United States
Department
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Forest Service

**Pacific Southwest
Research Station**

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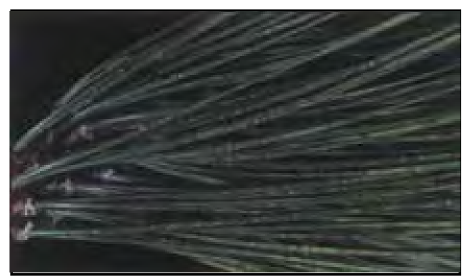
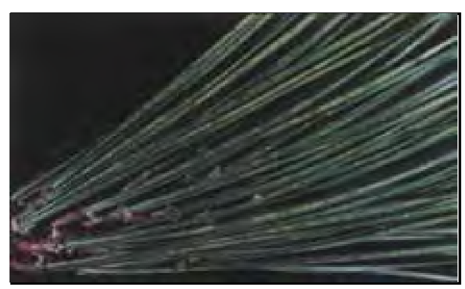
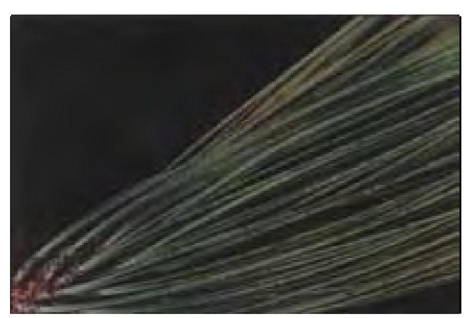
General Technical
Report PSW-GTR-155

Evaluating Ozone Air Pollution Effects on Pines in the Western United States

Paul R. Miller Kenneth W. Stolte Daniel M. Duriscoe John Pronos







Publisher:

Albany, California
Mailing address:
PO Box 245, Berkeley CA
94701-0245

510 559-6300

http://www.psw.fs.fed.us/

July 1996

Pacific Southwest Research Station

Forest Service
US Department of Agriculture

Abstract

Miller, Paul R.; Stolte, Kenneth W.; Duriscoe, Daniel M.; Pronos, John, technical coordinators. 1996. **Evaluating ozone air pollution effects on pines in the western United States**. Gen. Tech. Rep. PSW–GTR–155. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture; 79 p.

Historical and technical background is provided about ozone air pollution effects on ponderosa (*Pinus ponderosa* Dougl. ex Laws) and Jeffrey (*P. jeffreyi* Grev. and Balf.) pines in forests of the western United States. The principal aim is to document the development of field survey methods to be applied to assessment of chronic ozone injury by government agencies responsible for management of forest and air resources, primarily in the Sierra Nevada and mountains of southern California. Detailed procedures are supplied for the selection of sample plots, collection of plot and tree data, computation of an injury index for each tree and execution of quality assurance procedures. The methods have been field tested in California National Forests and National Parks during 4 consecutive years.

Retrieval terms: ozone, injury, ponderosa, Jeffrey, pines, methods

Technical Coordinators

Paul R. Miller is Plant Pathologist in the Atmospheric Deposition Effects Research Work Unit at the Pacific Southwest Research Station, USDA Forest Service, 4955 Canyon Crest Drive, Riverside, CA 92507.

Kenneth W. Stolte is Forester and Deputy Leader of the Forest Health Monitoring Program, Southern Research Station, USDA Forest Service, 3041 Cornwallis Rd., Research Triangle Park, NC 27709.

Daniel M. Duriscoe is Biological Technician, Resources Management, Sequoia and Kings Canyon National Parks, USDI National Park Service, Three Rivers, CA 93271.

John Pronos is Plant Pathologist, Forest Pest Management, Stanislaus National Forest, USDA Forest Service, 19777 Greenley Rd., Sonora, CA 95370.

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Contents

In Brief

A standard method is needed to evaluate the effects of chronic ozone air pollution on the health of ponderosa and Jeffrey pines in California and other western States. These species are ideal bioindicators of ozone damage because they are ozone sensitive, widely distributed, and an important element of the forest ecosystem. The first two papers of this report describe the current knowledge of extent and severity of ozone injury to conifer forests in the West and present the reasons why government agencies need a continuing assessment of this problem for the implementation of sound ecosystem management. The next three papers describe the biological mechanism of ozone injury, review the development and application of early methods for assessing injury to pines, and present the statistical criteria for plot design and sampling procedures. These papers provide the basis for the design of a recommended standard procedure. The last three papers present detailed instructions for establishing sample plots, collecting data, processing data, and applying quality assurance procedures. The appendix provides very precise detail for tasks relating to data gathering, data processing and expressing injury in the form of an injury index. The method has been field tested by collecting data from 1,700 trees at 33 plots in California from 1991 to 1994. Although this report provides a fully workable method for measurement and description of chronic ozone injury to pines, the authors encourage further development of methods for data collection and interpretation.

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Retrieval terms: ozone, injury, ponderosa, Jeffrey, pines, methods

Preface

The high rate of fossil fuel consumption needed to sustain the economies of western States, coupled with ever–increasing population densities, result in air pollution. The climate and topography in California are particularly conducive to the accumulation of ozone in urbanized air basins and the subsequent long–range transport to forested mountains downwind. Ozone damages many plant species including ponderosa and Jeffrey pines. The natural range of these species in the Sierra Nevada and southern California mountains is extensive. Chronic ozone exposure damages the foliage, reduces growth and makes trees more vulnerable to natural pests like the pine bark beetle. Protection of this forest resource requires knowledge about the extent of ozone damage and its change in severity over time.

The purposes of this report are to (1) examine the current effects of ozone air pollution on mixed conifer forests in the western United States; (2) review the mechanisms by which ozone injures leaf tissue and diminishes tree health; (3) describe and evaluate the historical methods used by different groups to evaluate ozone injury to pines and to assign a numerical index of injury for the purpose of tracking injury trends; (4) prescribe the statistical principles that must be applied for sampling; (5) propose an improved method for measurement and evaluation of ozone response variables to develop an injury index for ponderosa and Jeffrey pines; (6) provide detailed instructions for establishing monitoring plots, measuring ozone injury and other essential plot and tree variables; and (7) outline the procedures for handling the data set derived from annual measurements, including the computation of the current injury index.

Representatives of Federal land management agencies, and the California Air Resources Board began the process of implementing these objectives at a meeting held in 1989 at the USDA Forest Service's Pacific Southwest Research Station, Riverside, California. Preliminary drafts of this report have been prepared and circulated within a small interagency committee of workers with responsibilities to assess the prevalence and severity of ozone injury to conifer forests using ponderosa and Jeffrey pines as bioindicators. Agreement on basic methods was available by 1991 so that monitoring plots were established and tree injury data collected at approximately 33 locations in the Sierra Nevada and San Bernardino Mountains. This activity was named the Forest Ozone REsponse Study (FOREST). Each year from 1991 to 1994 summer training sessions were held to certify data gatherers and winter debriefing sessions were implemented to evaluate methods and examine data summaries from the preceding summer. These iterations have lead to standardization and refinement of the procedures included in the data collection and analysis portions of this document.

The guiding principle of this effort has been to establish a methodology leading to the computation of a single injury index on the basis of a diverse data base of plot, tree, branch, and needle data that will facilitate exploration of other methods for describing and assessing chronic ozone injury. Some measured attributes must serve as common denominators allowing interpretation between different numerical expressions of chronic tree injury. The index proposed by the authors represents the current state–of–the–science; future modifications and improvements may result from further analysis of the data.

Acknowledgments

We are grateful to colleagues for advice, including Diane Ewell, Judy Rocchio, Steve Cline, Charley Peterson, John Moore, Chip Harvey, Bruce Nash, Tamara Franklin, Kathy Tonnesson, Deborah Mangis, and Mark Scruggs. We thank Anthony Gomez and David Jones for providing photography. We appreciate the help of external reviewers including John Kliejunas, Patrick Temple, and Art Chappelka. Editorial advice and manuscript editing were provided by Vince Dong and Laurie Dunn, Pacific Southwest Research Station, USDA Forest Service.

Extent of Ozone Injury to Trees in the Western United States

Paul R. Miller[1]

The widespread nature of ozone air pollution in California and the suspected potential for ozone damage to forests near cities in other Western States led to field surveys and establishment of monitoring plots beginning in the mid–1960's. The surveys and plot results were divided into three categories depending on the evidence of injury that was documented, including (1) surveys that failed to reveal any evidence of injury even though ozone air pollution was suspected; (2) visible foliar injury that was detected although no structural or functional changes were evident in the forest ecosystem; and (3) the presence of both foliar injury and structural and functional changes. Recent monitoring has shown that ozone air pollution has not caused damage to pines in Colorado or western Washington. Slight damage was found in southern Arizona, slight to moderate damage was extensive in the Sierra Nevada, and moderate to severe damage was evident in the San Bernardino Mountains of southern California.

Injury Undetected

Front Range of Colorado

Ozone air quality for the Colorado Front Range country as summarized by Bohm (1992) showed daily peak concentrations sometimes exceeding 100 ppb. Highest concentrations generally occurred at valley and foothill locations of the central part of the Front Range. Rocky Mountain ponderosa pine (*Pinus ponderosa* var. *scopulorum*) dominated the lower foothills beginning at about 2,000 m. This species was the subject of extensive surveys for visible ozone symptoms and sampling of cores for tree ring analysis in two related studies (Graybill 1992). For the unmanaged old–growth stands distributed along the full north to south extent of the Front Range, no evidence of visible injury was found and tree–ring chronologies showed a consistent relationship with precipitation without any evidence of a departure that could be implicated with ozone exposure in recent decades. This result was also obtained by sampling second–growth stands in the same region (Peterson and Arbaugh 1992). Exposure of seedling *P. ponderosa* var. *scopulorum* to ozone in fumigation experiments did not indicate sensitivity to ozone at concentrations up to four times higher than the highest ambient concentrations observed at Front Range monitoring stations (Miller and others 1983).

Western Washington

Parts of the Douglas–fir (*Pseudotsuga menziesii*) region of western Washington, particularly Olympic National Park, have been characterized by the lowest ozone concentrations (< 60 ppb) in the West (Bohm 1992). However, Edmunds and Basabe (1989) observed transported peaks of ozone southwest of Seattle–Tacoma that reached 100 ppb. In the latter case no foliar symptoms of ozone injury were observed on Douglas–fir. The results of an extensive tree–ring study of old–growth Douglas–fir in western Washington revealed that the dominant pattern was one of growth increase (Brubaker and others 1992). Although examples of growth decrease were found, spatial patterns in these results did not suggest an impact by ozone or other air pollutants in the Puget Sound region.

[1]Plant Pathologist, Pacific Southwest Research Station, USDA Forest Service, 4955 Canyon Crest Drive, Riverside, CA 92507

Visible Injury Without Structural or Functional Changes

Southern Arizona

A cruise survey was done of ponderosa pine stands (both *P. ponderosa* var. *scopulorum* and *P. ponderosa* var. *arizonica*) in the Rincon Mountains of Saguaro National Monument (Duriscoe 1990). A total of 660 trees were observed (20 trees per sampling point at 33 points). The two varieties of ponderosa pine were intermixed, and the survey results were not described separately for each variety. Foliage was inspected on a sample of branches cut from the lower crown of each tree. Fifteen percent of the trees (99 out of 660) were diagnosed as having ozone–induced chlorotic mottle on the foliage. Twenty–six of the 33 sample points contained at least one tree with ozone injury symptoms. A significant difference was found in number of whorls retained, with ozone sensitive genotypes retaining fewer whorls. The individual point showing the most injury was on Heartbreak Ridge, between Mica Mountain and Happy Valley Lookout. The average ozone injury for the whole sample was considered slight. Because Tucson is located 30 miles west–northwest, it is the most probable source of ozone–polluted air.

Graybill and Rose (1989) examined the tree ring chronologies of 11 ponderosa pines from Saguaro National Monument that had evidence of needle injury. Only three of these trees showed evidence of a post 1950 decline in ring growth; thus, many other variables must be considered as participating causes of changes in the observed chronologies. Further studies by Graybill (1992) of tree ring chronologies from the Santa Catalina and Rincon Mountains showed four of five chronologies from ponderosa pine sites that had extreme growth suppression after 1950. No evidence of a correlation between ozone injury to foliage and change in ring growth for these sites was found, and the possible factors contributing to post–1950 growth changes are being investigated further.

Joshua Tree National Monument

Temple (1989) conducted surveys of native vegetation for ozone injury in 1984 and 1985 at Joshua Tree National Monument—which is located about 100 km east of the Los Angeles basin in southern California with maximum daily concentrations of ozone recorded after 8 p.m. because of the extra time needed for transport from the basin through San Gorgonio Pass. During 1984 and 1985 the peak value frequently exceeded 100 ppb. Permanent plots were established to include woody riparian species, namely *Acacia greggii*, *Chilopsis linearis*, *Rhus trilobata*, and *Salix gooddingii*. No symptoms were observed on any species under field conditions, however, *R. trilobata* was sensitive when fumigated with ozone (100 ppb, 4 hours/day, for 4 days). Subsequent field surveys revealed ozone injury symptoms on squaw bush by ambient levels of ozone when soil moisture was not a limiting factor (Stolte, personal communication).

