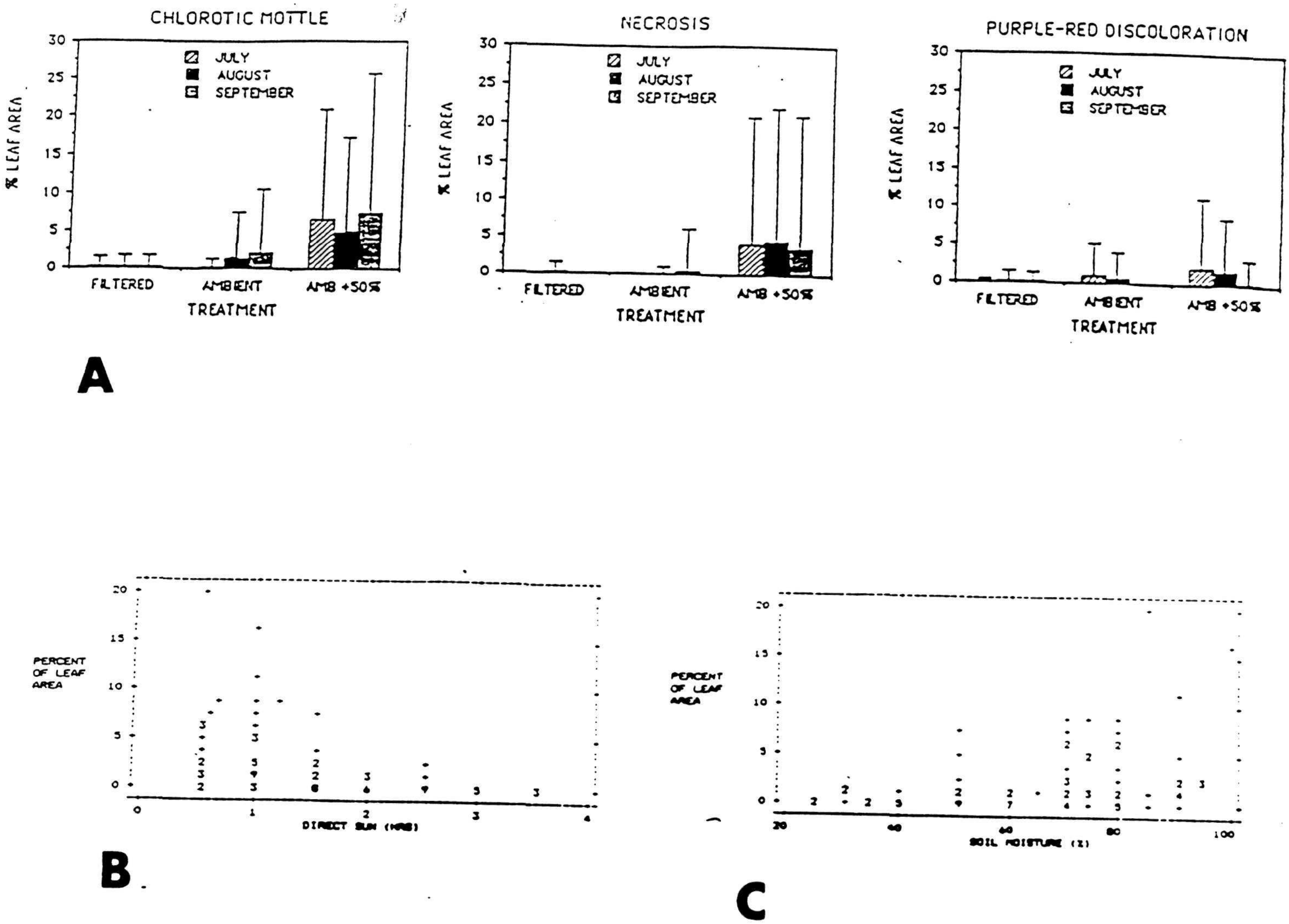

Figure 1. A. Visible injury to seedlings growing under conditions of short duration of sunlight (< 2 hrs) in supercells in racks above ground (Group B). Seedlings were measured for visible foliar injury during July 6-13, August 4-12, and September 12-16, 1988 in open-topped chambers at Highlands in SEKI. B. Relation of average percent of sequoia seedling leaf surface area occupied by chlorotic mottle symptoms in relation to an estimate of the daily duration of direct sunlight at 87 1 m^2 plots located in a recent prescribed burn area (keyhole) at Giant Forest. C. Relationship between severity of foliar injury to saturation at 87 1 m^2 plots located in a recent prescribed burn area (keyhole) at Giant Forest.

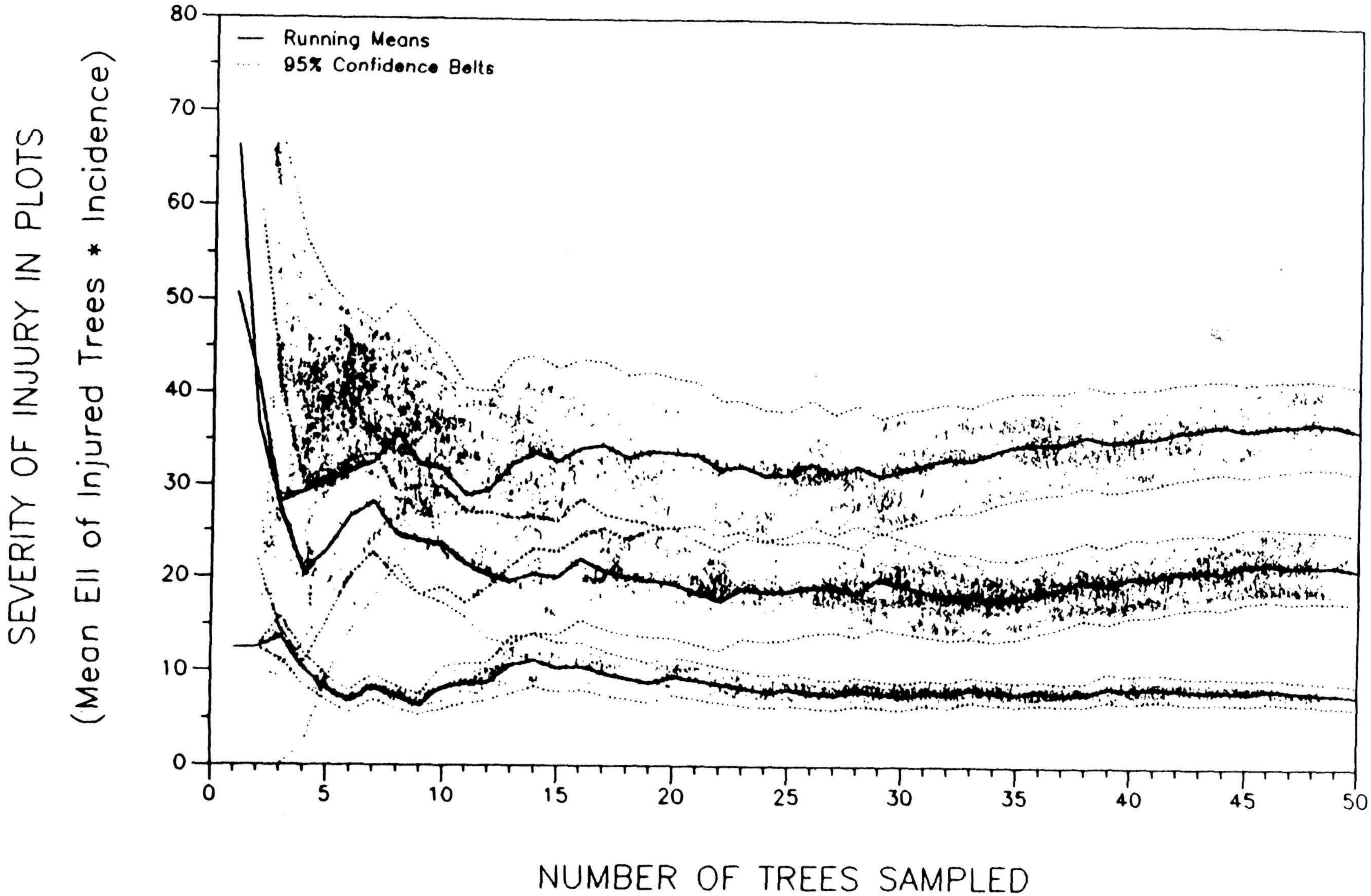

Figure 2. Number of trees that should be sampled in stands of Ponderosa and Jeffrey pines in Sequoia National Park to delineate stands (3 each) of slight, moderate, and severe ozone injury (bottom, middle, and upper lines, respectively). Dotted lines mark boundary of 95% confidence intervals. Severity of ozone injury defined by average injury index (EII) of trees times the incidence of injury within the stand. Highest variability occurs in stands where injury is most severe (upper line). Information used to determine number of trees in long-term monitoring plots and in cruise surveys.