Western Slope of the Sierra Nevada

Detection of ozone injury symptoms to ponderosa and Jeffrey pines in the Sierra Nevada, California (Miller and Millecan 1971) and subsequent surveys by Forest Service pest management specialists using 52 trend plots (Allison 1982, 1984a, 1984b, Pronos and Vogler 1981) provided the earliest data describing the extent of ozone injury and the early trends of the severity of injury. For example, Pronos and Vogler (1981) reported that between 1977 and 1980 the general trend was an increase in the amount of ozone symptoms present on pine foliage.

Peterson and others (1991) sampled crown condition and derived basal area growth trends from cores collected from ponderosa pines at sites in seven Federal administrative units (National Forests and National Parks) located from north to south in the Sierra Nevada including Tahoe National Forest, Eldorado National Forest, Stanislaus National Forest, Yosemite National Park, Sierra National Forest, Sequoia–Kings Canyon National Park, and Sequoia National Forest. In July–August 1987, four symptomatic and four asymptomatic sites were visited in each unit and only sites with ponderosa pines greater than 50 years old were selected for sampling. The symptomatic plots generally indicated increasing levels of chronic ozone injury (reduced numbers of annual needle whorls retained and chlorotic mottle symptoms on younger age classes of needles) from north to south. In general, the results of this study documented the regional nature of the ozone pollution problem originating primarily from the San Joaquin Valley Air Basin (SJVAB), as well as the San Francisco Bay Air Basin further to the west. The study found no evidence of recent large–scale growth changes in ponderosa pine in the Sierra Nevada mountains; however the frequency of trees with recent declines of growth did increase in the southernmost units. Because these units had the highest levels of ozone (and more chlorotic mottle symptoms on needles of younger age classes) strongly suggests that ozone is one of the contributing factors to decline in basal area increase. Other factors limiting tree growth in this region include periodic drought, brush competition and high levels of tree stocking.

A region–wide survey (Peterson and others 1991) of ponderosa pine provided a useful backdrop for reporting a number of other studies or surveys in the Sierra Nevada that were more narrowly focused. Another tree ring analysis and crown injury study was focused on Jeffrey pines in Sequoia–Kings Canyon National Park (Peterson and others 1989). This study suggested that decreases of radial growth of large, dominant Jeffrey pines growing on xeric sites (thin soils, low moisture holding capacity) and exposed to direct upslope transport of ozone, resulted in as much as 11 percent less in recent years compared to adjacent trees without symptoms.

Both permanent plots and cruise surveys have been employed in Sequoia, Kings Canyon (SEKI) and Yosemite (YOSE) National Parks to determine the spatial distribution and temporal changes of injury to ponderosa and Jeffrey pine within the Parks (Duriscoe and Stolte 1989). Comparisons of the same trees at 28 plots between 1980–82 and 1984–85 in SEKI showed increases of ozone injury to many trees and increases of the total number of trees with ozone injury. Ozone injury was found to decrease with increasing elevation of plots. The highest levels of tree injury in the Marble Fork drainage of the Kaweah River at approximately 1,800 m elevation were associated with hourly averages of ozone frequently peaking at 80 to 100 ppb, but seldom exceeding 120 ppb.

A cruise survey in 1986 evaluated 3,120 ponderosa or Jeffrey pines in SEKI and YOSE for ozone injury (Duriscoe and Stolte 1989). More than one–third of these trees were found to have some level of chlorotic mottle. At SEKI symptomatic trees comprised 39 percent of the sample (574 out of 1,470) and at YOSE they comprised 29 percent (479 out of 1,650). Ponderosa pines were generally more severely injured than Jeffrey pines. The Forest Pest Management (FPM) score (low score equals high injury) was 3.09 for ponderosa and 3.62 for Jeffrey (Pronos and others 1978). These cruise surveys identified the spatial distribution of injury in SEKI and YOSE, and indicated trees in drainages nearest the San Joaquin Valley were most injured.

In SEKI field plot observations of seedling health and mortality in natural giant sequoia groves from 1983 to 1986 showed that emergent seedlings in moist microhabitats had ozone–induced foliar symptoms. Seedling numbers were reduced drastically from drought and other abiotic factors during this period. A variable such as ozone that could injure seedling foliage sufficiently to reduce root growth immediately after germination could increase vulnerability to late summer drought. After fumigation giant sequoia (*Sequoiadendron giganteum* Bucch.) seedlings developed chlorotic mottle following *in situ* exposure to both ambient ozone concentrations and 1.5× ambient ozone in open top chambers during the 8 to 10 weeks after germination (Miller and others 1994). Significant differences in light compensation point, net assimilation at light saturation, and dark respiration were found between seedlings in charcoal filtered air treatments and 1.5× ambient ozone treatments (Grulke and others 1989). These results could mean that ozone has the potential to be a new selection pressure during the regeneration phase of giant sequoia, possibly reducing genetic diversity.

The Lake Tahoe Basin is located at the northern end of the Sierra Nevada sampling transect (near the Eldorado National Forest) (Peterson and others 1991). Because the Basin is distinct, its air quality is the product of mostly local pollution sources in contrast to most other Sierra Nevada sites where pollution results from long–range transport. In 1987 a survey of 24 randomly selected plots in the basin included a total of 360 trees of which 105 (29.2 percent) had some level of foliar injury (Pedersen 1989). Seventeen of these plots had Forest Pest Management (FPM) injury scores (Pronos and others 1978) that fell in the slight injury category. Of 190 trees in 16 cruise plots that extended observations to the east outside the basin, 21.6 percent had injury—less than in the basin.

Visible Ozone Injury with Structural and Functional Changes

San Bernardino Mountains

The source area for pollutants transported to the conifer forests of the San Bernardino Mountains is the South Coast Air Basin of southern California. Confirmation that ozone was the cause of foliar symptoms on ponderosa pine was reported by Miller and others (1963). Between 1976 and 1991 the weather adjusted ozone data for the May through October "smog season" showed that the number of Basin days exceeding the Federal Standard (> 120 ppb, 1 hr average) declined at an average annual rate of 2.27 days per year (Davidson 1993). The number of days with Stage I episodes (> 200 ppb, 1 hr average) declined at an average annual rate of 4.70 days per year during the same time period. For the Federal Standard the total days per year with exceedances was as high as 159 in 1978 with the lowest at 105 days in 1990. Regarding Stage I episodes the high was 108 in 1979 and the low was 33 days in 1990. The 1974 to 1988 trends of the May through October hourly average and the average of monthly maximum ozone concentrations for Lake Gregory, a forested area in the western section of the San Bernardino Mountains, also showed a decline (Miller and McBride 1989). Similarly the injury index has shown an improvement in chronic injury to crowns of ponderosa and Jeffrey pines between 1974 and 1988, in 13 of 15 plots located on the gradient of decreasing ozone exposure in the San Bernardino Mountains (Miller and McBride 1989). The two exceptions were plots located at the highest exposure end of the gradient.

Miller and others (1991) reported that for the 1974 to 1988 period the basal area increase of ponderosa pines was generally less than competing species at 12 of the 13 plots evaluated. The total basal area for each species, as percent of the total basal area for all species (*fig. 1*), shows that ponderosa and Jeffrey pines lost basal area in relation to competing species that were more tolerant to ozone, namely, white fir, incense cedar, sugar pine and black oak at plots with slight to severe crown injury to ponderosa or Jeffrey pine. The accumulation of more stems of ozone tolerant species in the understory presents a fuel ladder situation that jeopardizes the remaining overstory trees in the event of a catastrophic fire. The ozone tolerant species are inherently more susceptible to fire damage because of thinner bark and branches close to the ground.

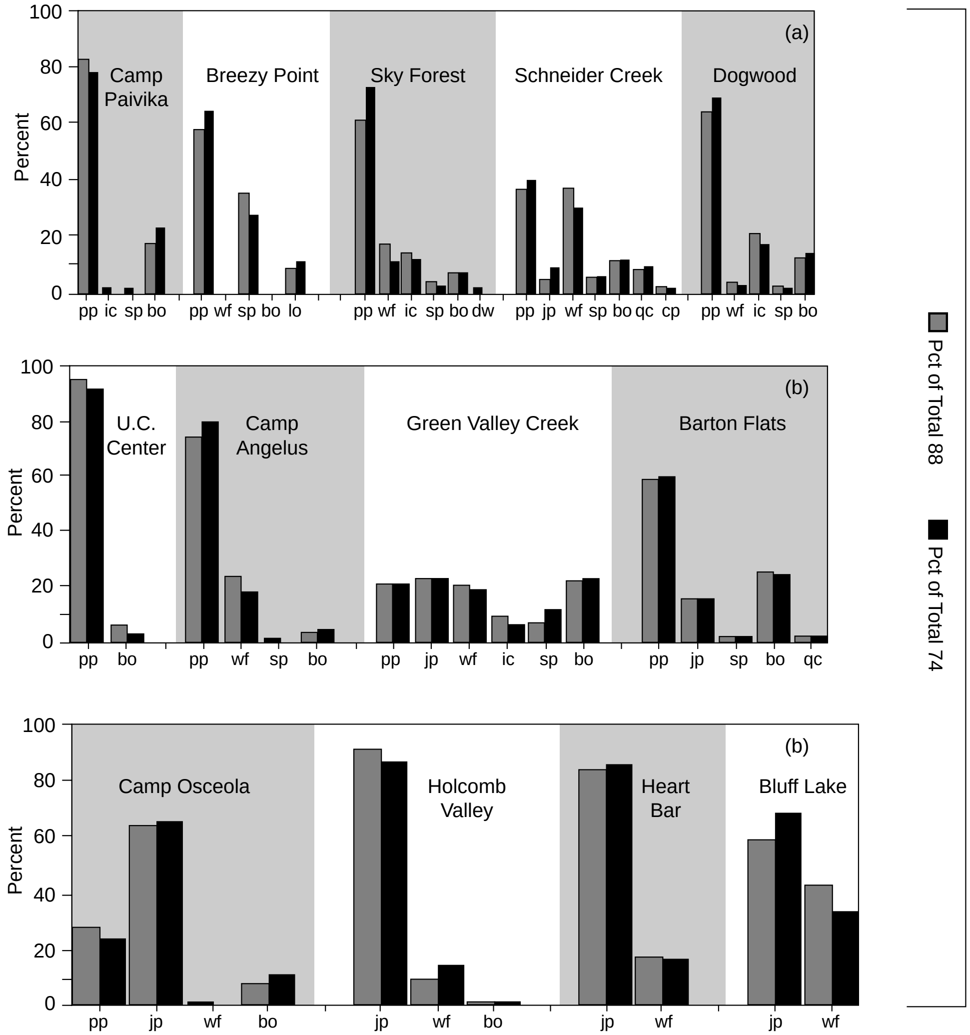


Figure 1—Total basal area for each species as percent of the total basal area for all species at 12 sites in the San Bernardino Mountains in Southern California (Miller and others 1991);
pp= ponderosa pine
ic= incense cedar
sp=sugar pine
bo=black oak
dw=dogwood
qc=Chrysolepis oak
cp=Coulter pine
jp=Jeffrey pine
lo=live oak.
wf=white fir

Conclusion

On the basis of these investigations of ozone injury in California, Colorado, Arizona, and Washington, the mixed conifer forest type in the Sierra Nevada and southern California mountains has become the major focus of interest and concern now and in the immediate future because of extensive ozone injury to ponderosa and Jeffrey pines. If ozone levels should increase with the expansion of urban areas in other regions of the West, a monitoring protocol should be established with baseline plots observed at long–term intervals of 5 or 10 years to track forest health.

Government Agencies' Need for Data on Ozone Injury to Western Pines

Brent Takemoto and Trent Procter[1]

Since the 1970's, researchers from the USDA Forest Service and USDI National Park Service have conducted field surveys to measure the presence or absence, amount, and changes of ozone–caused injury to ponderosa and Jeffrey pines (Pronos and others 1978, Pronos and Vogler 1981, Stolte and others 1992). However, differences in measurement protocols among studies and the lack of site–specific ozone concentration data have limited the extent that data can be used to project long–term or regional–scale trends. Although the effects of ozone on ponderosa and Jeffrey pines are still an important ecological concern in California, efforts to determine temporal or spatial trends in needle injury and efforts to relate injury to ambient concentrations of ozone would be enhanced if researchers used the same method to quantify amounts of ozone–caused needle injury.

In March 1989 a workshop was held at the Forest Service's Pacific Southwest Research Station, Riverside, California, to discuss Federal land management and State environmental protection agency needs regarding the effects of ozone on western pine tree species. The purpose of the workshop was to develop and document a recommended methodology for measuring ozone injury on pine trees in the western United States. This method would be used to collect quality–assured pine needle injury data. It would meet the needs of the participating organizations to monitor the condition of air quality–related values in class I areas (Forest Service, National Park Service), forest health (Forest Service) or the welfare effects of ozone in California (Air Resources Board). The participants agreed to prepare a document that described the methodology in detail, contained overviews of the effects of ozone on native vegetation, and reported methods or practices used to measure the amount of ozone–caused injury to ponderosa and Jeffrey pines.

Federal land managers (e.g., USDA Forest Service, USDI National Park Service) are responsible for assessing and preventing injury to forest trees and other air quality–related values in wilderness areas, National Parks, and National Monuments (cf. Clean Air Act of 1970, Clean Air Act Amendments of 1977 and 1990). The Clean Air Act Amendments of 1977 ensure that air quality in some regions of the United States, which is cleaner than required by National Ambient Air Quality Standards, would be protected from deterioration in future years. Natural resource areas, such as national wilderness areas and National Parks, were prioritized for protection. Class I areas, characterized by specific areal and formal designation criteria, were afforded the greatest degree of air quality protection (Bunyak 1993).

Air pollutant data showing the effects of ozone on ponderosa and Jeffrey pines are of interest to Federal land managers in California, particularly in the Sierra Nevada and San Bernardino Mountains. Because the harmful effects of ozone on western yellow pines are well– documented (Miller 1989), ozone is still considered by Federal land managers to be a regional– scale ecological stress to air quality–related values in wilderness areas in California (Peterson and others 1993). Pine needle injury–response data are an example of the kind of information Federal land managers may use to evaluate the impacts of current and future exposures to ozone on air quality–related values in their administrative domain.

In the State of California, the Air Resources Board of the California Environmental Protection Agency (Cal EPA) is responsible for planning and implementing air quality–related activities (cf. Title 17, California

[1]Air Pollution Specialist, California Air Resources Board, Research Division, 2020 L Street, Sacramento, CA 95814; Air Resource Management Specialist, USDA Forest Service, Regional Air Resource Program, Sequoia National Forest, 900 W. Grand Ave., Porterville, CA 93257

Administrative Code, Section 70101). This Board sponsors research on the causes and effects of air pollutants (Air Resources Board 1993), operates a network of air monitoring stations (e.g., Air Resources Board 1991), and sets policies for controlling pollutant emissions from mobile sources (Air Resources Board 1990). In addition, the Air Resources Board has sponsored research to characterize ozone concentrations in forests on the western slope of the Sierra Nevada (Carroll 1991, 1992) and supported studies to document the adverse ecological effects of air pollutants on ponderosa pine seedlings (Bytnerowicz and Temple 1993). Information on the effects of ozone on ponderosa and Jeffrey pines is considered by Air Resources Board staff when reevaluating the welfare effects component of existing State ambient air quality standards (Stromberg and others 1987).

Rationale for Federal and State Agency Involvement

Federal land managers have the responsibility to protect the air quality–related values in wilderness areas in their jurisdiction from the adverse effects of air pollutants (cf. Organic Act of 1916, Clean Air Act of 1970). Air quality–related values are air pollution–sensitive values (e.g., biological diversity, water quality, vegetation, wildlife) that characterize or define the basis for preserving lands as wilderness areas (Fox and others 1989). The Wilderness Act of 1964 (cf. Public Law 88–557) defines a wilderness as "an area untrammeled by man" and "an area of undeveloped Federal land retaining its primeval character and influence." Air quality–related values in wilderness areas include visibility, flora, fauna, cultural and historical resources, odor, soil, water and virtually all resources that are dependent upon and affected by air quality (Bunyak 1993).

The Clean Air Act Amendments of 1977 authorized Federal land managers to protect air quality–related values in designated wilderness areas from the adverse effects of air pollutants. Sections 160–169A of the Act Amendments established a program for the prevention of significant deterioration (PSD) of air quality. The principal objective of the PSD program is to prevent the degradation of air quality in areas that are in compliance with National Ambient Air Quality Standards, while maintaining a margin for pollutants generated by future emission sources (Peterson and others 1993). To implement the PSD program, Federal land managers review applications for new and modified sources of air pollutants that may impact their administrative domain (e.g., proposed sources within 100 km of a wilderness area).

The review of a PSD application requires Federal land managers to perform a rigorous analysis to: (1) determine which control technology may need to be used to minimize pollutant emissions, (2) estimate the amount of pollution contributed by a proposed project to existing ambient pollution levels, and (3) assess impacts to air quality–related values. On the basis of the findings of their analysis, Federal land managers advise the U.S. Environmental Protection Agency (EPA) or the state permitting authority if emissions from a proposed project may adversely impact a wilderness area (Bunyak 1993).

U.S. Department of Agriculture, Forest Service

In the U.S. Department of Agriculture, the Forest Service has delegated the responsibility to review PSD applications to its nine regional offices. For example, PSD applications in California are reviewed by staff in the Pacific Southwest Region, headquartered in San Francisco, California. The Pacific Southwest Region is also responsible for reviewing applications submitted in Hawaii, Guam and other islands with Trust Territory status. In addition to the PSD program, the Pacific Southwest Region of the Forest Service also conducts research on the long–term effects of air pollutants on vegetation in the western United States (Miller 1989). Ozone injury surveys have been conducted since the 1960's in the San Bernardino Mountains (Miller and Millecan, 1971) and since the 1970's in the Sierra Nevada (Pronos and others 1978).

U.S. Department of the Interior, National Park Service, Air Quality Division

In the U. S. Department of the Interior, the National Park Service, Air Quality Division reviews PSD applications for the National Park Service. Because clean air is fundamental to visitor enjoyment, environmental quality, scenic vistas and the preservation of natural and cultural resources in National Parks and wilderness areas, the National Park Service has incorporated air resource management into park operations and planning. In support of its resource management goals, the National Park Service has also sponsored ambient ozone monitoring and research in some National Parks where ozone–caused injury to ponderosa and Jeffrey pines has been observed (Patterson and Rundel 1990). Currently, the National Park Service continues its efforts to identify symptoms of air pollution injury, examine the mechanistic basis of air pollution injury, and understand its significance to individual species and plant communities.

State Environmental Protection Agencies— California Air Resources Board

National Ambient Air Quality Standards are established by the U.S. Environmental Protection Agency to protect human health and welfare from the harmful effects of air pollutants on a national–scale. However, because of the severity of the air pollution problem in California, more stringent state ambient air quality standards have been adopted for selected criteria air pollutants, including ozone. The Air Resources Board (ARB) of California re–examines the technical basis for existing air quality standards (Stromberg and others 1987), sponsors research projects to investigate the causes and effects of a variety of air pollutants (Air Resources Board 1993), operates extensive criteria and toxic air pollution monitoring programs (Air Resources Board, 1991), develops rules/regulations to control pollutant emissions from motor vehicles (Air Resources Board 1990), and works with regional and local air quality management districts to attain and maintain state ambient air quality standards.

Summary

The effects of ambient concentrations of air pollutants on the health of forest trees is of concern to Federal land management and State environmental protection agencies. In California, ponderosa and Jeffrey pines are an important air quality–related value, as they are known to be sensitive to elevated concentrations of ozone. Data from field surveys to assess ozone–caused needle injury on ponderosa and Jeffrey pines, combined with quality–assured ozone concentration data from government agency–funded research and air monitoring programs, collectively provide valuable information for decision–makers concerned with forest ecosystem management and/or regulating sources of air pollutants in California. Quality–assured ozone concentration and pine needle injury response data are needed by Federal land managers to review PSD applications for new and modified sources that may impact wilderness areas, and by environmental protection agencies to evaluate the welfare effects of ozone in examining the basis for new or existing ambient air quality standards.

Symptomology of Ozone Injury to Pine Foliage

Kenneth Stolte[1]

Symptoms of ozone injury on western pines, ranging from effects on needles to effects on portions of ecosystems, can be differentiated from symptoms induced by other natural biotic and abiotic stressors occurring in the same area. Once identified in laboratory and field studies, quantification and monitoring of these symptoms can be used to provide reliable information on the effects of air pollution on forest trees and ecosystems. Monitoring symptoms of ozone injury can be used to determine status, changes, and trends in effects ranging from needle injury to tree growth. This chapter provides information on the types and patterns of ozone effects on pines, with emphasis on tree level effects in the western United States.

Identification of Ozone Symptoms

Ozone pollution leaves no elemental residue that can be detected by analytical techniques, as do fluorides (Carlson and Dewey 1971) and sulfur dioxide (Smith 1990). Therefore, the visible response of the needles and tree crown to ozone is the only detectable evidence of ozone as the causal agent for injury. These "ozone response variables," made at the needle, whorl, branch, tree, or stand level, and aggregated in analysis, can be an effective way to quantify ozone effects on pines (Stolte and others 1992). Ozone response variables include microscopic and macroscopic injury to the foliage (primary effect), and injury to branches and roots (secondary effects) that can be quantified within defined levels of accuracy and precision.

When exposed to elevated ambient ozone in excess of background levels common to the site (cumulative exposures greater than 60 ppb; Lefohn and others 1990, 1992), ponderosa and Jeffrey pine may show symptoms of decline that begin with invisible damage to mesophyll cells and subsequent visible discoloration of the foliage. Gaseous pollutants pass through the stomata of conifer foliage and cause direct damage to the photosynthetically active mesophyll cells, often producing a diagnostic visible injury pattern (Evans and Miller 1972b). Next, degeneration of essential biological processes in the needles occur that may eventually lead to a manifestation of other crown response variables, such as accelerated needle abscission, reduced crown vigor, increased susceptibility to other pathogens, and tree death (Miller and Elderman 1977).

Controlled exposures and field observations of ozone effects on western conifer species have confirmed that a distinct visible symptom known as chlorotic mottle typically occurs on needle surfaces (Miller and others 1963, Richards and others 1968). Chlorotic mottle begins as the walls of mesophyll cells below the epidermis degrade, causing the loss of cellular contents and the subsequent degradation of chlorophyll within the cell (Evans and Miller 1972a, Evans and Miller 1972b, Rice and others 1983). Microscopically this condition appears as amorphous staining of cellular contents, plasmolysis of cell contents, and cell death. The degradation of chlorophyll beneath the epidermis appears on the needle surface as amorphous chlorotic blotches with diffuse borders that occur in irregular patterns, giving a yellow "mottled" appearance; hence the terminology "chlorotic mottle" (*fig. 1*). This foliar injury symptom is visibly distinct from foliar symptoms induced by other air pollutants.

Chlorotic mottle frequently appears in the one-third of the needle surface nearest the tip on 1-year-old or older needles, and progresses basipetally until the entire needle is affected (Miller and others 1963, Richards and others 1968).

[1]Deputy Director, Forest Health Program, Southern Research Station, USDA Forest Service, 3041 Cornwallis Rd., Research Triangle Park, NC 27709

Figure 1—Healthy ponderosa pine needles are shown on the right one–third of the photo compared to chlorotic mottle caused by ozone injury that gradually increases in intensity on the left.

This pattern is observed mainly in southern California. In the Sierra Nevada the mottle tends to occur randomly along the entire needle length (Pronos and others 1978). The current–year's needles will show small amounts of chlorotic mottle only when summer ozone exposure levels are higher than usual and/or adequate soil moisture contributes to higher stomatal conductance and more ozone flux to needles, or both. This condition is usually the exception and not the rule for the response of current year needles to ozone exposures in the Sierra Nevada. Tip necrosis or necrotic bands can result from acute ozone exposures in fumigation experiments, but chronic field exposures typically induce only chlorotic mottling. Needle whorl loss can occur after chlorotic mottle injury, without the occurrence of tip necrosis, on the foliage of ponderosa pine is (*fig. 2 a–e*).

Foliar Symptoms Caused by Insects and Fungi

Foliar injuries resulting from biotic agents may appear on needle surfaces, confounding diagnosis of ozone injury. Chlorotic and necrotic spots or blotches caused by sucking insects, such as aphids and pine needle scale, may sometimes closely resemble ozone chlorotic mottle. The most common confounding pests are fungi (Scharpf 1993), and chewing (needle weevil) or sucking (scale or aphids) insects (Miller and Elderman 1977, Pronos and others 1978). Chlorotic mottle can be differentiated from injuries brought about by these diseases and insects by in-hand observation of the color and pattern of the symptoms on the needles. Careful observation may reveal the presence of fungus fruiting bodies (*fig. 3*). Close inspection of chlorotic islands of tissue (aided by a hand lens) often reveals a distinct necrotic point at the center of the discolored area, where the insect penetrated the epidermis with its piercing mouth parts (*fig. 4*). Remnants of the insects may also be present on the needles, for example aphid honeydew or the waxy or shell-like coatings that protects female scale insects throughout their adult life. Scales can cover 50 percent or more of the surface area of pine needles, with associated chlorotic and necrotic blotches on the opposite sides of the needles (*fig. 5*).