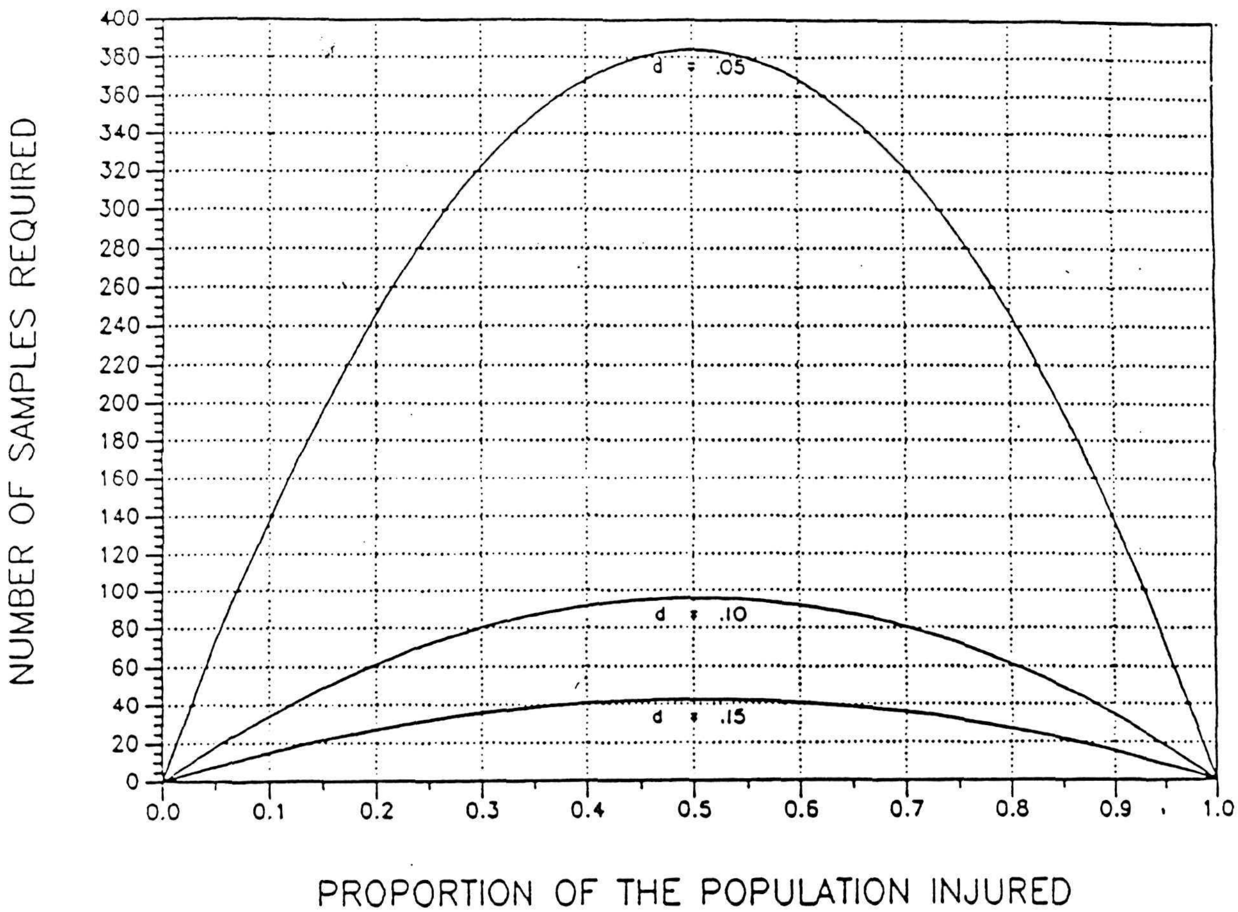

Figure 3. Required sample sizes at three levels of precision (.05, .10, .15) for populations with degrees of incidence of injury ranging from 0 to 100%. The largest sample sizes are for those populations where half the individuals are injured and half are not.