Elytroderma needle cast fungus (*Elytroderma deformans*) causes a reddish-brown foliar discoloration, extending from the tip to base of the needle and leading to premature abscission of two–year–old ponderosa pine needles (*fig. 6*). *E. deformans* infected needles can occasionally be identified by the presence of dark, elongated fruiting bodies on the needles, and entire crowns of trees may show the presence of witches brooms (*fig. 7*) (tightly packed profusions of short branches) (Sharpf 1993). Similarly, *Lophodermella cerina* (pine needle cast) causes reddish-brown discoloration of ponderosa pine needles with a distinct band

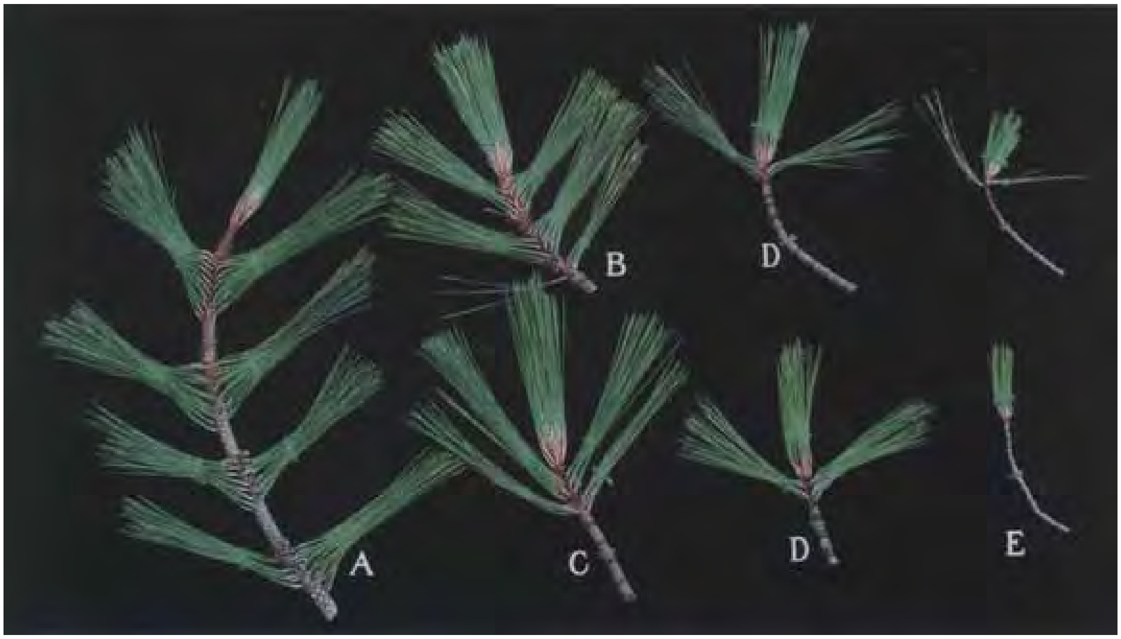


Figure 2—Progression of ozone injury on ponderosa pine branches sampled from different trees on the same site in 1994. The same progression could also occur on a single tree over time. A) A nearly healthy branch with five annual whorls of needles (numbered whorls 1-5 from youngest to oldest) with small amounts of chlorotic mottle near needle tips on whorls 4 and 5. B) A branch with four annual whorls with chlorotic mottle on whorls 3 and 4; abscission of needles in whorl 4 has begun. C) Another branch with three annual whorls retained, and with considerable mottle on whorl 3 and a small amount on whorl 2. D) [upper] A branch with only two annual whorls has slight development of mottle on needles of whorl 2. D) [lower] Severe chronic ozone injury results in mottle on whorl 1 needles. E) [upper and lower] In the most severe cases of injury at the highest ozone exposure, only the current year's needles remain; also, they are shorter and have over 30 percent mottle on them.

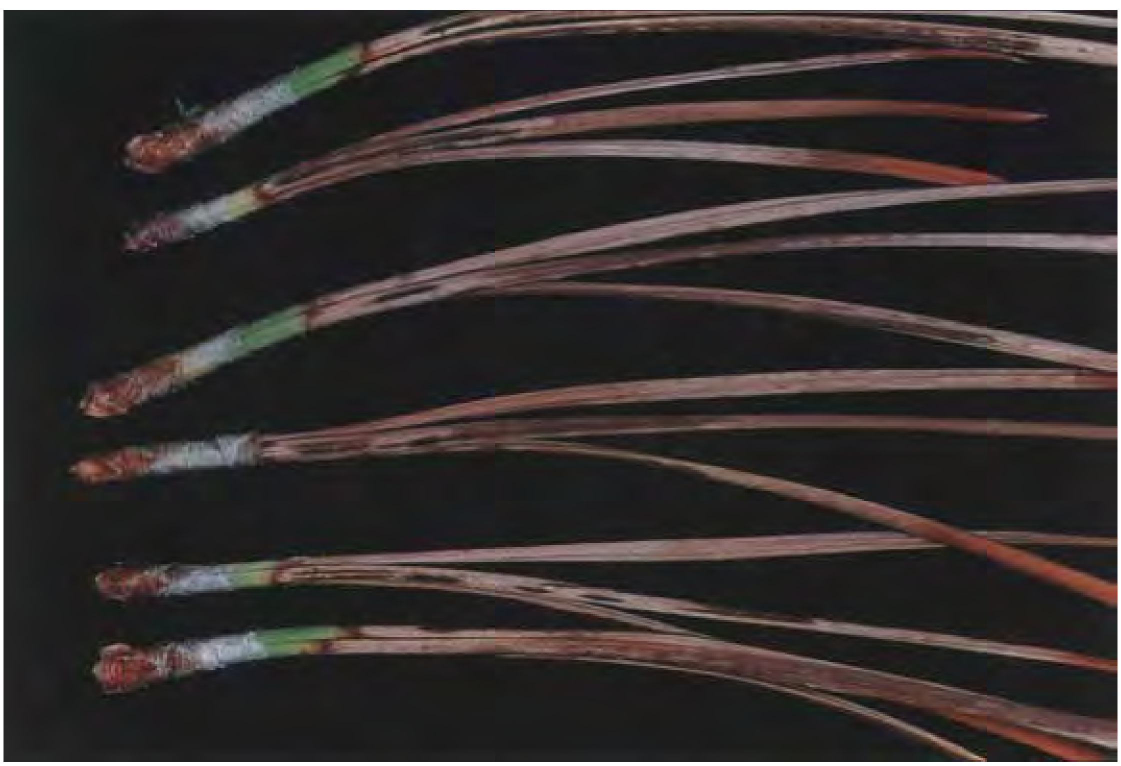


Figure 3—Elytroderma needle disease may be identified by the elongated dark fruiting bodies of the causal fungus.

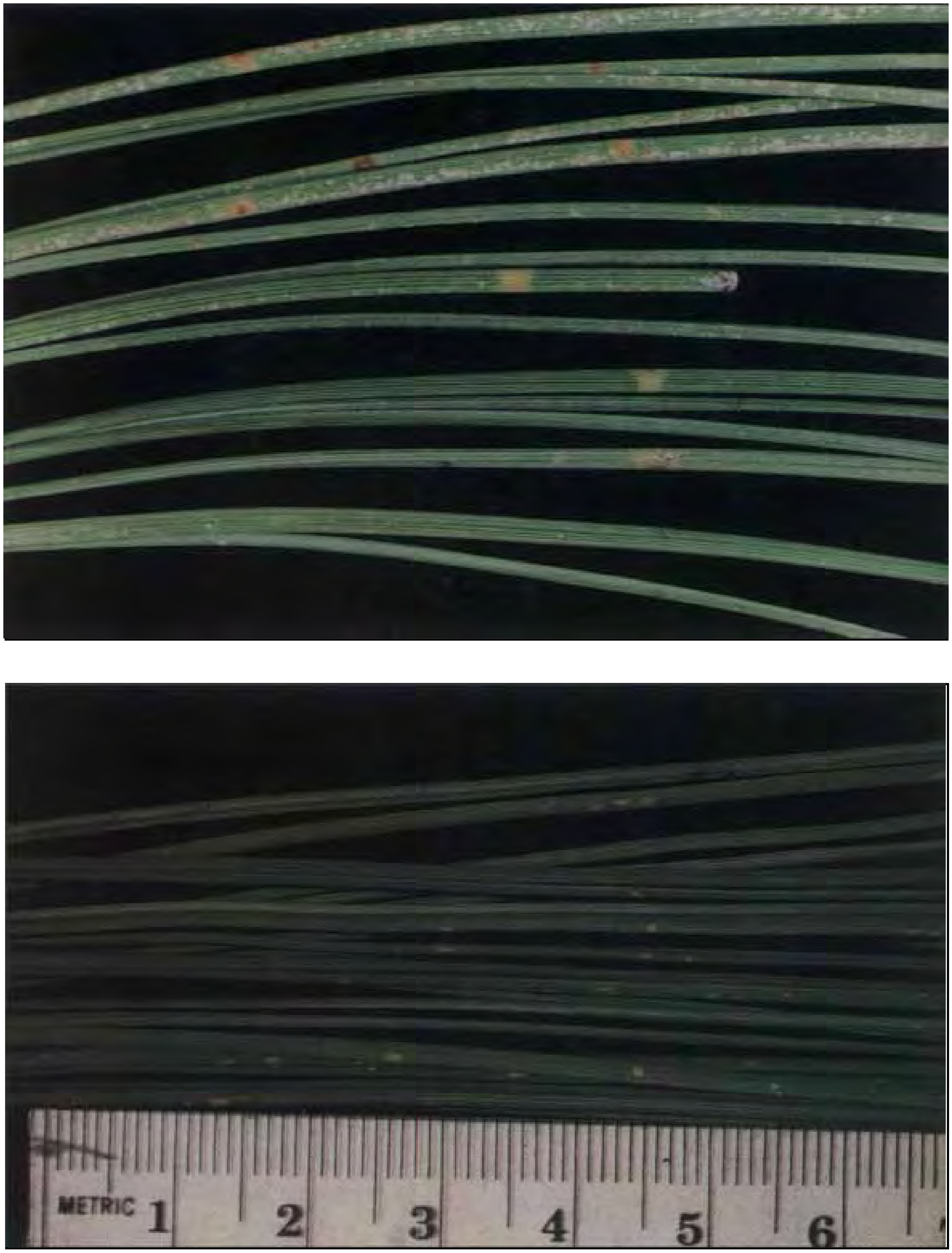


Figure 4—Yellow bands (upper photo) or yellow spots (lower photo) may reveal a distinct necrotic point in the center believed to be the mark left by the piercing mouthparts of sucking insects.

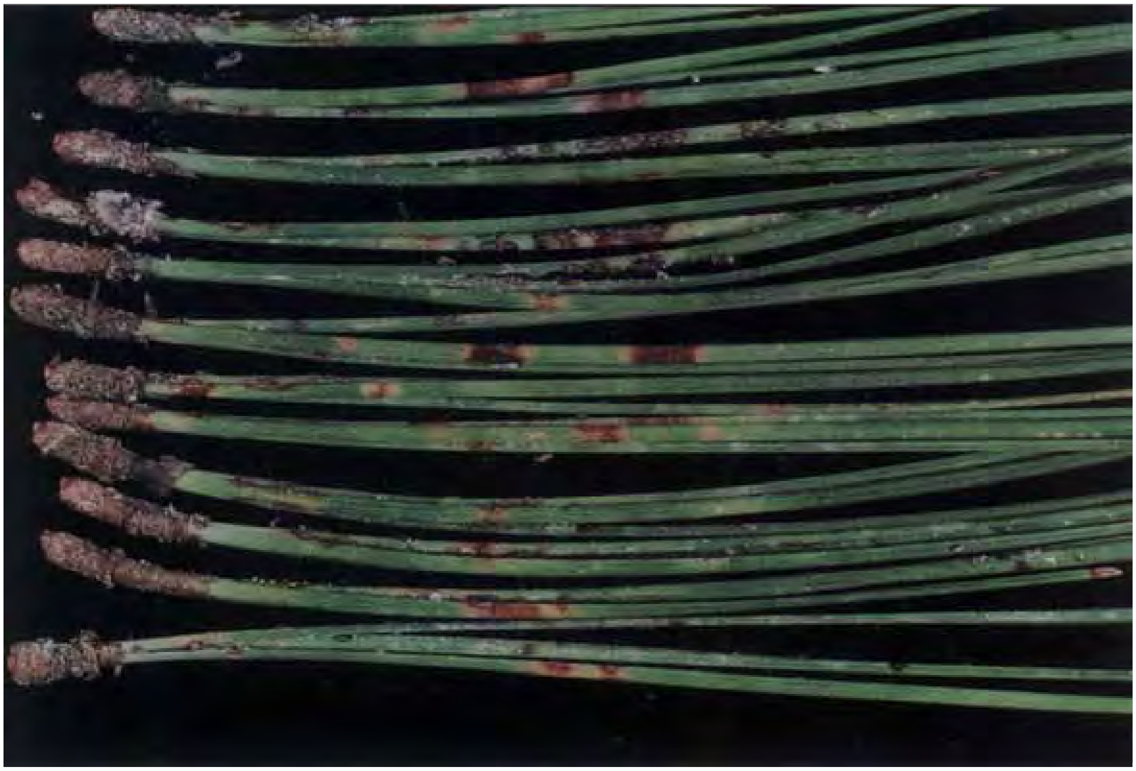


Figure 5—Yellow or brown bands or islands of needle tissue may be caused by the long–term residence of scale insects. In this photo the black scale insect still remains on the needle.

Figure 6—Elytroderma needle disease is identified on ponderosa and Jeffrey pines by the reddish brown color of remaining needles and the small "broom–like" structures formed by infected branches.

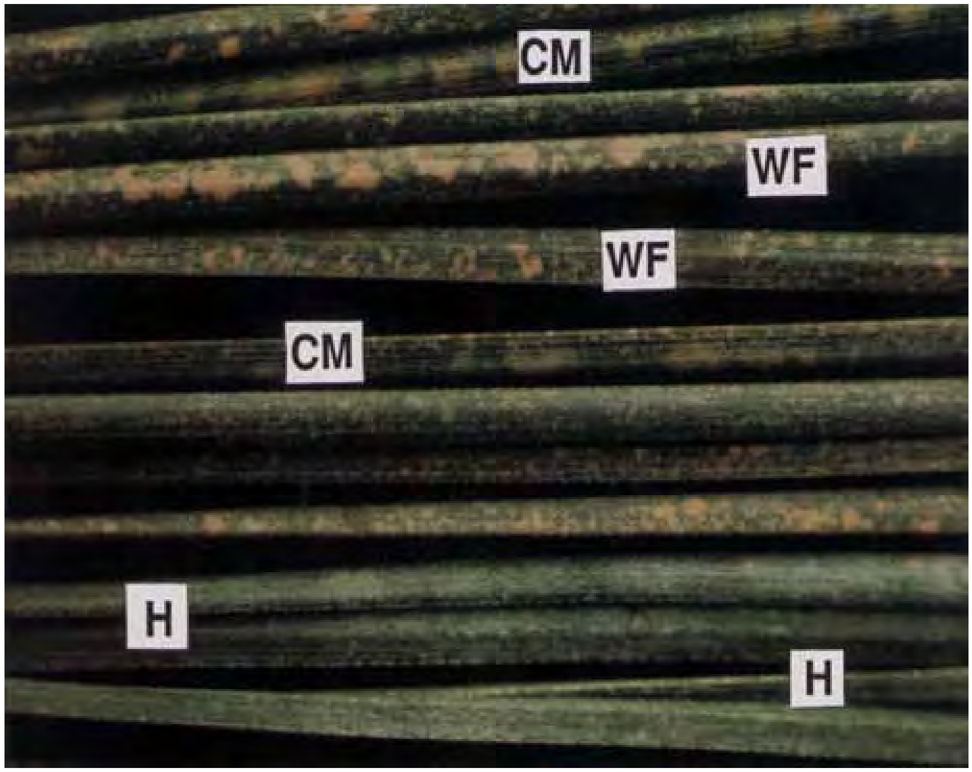


Figure 7—Three healthy (H) ponderosa pine needles (lower) are compared to those with a mixture of chlorotic mottle (CM) caused by ozone, best illustrated by the second needle from the top in which irregular chlorotic islands are visible. Weather fleck (WF) is displayed by the fourth and fifth needles from the top; note that weather fleck lesions are tan, not yellow, and borders are more irregular in shape. Weather fleck is found exclusively on the upper surfaces of needles (facing the sky). Chlorotic mottle can occur on all surfaces and is best evaluated on the lower surface where fleck is not present.

near the middle and premature needle abscission that results in thin crowns (Scharpf 1993).

Needle Symptoms Caused by Abiotic Factors

Foliar injuries from abiotic factors, other than ozone, may be present on pine needles as well. Winter fleck of pine needles is apparently caused by snow and cold temperatures and is a symptom that superficially resembles chlorotic mottle. The lesions, when large, can be irregular in size and shaped like chlorotic mottle, but are tan or brown and vastly more abundant on the adaxial surface of the needle, suggesting the role of sunlight in their formation (Miller and Evans 1974). Also, smaller weather fleck lesions tend to be more round in shape, with distinct margins to the lesions. Histological examination has shown that weather fleck results from a uniform plasmolysis of all mesophyll cells lying under the surface lesion and can cause collapse of the epidermis, but ozone injury affects only mesophyll cells under the epidermis randomly, usually without structural loss to the epidermis. Symptoms of chlorotic mottle and weather fleck can be differentiated even when they are found together in significant amounts on the same needles (*fig. 7*).

Needle injury from road salt accumulation, drought, desiccation, winter injury, and lightning can be visually distinguished from ozone injury by the color, shape, pattern of development on the needles, and effects on the tree crown. Salt accumulation and drought stress on conifers cause a discoloration of all the needles on the branch, with the tips usually turning brown. Salt accumulation affects the entire crown, but with drought stress the crown starts dying at the top first (Scharpf 1993). Ozone injures the older whorls of needles first on a branch, and typically affects the crown through mortality of branches starting at the bottom and progressing to the top (Miller and others 1963).

Crown Changes Associated with Chronic Ozone Injury

In many conifer species, foliage longevity can be measured by counting nodes on branches back from the branch tip to the oldest whorl, with each node separating a annual whorl of needles or needle fascicles corresponding to one year of growth. A direct relationship between the incidence of chlorotic mottle and accelerated abscission of needles has been shown for ponderosa pines in southern California and for ponderosa and Jeffrey pines in the Sierra Nevada of California (Duriscoe and Stolte 1989, Miller and Van Doren 1982). Reduction in needle longevity (*fig. 2d,e*) is recognized as an indicator of air pollution stress for these species when other factors leading to accelerated abscission of needles are taken into account. In unpolluted areas of the west coast, ponderosa pines may be expected to retain foliage for 3 to 5 years, with an average of four annual whorls retained at any given time (Sudworth 1908).

Foliage longevity is related to other factors, most particularly the elevation at which a tree grows (Ewers and Schmidt 1981), which is an indication of the length of the growing season at a particular site. Trees at higher elevations consistently retain their foliage longer (as high as 47 years for bristlecone pine, *Pinus aristata*). Interpretation of data on foliage longevity must consider other confounding factors, for example, persistent infections of needle cast fungi can lead to tree crowns that are extensively defoliated.

The bole and other crown variables that are associated with growth and overall tree vigor can respond to elevated ozone exposures. Branch mortality in the lowest portion of the crown has been observed in southern California (Parmeter and Miller 1968), leading to a decrease in vertical crown length, as measured by percent live crown (Stark and others 1968). Before lower branch mortality occurs, a decline in vigor in the lower crown may be observed as a reduction in needle length (Parmeter and others 1962), and the production of fewer numbers of needle fascicles (Duriscoe and Stolte 1989, Ewell and others 1989). A reduction in the vertical and radial growth of stems has been documented for ozone-stressed trees in southern California and southern Sierras (McBride and others 1975, Peterson and others 1991, 1995), Cone and seed production can also be reduced by ozone stress in ponderosa pines (Miller and Elderman 1977). Oleoresin exudation pressure, yield, and rate of flow were all substantially reduced in oxidant-injured ponderosa pines in southern California, while the crystallization rate was observed to increase (Cobb and others 1968). The moisture content of phloem and sapwood were found to be reduced, as well as a reduction in phloem thickness. These phenomena have been associated with susceptibility to cambium damage from the heat of fire and successful attack by bark beetles (Cobb and others 1968, Graban and Duriscoe 1992).

Interaction of the Effects of Drought, Low Temperature and Bark Beetles with Ozone Injury

Temple and others (1992) compared well–watered and drought stressed ponderosa pine seedlings exposed to ambient and 1.5× ambient ozone at a site on the western slope of the Sierra Nevada near Sequoia National Park. Drought stressed seedlings developed very low levels of visible injury compared to well–watered seedlings. Defoliation did not develop with drought stressed seedlings but did result with well–watered seedlings.

Peterson and others (1995) studied the long–term radial growth of bigcone Douglas–fir (*Pseuotsuga macrocarpa*) throughout its natural range in the San Bernardino Mountains of southern California. Sample points were located along a gradient with simultaneously decreasing ozone concentration and moisture availability. Short–term growth reductions induced by drought were an important component of longer term growth reductions at sites with high ozone exposure. Thus an ozone–climate stress complex may be responsible for recent reductions in the growth of bigcone Douglas fir in this region.

The widespread occurrence of weather fleck symptoms on upper surfaces of needles of Western pines is not well understood, but other conifers may provide clues. One of the mechanisms that may explain winter injury to Norway spruce foliage is prior exposure to ozone. Ozone exposure of seedlings in summer (6 hr/day for 60 to 70 days at concentrations up to 150 ppb) did not result in ozone symptoms. After freezing the older needles of saplings exposed to 100–120 ppb had more necrosis than needles kept in carbon–filtered air (Brown and others 1987 and Barnes and Davidson 1987). Ozone injury apparently increased water loss of excised needles from one of five clones tested (Barnes and Davidson 1987). Cumming and others (1988) have shown that twice ambient ozone exposure (Ithaca, NY) decreases the winter hardiness of the newest needles of red spruce seedlings. Laurence and others (1989) also observed a relationship between visible winter injury and ozone exposure on red spruce seedlings.

Severe pollutant injuries to tree crowns may eventually lead to weakening of symptomatic (pollutant sensitive) individuals. Ponderosa and Jeffrey pine trees that retain less than two whorls of needles and have high levels of chlorotic mottling on the remaining needles have been shown to have increased susceptibility to natural biotic and abiotic stresses. Stark and others (1968) reported that ozone stressed ponderosa and Jeffrey pines in the San Bernardino Mountains of southern California suffer from increased susceptibility to pine beetles (*Dendroctonus brevicomis*) as a result of increased needle defoliation, decreased photosynthetic capacity, suppressed radial growth, and reduced retention of nutrients in the foliage.

Stand–Level Changes Associated with Chronic Ozone Injury

The extent and cause of tree mortality, and subsequent effects upon the structure and composition of forests, has been shown to be related to elevated ozone exposures in the San Bernardino Mountains of southern California. All trees within a fixed-area plot were tallied and monitored for decades, and changes in successional patterns were found (Miller and others 1991). In the western portion of these mountains, the interaction of air pollution stress and a human-modified fire regime have brought about the alteration of ponderosa pine forests in favor of ozone-tolerant incense cedar and white fir.

History of Ozone Injury Monitoring Methods and the Development of a Recommended Protocol

Daniel Duriscoe, Kenneth Stolte, and John Pronos[1]

The minimum requirement for long–term monitoring of air pollution effects on forest stands is to develop methods for observers to locate, evaluate, and re–evaluate individual trees at intervals of one or more years. Studies of this nature have used permanent quadrats or "plots" in which individual plants are tagged or mapped. Multiple levels of information can be gathered from this approach depending on how the plots are structured, how many plots are established in a given area, the size and shape of plots, and how the plots are located. The appropriate level of complexity depends on the research or management objectives and resources available.

A simple objective in air pollution effects studies is to track changes in the crown condition of selected individual trees of one or more pollutant–sensitive species. More comprehensive assessments are possible, such as the inventory and monitoring of the population distribution and structure of the sensitive species and all associated plant species within the plot. Such information is valuable in describing plant community succession, which may be affected by air pollution stress.

This paper reviews previous work that describes long–term trends in crown condition of ozone–sensitive ponderosa and Jeffrey pines—such as the oxidant injury score (OIS) developed for use in the San Bernardino National Forest (SBNF) (Miller 1973); the USDA Forest Service, Forest Pest Management method (FPM) (Pronos and others 1978); the USDI National Park Service, Air Quality Division method (AQD) (Stolte and Bennett 1985), and the Eridanus injury index (EII), proposed by Duriscoe (1988)—and recommends a procedure for defining sub–populations and locating plots in future ozone injury evaluation efforts.