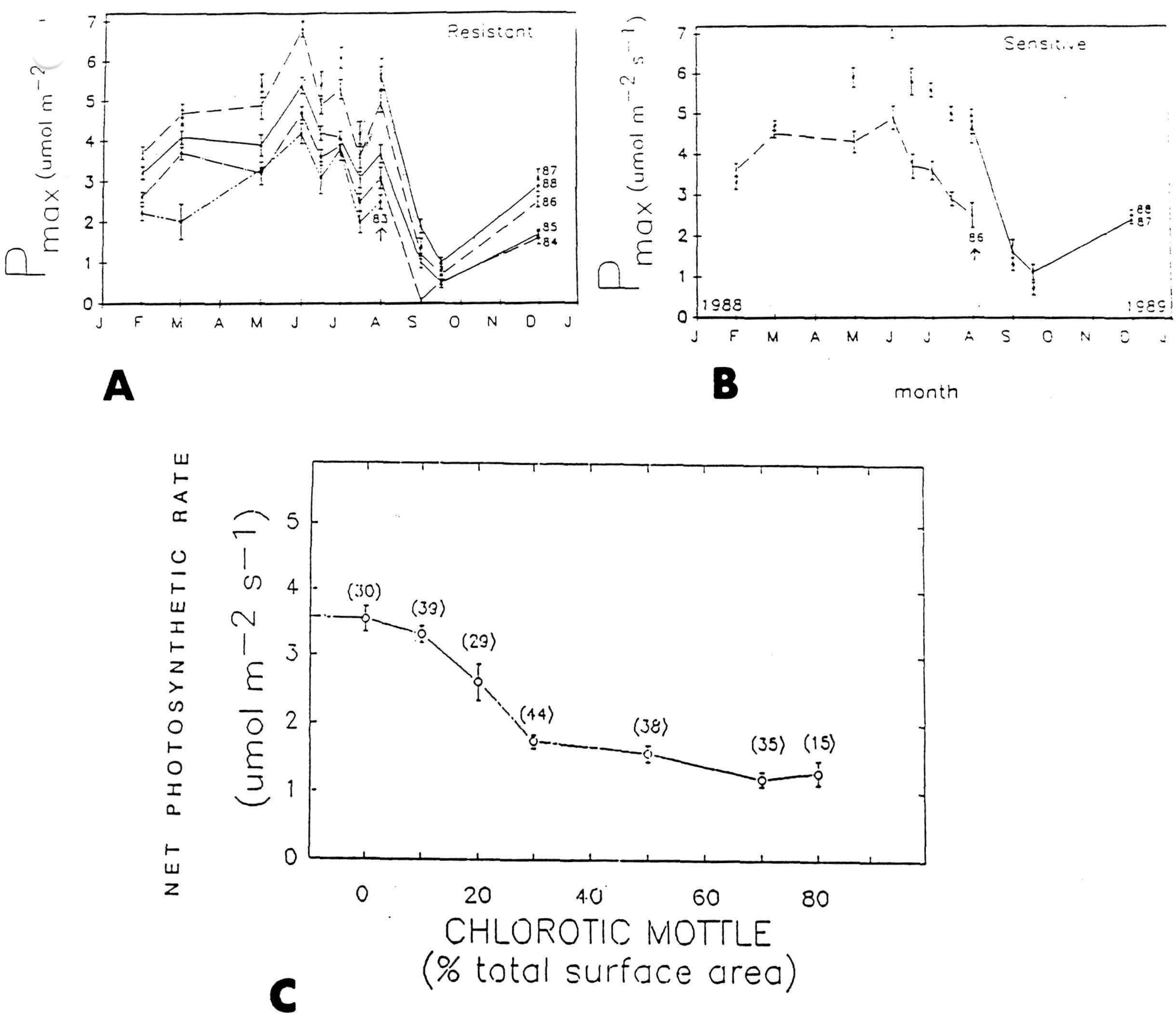

Figure 4. A. Annual course of photosynthetic contribution of six different-aged whorls of needles of Jeffrey pines without visible foliar ozone injury (resistant genotypes). B. Annual course of photosynthetic contribution of 3 different aged whorls of Jeffrey pines with visible foliar ozone injury (sensitive genotypes). In both sensitive and resistant genotypes, physiological activity peak occurs when ozone is low in SEKI (relative to doses recorded in August). C. Relationship between net photosynthetic rate of Jeffrey pine needles in Sequoia National Park in June 1988, and percentage of needle surface area affected with ozone-induced chlorotic mottle.