Methods of Assessing Crown Condition

Oxidant Injury Score

The major components of the Pacific Southwest Research Station oxidant injury score (OIS) or SBNF index (Miller 1973) include determination of the severity of chlorotic mottle, number of whorls retained, normal needle length, and branch mortality:

Portion of crown and attribute		*Numerical code*
Upper half:	Number of needle whorls	Count: 1,2,3, etc.
	Condition of each whorl:	
	Green, healthy	4
	Chlorotic mottle	2
	Brown, dead	0
	Needle length (estimate):	
	Average length	1
	Shorter than average	0

[1]Biological Technician, Resources Management, Sequoia and Kings Canyon National Parks, USDI National Park Service, Three Rivers, CA 93271; Deputy Director, Forest Health Monitoring Program, Southern Research Station, USDA Forest Service, 3041 Cornwallis Rd., Research Triangle Park, NC 27709; Plant Pathologist, Forest Pest Management, Stanislaus National Forest, USDA Forest Service, 19777 Greenley Rd., Sonora, CA 95370

Portion of crown and attribute		*Numerical code*
Lower half:	Repeat measurements described above, and estimate lower branch mortality	
	Branch mortality:	
	Average branch mortality	1
	Excessive mortality (Allowing for competition with neighboring trees)	0

The scores for each characteristic are added to obtain an ozone injury score for each tree. For example, consider a tree with 3 annual needle whorls in both upper and lower crown. The two youngest whorls were both rated as 4 and the older whorls each as 2, and the needle length and branch mortality were average (for the site and canopy position of the tree in the stand). This tree would have the summed score of 29 (whorl retention 3+3; upper and lower needle condition 4+4+2+4+4+2; upper and lower needle length 1+1; and branch mortality +1). Descriptive values associated with different score ranges were assigned as follows:

0–9 = Very severe
10–14 = Severe
15–21 = Moderate
22–28 = Slight
29–35 = Very slight
36+ = No visible symptoms

This method has been employed for monitoring ozone impacts on pines in the SBNF for 30 years (Miller 1963, Miller and others 1989). Needle retention and condition data were obtained by hands–on inspection of foliage in the lower crown (when it was within reach), and binocular evaluation of the lower and upper crown of tall trees. A minimum of 50 trees with DBH > 12 in were evaluated within the 50 ft wide plots of sufficient length to contain the 50 overstory trees. In addition, all other trees with DBH ≥ 4 in were tallied and evaluated for ozone injury.

This method has some important deficiencies, however, because needle condition is distinguished only as healthy, some level of chlorotic mottle, or as necrotic (dead); the intensity or severity of chlorotic mottle is not evaluated on each whorl. In addition, needle whorl retention, needle condition, and needle length on tall trees are determined by averaging several fields of view in the binoculars. This sampling method is not as amenable to determinations of precision and accuracy as are the same measurements made with the Air Quality Division (AQD) method because it does not permit statistical description of injury differences on the basis of chlorotic mottle, needle whorl retention, and needle length. Other problems with this method include branch mortality evaluations related to the best looking trees in the plot, which is estimated—not measured; and chlorotic mottle that is difficult to detect and distinguish from other foliar discolorations through binoculars when the sky is cloudy or overcast, or under windy conditions. Thus, the primary deficiency of this method is that it relies on relatively subjective evaluations.

Forest Pest Management Method

A second field method was developed by the USDA Forest Service's Forest Pest Management (FPM) Staff (Pronos and others 1978) to evaluate and monitor the long–term effects of ozone exposure on ponderosa and Jeffrey pines in the Sierra Nevada. The FPM method provides a means for making repeated estimates of ozone injury on the same trees at the end of each summer season. The FPM method obtains data from hands–on inspection of a sample of branches on small trees or pruned from the lower crowns of large trees. Ozone injury is quantified by noting the youngest whorl of needles showing chlorotic mottle symptoms from ozone:

Individual Tree Score	Youngest Needles with Symptoms	Average Plot Score	Severity of Injury
0	Current year	0 – 0.9	Very severe
1	Second year	1.0 – 1.9	Severe
2	Third year	2.0 – 2.9	Moderate
3	Fourth Year	3.0 – 3.9	Slight
4	Fifth or older	4.0	No injury

The FPM method has been used on both Forest Service and National Park Service lands. A considerable amount of data has been reported based on the FPM method (Allison 1982, Allison 1984a, 1984b, Duriscoe and Stolte 1989, Pederson 1989, Pronos and others 1978, Pronos and Vogler 1981, Wallner and Fong 1982, Warner and others 1982). The FPM method indicates the number of healthy needle whorls retained, but only up to a maximum of 4 and therefore does not describe the presence or the severity of chlorotic mottle on whorls 1 to 4. It also does not describe abnormal needle length or branch mortality in the lower crown, although all insects and diseases present on plot trees are included.

Air Quality Division Method

Experience gained from the OIS method and the FPM method was used to provide improvement and standardization of operational procedures in a method proposed by the National Park Service, Air Quality Division (AQD) (Stolte and Bennett 1985). This method described the complete process of establishing long–term monitoring plots and evaluating ozone injury in the crowns of trees, including locating plots on the landscape, criteria for accepting the plot as a valid unit, means for permanently marking plots and individual trees, and procedures for evaluating and analyzing data. This method involved quantification of:

- Visible ozone injury symptoms (chlorotic mottle)
- All abiotic and biotic injuries on needles of each whorl
- Needle retention per whorl and number of whorls per branch
- Modal needle length per whorl
- Upper and lower crown density.

The foliar injury symptoms and retention of needles and whorls were summed into a non–additive index.

The AQD method used 15 suitable (not too large to prune) ponderosa or Jeffrey pines per plot. Needle–level observations were based on five branches pruned from the lower crown. The systematic improvement represented by the AQD method has provided a basis for a synthetic approach to monitoring crown condition of trees that allows for the identification and analysis of many contributing factors. Replicate measurements of as many variables as possible led to a better understanding of the variance that can be expected in such observations at several levels of biological organization. This is important because replicate observations of lower levels (e.g. branches) are often averaged to derive an estimate for a higher level (e.g. tree), and the precision of this estimate will depend upon the variance in the observations.

This method has some disadvantages, however. Because only 15 trees were included in each plot, subsequent work by Duriscoe (1988) has shown that, in areas where injury levels are moderate to high, 30 to 50 trees are required for plot–to–plot comparisons to be statistically meaningful because of large within–plot variability. In addition, percent live crown was not included in the tree–level index. This tree characteristic is considered to be important to characterize the response of trees to competition from surrounding trees, determine the volume of crown subject to ozone stress, and estimate lower crown branch mortality, which is related to ozone stress. And finally, because it was first used in the Sierra Nevada in established plots that had been evaluated using the FPM method, time–series comparisons used only the components of the AQD index that were parallel with the FPM method. The AQD index was generally not reported.

Studies Evaluating the Efficiency of Crown Evaluation Methods

Duriscoe (1988) and Muir and Armentano (1987, 1988) conducted studies about improvement and modification of plot design, tree selection method, numbers of trees within plots, and kinds of crown variables to be measured. Muir and Armentano (1988) performed a study comparing data derived from hands–on evaluation of pruned branches with data from branches of the same tree inspected with binoculars and spotting scopes as a means of discovering biases and sources of error inherent in the use of optical instruments. Evaluations with optical instruments of the upper crowns of ponderosa pines were not highly correlated with hands–on observations. The former method underestimated chlorotic mottle and needle retention. After adjustment for bias the optical methods met criteria for accuracy and precision in the lower crown for needle retention, but not chlorotic mottle. Muir and Armentano (1988) concluded that under the conditions of this test, with observers generally unfamiliar with the use of scopes and binoculars for this purpose, the method was inadequate for characterizing injury. Thus, the OIS or SBNF method apparently could not be reliably used by novice observers with minimal training or experience. Binocular observations by experienced observers were not compared to hands–on observations, however.

Duriscoe (1988) evaluated the between–tree variability in stands of ponderosa and Jeffrey pines where stands were characterized as slight, moderate, and severe for ozone injury. He found that in order to distinguish a moderately injured stand of trees from a slightly injured stand it was necessary to evaluate at least 30 trees in each stand (*fig. 1*). The separation between stands was most pronounced when 50 trees were evaluated, but little additional statistical power was gained beyond the evaluation of 30 trees.

Standardized Crown Condition Evaluation Method for Ponderosa and Jeffrey Pines

Further development of a standardized crown evaluation method was reported by Duriscoe (1988), who proposed an additive tree–level index combining data from four crown condition variables collected according to the protocol established by the AQD method. An important advantage of this method is that variables are measured directly when possible (e.g. needle length with a centimeter rule) or estimated at close range from pruned branches, reducing subjective judgment by the observer as much as possible (Stolte and Bennett 1985). The index has been known as the Eridanus injury index (EII) and more recently has been modified and renamed the ozone injury index (OII) (Guthrey and others 1993). It includes many of the desirable characteristics described by

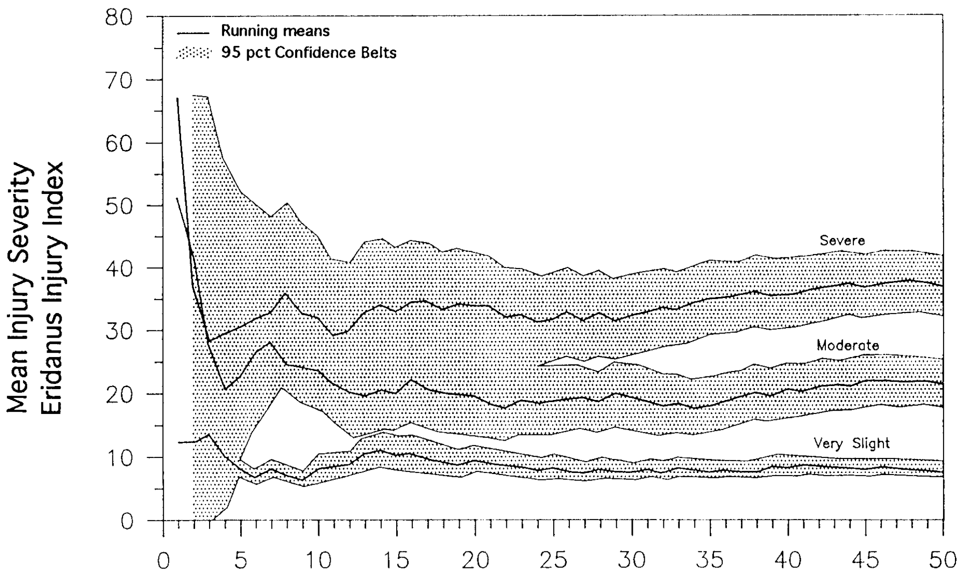


Figure 1—Variability in ozone injury between stands of ponderosa and Jeffrey pine in the Sierra Nevada (Duriscoe 1988). Line represents mean injury and shaded area represents the 95 percent confidence interval associated with the mean. (a) Severely injured stand with high variability in injury between trees within the stand. (b) Moderately injured stand with less variability in injury between trees. (c) Slightly injured stand with little between–tree variability and narrow confidence interval. Injury differences between stands are most obvious when at least 30 trees are sampled.

Muir and McCune (1987) for an index that quantifies air pollution effects on crown condition by including:

- Equal weights for component symptoms or carefully chosen differential weights
- Non–subjective (quantitative) symptom categorization
- Additive index
- Zero–minimum and maximum at the highest possible injury level
- Monotonic, nearly linear relationship to dose across the widest possible pollution gradient.

An interagency task group, convened in 1989 at the USDA Forest Service, Pacific Southwest Research Station, Riverside, California, also concluded that the EII was the most effective method. In summary, four crown response variables were selected and each was weighted so that it represented a specified percentage of the total index value as follows:

- Number of whorls retained (weighted 40 percent), including quantification of whorls with less than full needle retention (averaged from at least five representative branches pruned from the lower crown)
- Relative proportion of the surface area of each needle whorl covered with chlorotic mottle (weighted 40 percent) at a level of precision that can be realistically expected of a visual estimate (also averaged from the same pruned branches)
- Modal needle length of each whorl (weighted 10 percent) measured to the nearest centimeter with a ruler
- Percent live crown (weighted 10 percent) measured with a clinometer.

The EII aggregates field data on chlorotic mottle and needle retention that was collected and summarized to the tree level using the AQD method of branch sampling and evaluation. Some refinements to these methods were made for interagency project FOREST (Guthrey and others 1993); for instance, an acceptable level of precision in estimated number of whorls retained was obtained when the proportion of fascicle retention present in each needle whorl was estimated in one–third increments (<34 percent, 34–67 percent, and >67 percent fascicle retention). A six–class system of estimating the amount of chlorotic mottle on foliage of needle whorls was adopted. This system was based on the rationale that the amount of chlorotic mottle present on needles in each whorl (mottle severity) is related to net photosynthesis (Pnet), in Jeffrey pine trees (> 12.7 cm DBH) (Patterson and Rundel 1990), in early summer months before the summer drought in the southern Sierra Nevada (*fig. 2*). This relationship can be interpreted by two piecewise linear fits. The first fit is a sharp slope between 0 and 30 percent, and the second fit a gradual slope between 31 and 100 percent. Thus at least one half (about 48 percent) of Pnet in Jeffrey pine is lost when 30 percent of the total needle surface area is affected by chlorotic mottle. More research is needed with trees representing different age classes and crown positions to better define the relationship between visible injury symptoms and reduction in net photosynthesis.

Horsfall and Barratt (1945) have shown that the human eye perceives visual patterns in logarithms, that is, the human eye can most precisely perceive differences between two patterns (e.g. chlorotic and green needle tissue) when the ratio of the two is either low (1–12 percent) or high (88–100 percent). The Horsfall–Barratt (HB) system of measuring plant disease was proposed as a logarithmic (base 2) scale comprised of 12 increments (*table 1*). Classes of narrower width are at the extremes (near 0 percent and 100 percent injured) and the widest classes are near the midpoint (50 percent injured). Essentially, the entire area observed is repeatedly split in half, to 50 percent, 25 percent, 12.5 percent, 6.3 percent, and 3.2 percent (the smallest increment readily detected by the human eye). Because most of the rapid decreases in net photosynthesis occurs in the range 1–30 percent chlorotic, precision in estimating chlorotic mottle in this range is more important than for foliage that has 30–100 percent chlorotic mottle. The categories for estimating injury severity proposed, therefore, are a modification of the HB system which lumps the two lowest classes and six of the highest classes into three distinct groups (*table 1*). Illustrations of the break points in the proposed six–class system (6.25 percent, 25 percent, 50 percent, and 75 percent), as photographs of actual foliage are valuable when used for comparison in field data collection.

A suggestion to weight the appearance of chlorotic mottle on younger foliage more heavily than that found on older whorls was considered by the interagency task group in 1989 (*table 2*). The rationale was that, after the needles mature, the current year's foliage is the physiologically most active of all needle whorls on the tree and inhibition of photosynthesis on these needles would represent a more serious injury to overall tree physiology (Patterson and Rundel 1989; *fig. 3*). In addition, review of previously collected data showed that when ozone injury is so severe that chlorotic mottle appears on the current or 1–year–

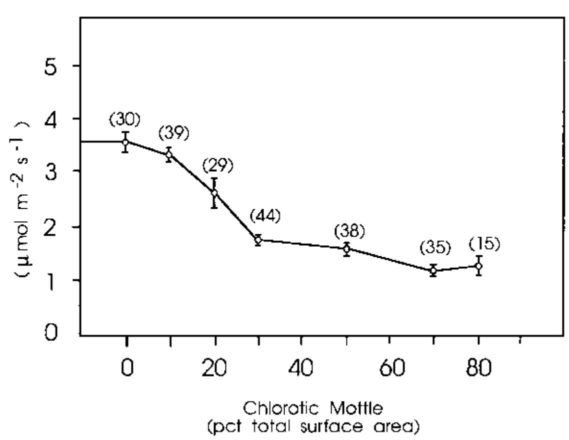


Figure 2—Relationship between severity of chlorotic mottle, represented by percent surface area with mottle, and net photosynthesis in Jeffrey growing under conditions of adequate soil moisture in early summer 1988, Sequoia National Park (Patterson and Rundel 1989).

Table 1—*Chlorotic mottle injury is scored in six injury classes that relate to observed relationships between percentage of lower surface area of needles covered with chlorotic mottle and net photosynthesis in Jeffrey pine trees under field conditions. These injury classes are comparable to the Horsfall–Barrett scale. Percent fascicle retention and fascicle retention classes are also shown. This variable is estimated as one of three classes.*

OII Scale		Horsfall–Barrett Scale		OII Scale	
Class	Pct CM	Injury Class	Pct Injury	Retention Class	Pct Fascicles Retained per Whorl
1	0	1	0	1	<34
2	1–6	2	1–3	2	34–66
3	7–25	3	4–6	3	>66
4	26–50	4	7–12		
5	51–75	5	13–25		
6	>75	6	26–50		
		7	51–75		
		8	76–88		
		9	89–94		
		10	95–97		
		11	98–99		
		12	100		

Table 2—*Weighting of the photosynthetic contribution of needle whorls of different ages.*

Whorl Number	Number of Whorls[1]									
	1	2	3	4	5	6	7	8	9	10
W1	100	50	35	28	25	23	23	22	21	20
W2		50	35	28	25	23	22	22	21	20
W3			30	24	22	20	19	18	18	17
W4				20	17	16	15	14	13	12
W5					11	10	9	8	7	6
W6						8	7	6	5	5
W7[2]							5	5	5	5
W8								5	5	5
W9									5	5
W10										5
Totals	100	100	100	100	100	100	100	100	100	100

[1]Based on Patterson and Rundel (1989), weighting of the relative contribution of whorls to the carbon fixation of asymptomatic Jeffrey pine needles (*fig. 2 and 3*). Whorl one is given greater weight than indicated in the figure because of an increase in carbon fixation that will occur as whorl one matures to whorl two status. All other whorls will decrease in carbon fixation ability as they age.

[2]Injury on whorls seven or greater recorded and computed with an estimated 5 percent weight factor. Each preceding whorl decreased by 1 percent for each additional whorl over six so that total for all whorls equals 100 percent. No whorl would have a weighting factor less than 5 percent.

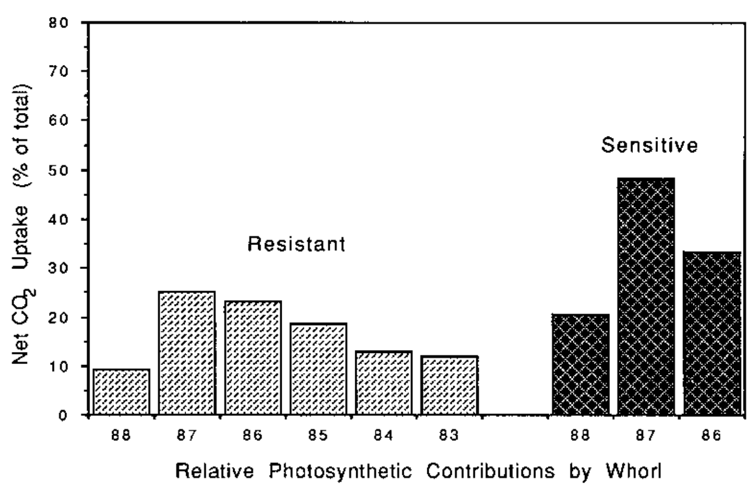


Figure 3—Relative photosynthetic contributions of different age needle whorls in sensitive (with ozone injury) and resistant (without ozone injury) genotypes of Jeffrey pine in 1988 in Sequoia National Park (Patterson and Rundel 1989).

old needles, in almost all cases no more than two or three whorls remain on branches (Duriscoe and Stolte 1989). The needle whorl retention component of the EII (also weighted 40 percent) becomes the single most important factor in determining tree score. The program differentially weights the mottle by the age–class of the whorl it appeared on. This is how the contribution to the index is computed. The maximum contribution is 40 and the minimum is 0 (*table 2*).

Weighting the premature abscission of progressively younger needle whorls more heavily is desirable. The EII utilized a linear relationship between the retention portion of the total score (maximum 40, minimum 0) and the mean number of whorls retained on sampled branches.

We recommend the OII weighting for trees in the low–to–middle portion of the mixed conifer belt of the Sierra Nevada and San Bernardino Mountains be based on the curve represented by the percent of a 1,700 tree sample of ponderosa and Jeffrey pines retaining whorls of ages 0 to 9 (or 1 to 10 years old) at plots located in the Sierra Nevada and San Bernardino Mountains (*fig. 4*). The higher frequency of whorls present for younger whorls represents a higher importance or weighting that is used when calculating the injury index.

Conclusion

The recommended standardized procedure for evaluating ponderosa and Jeffrey pines for ozone injury uses a slightly modified version of data collection methods proposed by the NPS Air Quality Division (Stolte and Bennett 1985), and a data summarization method of a tree–level index reported by Eridanus Research Associates (Duriscoe 1988). The raw data on each index component (mottle, retention, needle length, and percent live crown) are the most important products of the monitoring effort. Many indexes can be contrived and tested over time, and each index component can also be analyzed separately to test different hypotheses regarding chronic ozone injury.

Sampling of a spatially distributed population of stationary individuals requires that the location of individuals be defined. The objectives of the study, probability theory, and the level of confidence in inferences from the sampled population should determine where to locate the sampling and how many subjects to observe. The estimation of the effects of air pollution on a defined population of trees is the main objective of monitoring. Previous work on ponderosa and Jeffrey pines has been focused on long–term monitoring (Guthrey and others 1993, Pronos and others 1978, Warner and others 1982), relating crown condition to ozone exposure or radial stem growth (Miller and

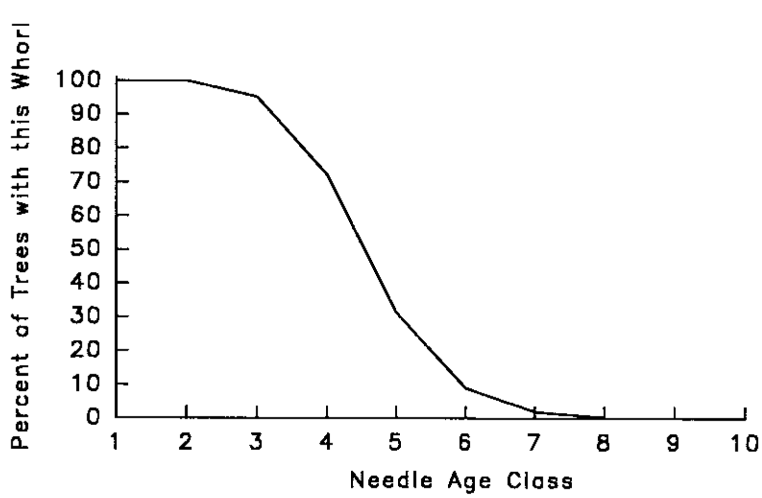


Figure 4—Weighting of the importance of needle whorls based on a curve showing the percent of 1,700 trees from the Sierra Nevada and San Bernardino Mountains with complete whorls of different ages.

others 1991, Peterson and Arbaugh 1992), and surveying broad areas in an attempt to estimate population–level effects and describe spatial and temporal variation in effects (Duriscoe and Stolte 1989, Peterson and Arbaugh 1992, Pronos and others 1978, Warner and others 1982). Each study required a different type of sampling design for optimizing plot and tree locations and numbers.

A systematic method of locating plots has been employed in the establishment of long–term monitoring plots in the Sierra Nevada (Pronos and others 1978, Warner and others 1982). Plots were located at each 1,000–foot contour interval along roads and/or foot trails within agency area boundaries, where ponderosa and/or Jeffrey pines were found in sufficient density to yield 10 sample trees within a given defined area. The advantages of this system are its ease of implementation and wide geographic coverage. Systematic sampling is often used to accurately describe characteristics of populations that are randomly distributed or completely unknown. The uniformity of areal coverage in such designs allow an unbiased spatial description of the phenomenon investigated. Unfortunately, the use of the 1,000–foot elevational contour and roads or trails as a locational device lead to a somewhat biased sample of the pine population. Areas of great vertical relief or landforms that undulate around a contour line should be sampled more intensively than areas of gentle uniform elevational gradient or relatively flat areas falling mostly between sampled contours. Large contiguous roadless and/or trail–less areas are unsampled unless the road and/or trail network is relatively uniform and sufficiently dense, then this method provides a reasonably accurate estimate of the population and allows easy relocation of plots.

To test the hypothesis that ozone effects are correlated to a known ozone gradient, other studies (Guthrey and others 1993, Miller and Elderman 1977) have located plots near ambient atmospheric ozone monitoring stations. The advantages to this type of sampling design include the contribution to dose–response modeling efforts, and the ability to test for interactions between ozone exposure and other environmental factors such as climate, site index, and topographic position. The disadvantage to this design is that information is obtained from a restricted population (pines growing near an ozone monitor) and estimates of the overall population parameters can be made only with low confidence.

Detecting variations in air pollution effects to pines across landscape features has been attempted with areally stratified randomized designs in Sequoia, Kings Canyon, and Yosemite National Parks (Duriscoe and Stolte 1989). These “cruise surveys” were intended to cover as much area and observe as many trees as possible with a single crew in a single field season. Sample points were located randomly within areal strata defined systematically. By using FPM methods (Pronos and others 1978), trees were evaluated only for the crown condition parameters important to ozone injury response. In this manner, hundreds of square kilometers were covered with a much finer resolution than had previously been obtained. Trees were not tagged, mapped, or located in a manner that would allow relocation, as this would have increased the time required at each sample point. Advantages to this method include randomization of sample point locations, both fine geographic resolution and extensive coverage of the entire population, and elimination of variance in the application of evaluation techniques that might result from using different crews. Disadvantages include an inability to re–visit the same trees for long–term monitoring, visit all locations within a narrow time interval (2 weeks or less), and investigate interactions between air pollution effects and other environmental factors.