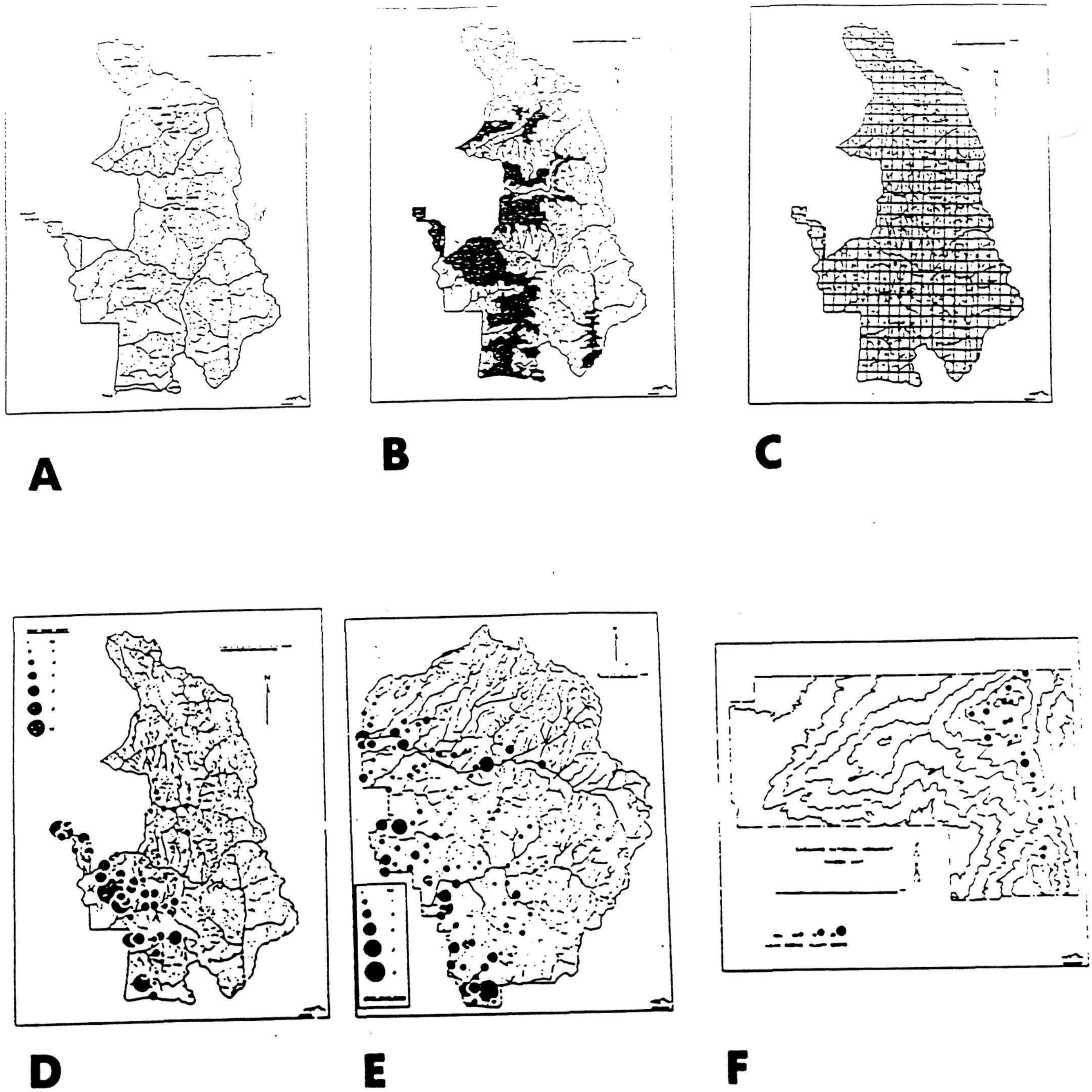

Figure 5. **Stratified random sampling method used to evaluate the extent and severity of foliar injury from chronic ozone exposure to Jeffrey and ponderosa pines in Sequoia and Kings Canyon National Parks, Yosemite NP, and Saguaro NP. A. Major river drainages in Sequoia and Kings Canyon National Parks. B. Distribution of mixed conifer forests containing Jeffrey and ponderosa pine. C. North-south oriented grid (3.2 km square) with randomly-selected sample point in each grid. D. The distribution and severity of foliar injury that resulted from chronic ozone stress are shown in this figure. E. Relative severity of foliar ozone injury throughout Yosemite in 1986. F. Relative severity of foliar ozone injury throughout the Rincon Mountains of Saguaro in 1986.**

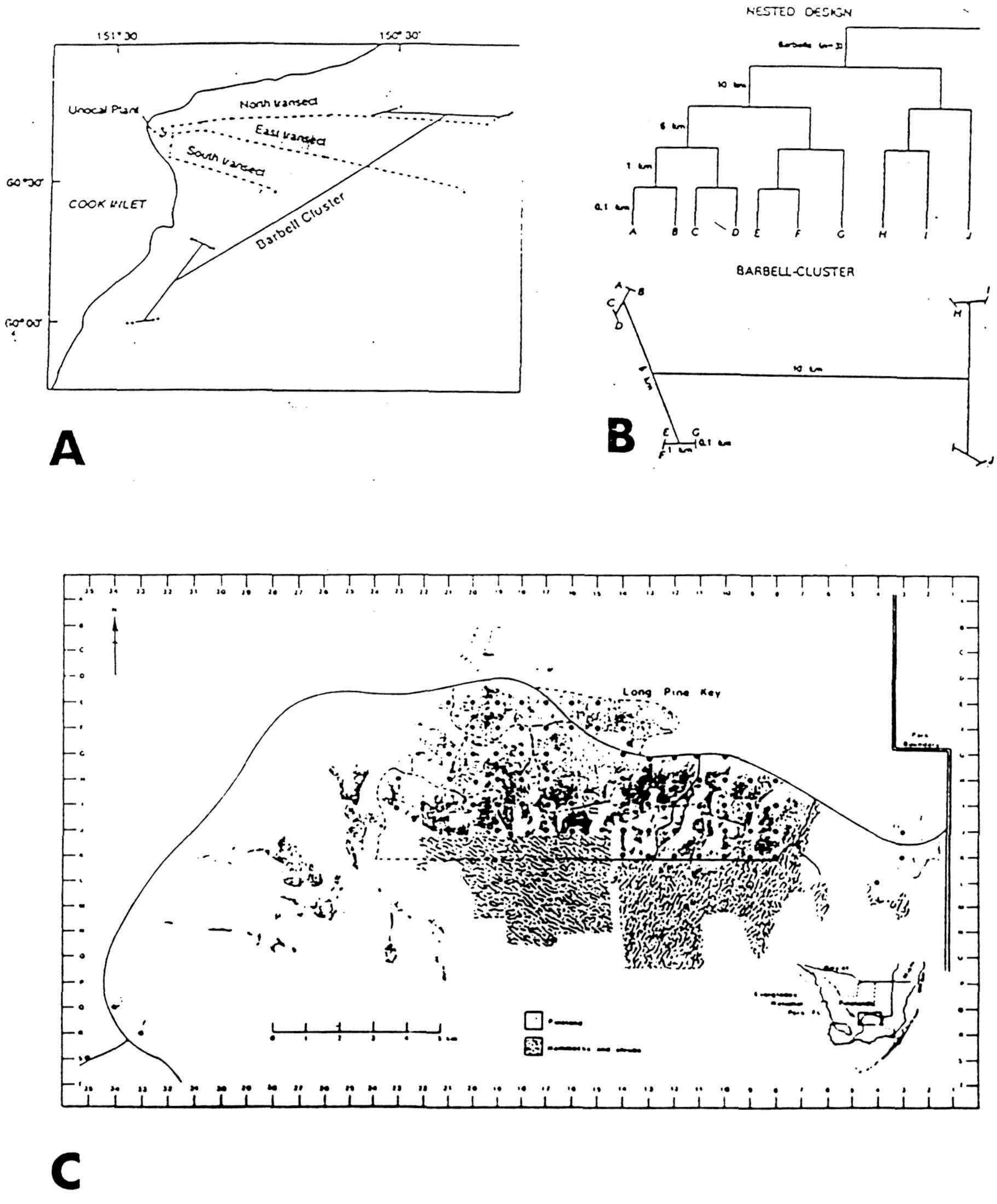

Figure 6. Methods employed to assess elemental concentrations in soils and plants in the United States. A. Geometric radial transects near point sources of pollution in Alaska to assess elemental gradients. B. Barbell sampling design to assess spatial variability of selected elements. C. Grid sampling (0.75 km grid size) of slash pine (Pinus elliottii var. densa) in Everglades National Park (south Florida) for sulfur and toxic elements. Each grid sampling point became the focal point for establishment of long-term elemental trend plots.