In other studies, trees have been selected at sample points using belt transects (Miller and Elderman 1977, Pronos and others 1978) and circular plots (Duriscoe and Stolte 1989). Rectangular belts have a greater chance of encompassing the entire range of variability in tree crown condition found in the area because the long axis of the rectangle stretches across a greater distance on the ground and thus includes more micro–sites. If spatial autocorrelation in crown condition exists because of environmental factors, the sampling of nearest neighbor trees in a circular plot would tend to emphasize the effect. If trees are randomly selected from a long belt transect that can cross several microhabitats, a sample more representative of the population as a whole would be obtained.

In ponderosa and Jeffrey pines, a major factor governing tree response to ozone air pollution is the individual plant's genetic sensitivity or tolerance to the pollutant (Temple and others 1992). This sensitivity is randomly distributed throughout a given stand of trees so that within a 50 m radius extreme variation in individual tree response can be found (Miller and others 1989). Therefore, spatial autocorrelation of symptom expression is not expected; in fact, field experience has indicated the opposite. The entire range in crown condition expression of air pollution exposure present in the population experiencing that exposure can be found within a relatively small quadrat or belt transect. Thus, such a sample (a *cluster* sample) would be representative of the population.

Statistical Considerations for Plot Design, Sampling Procedures, Analysis, and Quality Assurance of Ozone Injury Studies

Michael Arbaugh and Larry Bednar[1]

The sampling methods used to monitor ozone injury to ponderosa and Jeffrey pines depend on the objectives of the study, geographic and genetic composition of the forest, and the source and composition of air pollutant emissions. By using a standardized sampling methodology, it may be possible to compare conditions within local areas more accurately, and to apply the results to larger geographical contexts, such as pine populations within and among watershed areas.

We present recommendations in this chapter to develop plot design and sampling strategies that follow established principles of observational studies, and we discuss the usefulness of indices and need for quality assurance for long–term monitoring of ozone injury to ponderosa and Jeffrey pine. The recommendations pertain only to the goals and conditions unique to ozone pollution impacts on mixed conifer, ponderosa and Jeffrey pine forests of the Western United States. Differences in climate, physiology, and air pollutant composition and exposure patterns will likely alter sample design and analysis needs for other forest types and locations.

One of the principal objectives of long–term sampling by repeated observations is to document tree and stand changes through time. In this paper sampling methods will be chosen to optimize long–term, repeated observations of tree populations within a watershed area, and to compare damage between watersheds. The specific questions will be addressed about plot design, the method used to locate plots, and the optimal sample size of branches per tree and trees per plot needed to estimate summary statistics and indices.

Considerations of Observational Studies

Cochrane (1983) defines observational studies as those in which the objective is to determine the causal effects of certain agents, procedures, treatments or programs that the investigator cannot subject to controlled experimentation. The lack of ability to impose treatments or procedures distinguishes observational studies from experimental studies. Nearly all long–term or regional surveys are observational because of the uniqueness of history, climate, soil and topography of each area. These data are also the most valuable, since most issues of forest health involve large areas and long periods (50 to 250 years).

Statistical principles are well–established for the analysis of observational studies and include:

- Comparison of "quasi–treatment" and "quasi–control" groups to resemble a designed experiment.
- Comparison of "treatment" group(s) with more than one "control" group to develop different contrasts with the "treatment" group.
- Comparison of "treatment" and "control" groups with important exogenous variables if feasible; if infeasible, groups should be adjusted for differences using covariates in the analysis.
- Use of a variety of measures and comparisons to reduce the dependence of study results on single aspects of the data and on

[1]Statistician, Pacific Southwest Research Station, 4955 Canyon Crest Drive, Riverside, CA 92507; and Mathematical Statistician, Pacific Northwest Research Station, P.O. Box 3890, Portland, OR, 97208

assumptions inherent for single methods of analysis (Strauss 1990).

The underlying theme behind observational studies is that the system cannot be completely defined or manipulated, and large sources of unknown variation may be present that violate the assumptions of a single analysis approach. Thus, repetition of the comparisons, measures and approaches is used to reduce the dependence of conclusions on incorrect assumptions associated with single measures.

Stratification of Target Populations

The technique of dividing a target population with high variation into smaller non–overlapping populations (strata) that are more homogenous is used whenever possible as a part of a sampling scheme. This division allows separate estimates to be developed for each stratum, and usually allows estimates for the entire population to be developed with a higher degree of precision for a given level of expense. To attain these benefits, strata are designed so that within–stratum variance is less than the variation in the target population. Ideally, strata will have meaningful interpretations, such as division by species composition, age groups, or density. In addition a long–term study should also be included to define strata stable through time.

The absence of reliable information is the most limiting factor for describing strata for visible injury surveys. Few studies provide information about the effects of density, topography, and stand structure on visible injury levels. Strata, therefore, need to be defined by coarse criteria using known information.

Drainage basins are commonly used as geographical stratifications because they often contain similar soils, history, air flow, and hydrological dynamics. Thus, one possible stratification scheme for visible ozone injury would be to divide populations by watersheds, and then subdivide watersheds into three strata: Jeffrey pine, ponderosa pine and a stratum with mixes of these two species.

Plot Considerations

Design

Plots are generally designed to yield a constant amount of information. This design simplifies aspects of statistical comparisons. For long–term monitoring an important consideration is whether plots should have constant area or a constant number of trees. The choice of approach used depends on the intended uses of the survey information. Defining plots with constant area facilitates extrapolation of survey estimates to the entire area of the target population. Defining plots to contain equal numbers of trees allows survey results to be used in making statements about the injury found on the tree level. Equal numbers give equal resolution of information about the average tree response within a plot.

Because visible injury monitoring is oriented to average tree response within watersheds rather than to area oriented watershed-level assessments, plots consisting of a constant number of trees are appropriate. This requirement is especially important because severe visible injury is rare. Often only a few trees in a stand will be severely affected by ozone injury. Reducing the number of trees per plot would reduce the ability to detect these effects.

Consistency with prior studies is also desirable. Information that is comparable to historical work allows a temporal picture to be developed that is important for evaluating trends. Previous pine monitoring has used both circular and rectangular plots. Circular plots, such as those used in the Eridanus injury index (EII) procedures, have several disadvantages. First, if plots containing a fixed number of trees are needed, then the radius of a circular plot must be uniquely selected for each plot. Several trial values for plot radius may be needed to arrive at the correct plot size. This process may be quite time–consuming if the number of trees needed is not small. Second, the selection of trees falling within a circular plot is identical to selecting nearest neighbors to a point. This results in biased estimates of stand density if trees occur in a clustered spatial distribution, as ponderosa and Jeffrey pine often do. Circular plots also increase the chance of including spatial correlations that may confound results by using the most compact possible plot layout.

Other types of pine monitoring include fixed area belt transects, as used in the USDI National Park Service Air Quality Division Procedure (Stolte and Bennet 1985), and variable area belt transects used in the San Bernardino National Forest (Miller 1983). Belt transects avoid disadvantages associated with circular plots. The longer plots sample across clusters of similar age, size, and genotypes and reduce spatial correlation effects associated with nearest neighbors. Thus, for our design belt transects are more appropriate than circular plots.

Number of Plots per Stratum

The results of a monitoring, or observational, study are estimates of representative conditions in the target population(s). This type of result contrasts with experimental design, which results in hypothesis testing and statements concerning treatment differences. Monitoring designs are less useful for comparisons, but yield estimates of the general target population (for which experimental data are sometimes poor). Sampling design cannot optimize for experimental and monitoring goals simultaneously, but our long–term monitoring approach can be used to develop population estimates with accompanying confidence levels.

Estimates derived from a survey consist of point estimates of the population value, with stated range of values for those estimates expressed as a confidence interval. Higher levels of confidence result in conservative (wide) intervals, while lesser degrees of confidence result in narrower intervals. Although other confidence levels are valid, it is customary to use 95 percent confidence levels.

Specification of the Desired Precision Level

Sample Size Determination

If sensitivity to a change from one level of a population characteristic to another is of interest, the survey can be designed to provide a confidence interval width that will reveal a change of that size with a stated reliability. Increased sample size (number of plots) will result in narrower confidence intervals, despite the confidence level chosen. It is necessary to postulate a difference of interest (the precision) before sample sizes can be determined. The level of precision should be based upon the desired size of the difference to be detected, and should be linked to a biologically significant indicator, such as loss of foliage and productivity.

Given a choice of confidence level, desired precision level, and some preliminary information about the variance, a sample size (number of plots) expected to give a confidence interval of the desired width can be estimated. The resulting sample size should be considered a minimum number, the actual number being 10 to 25 percent larger for long term surveys. This will insure the design against loss of plots due to fire or other causes.

Index Construction

Index Characteristics

The use of indexes to summarize information has several advantages: it reduces complex results to an understandable form, combines several diverse measures into a single measure, and enables policy–makers to have readily available numbers for justifying legislation. Indexes also have several negative features when used as scientific findings. Information is lost when measures are summarized, especially when several measures are combined, so that interactions occurring between the differing measures are missed. Indexes also involve some arbitrary decisions based on investigators expert knowledge. Although these estimates incorporate all knowledge available at the time of formulation, new information may change index construction decisions.

Inclusion of arbitrary, or rough estimates of tree biology, based on expert knowledge cannot be avoided, especially for visible injury assessments. In observational studies this weakness is compensated by using several measures, each depending on differing assumptions to increase the reliability of study results.

Thus, we recommend that several measures be developed, each using different assumptions, instead of a single index. A primary measure can be designated, but several other measures should be developed as supporting information. These measures may be other indexes, or simple summaries of measures used to construct the primary index. For example, the Forest Health Monitoring Program includes crown indices for crown transparency, and crown structure. It is important that the indices used depend on different assumptions because this information makes each measure useful. In addition, data used for index development should also be retained for future index refinement. Retention of the data used to construct indices will also allow alternatives to be calculated retroactively in the future if only one index is presently available.

Primary Index

The OII seems to satisfy the five 'requirements' stated by Muir and McCune (1987). The most important primary index component is the presence or absence of chlorotic mottle since it is the most direct evidence of ozone effects on pine foliage. Additional measures, multivariate graphs, principal components of the measures, and alternative indices can be also be developed. They should be alternate presentations of the data not dependent on the assumptions used to construct the OII index. The type of summarizations needed should be decided by experts familiar with the effects of ozone injury on sensitive tree species. Statisticians can help by enumerating the choices of summary presentation.

Within–Plot Sampling Considerations

Tree Sample Size

Previous studies considered all trees with DBH > 10 cm (Miller 1973, Muir and McCune 1987, Stolte and Bennett 1985). The OII method considers only trees with prunable crowns, possibly excluding large co–dominant trees. This exclusion may bias stand estimates if visible injury differs for this group. Trees with DBH < 10 cm are less important for stand estimates because they usually comprise a small portion of the stand, are highly stressed, and ozone exposure conditions differ from larger DBH classes.

Because the analysis goal is average tree damage, the number of trees included in the sample should be large enough to allow confident application of the Central Limit Theorem. Sufficiently large numbers will guarantee that averages will be distributed according to the normal (Gaussian) distribution, simplifying later analysis. The choice of any particular number is arbitrary, but inclusion of 30 or more trees per plot is likely to be sufficient for monitoring purposes.

In a general sense, the plots should be large enough to include sufficient sample trees for the categories of interest. This requirement is important if small numbers of sensitive trees are present. If these trees are randomly distributed, the probability that a group of n trees contain no sensitive trees is approximated by the binomial distribution, so

$$\text{Prob}\{\text{no sensitive trees}\} = (1\text{-}p)^n,$$

in which p is the proportion of the general population that is sensitive. The number of sample trees should be chosen to obtain an acceptably low probability of exclusion for these trees if sufficient knowledge is obtainable. If this knowledge is not available, then standard sample size formulas should be used.

During long–term monitoring, trees will be lost at times, so the initial plot layout must include enough trees to reasonably ensure that future mortality will not reduce the tree numbers to below that needed. The number of trees per plot should be chosen to match or exceed the foreseeable maximum required. Estimates of the sample size needed should be obtainable from the information in Duriscoe (1988). The standard sample size determination formula can be applied using several precision levels, and the standard deviation estimates obtained from graphs of the trees.

Branchlet Sample Size

Muir and Armentano (1987) determined optimal number of branchlets removed from a sampled tree, estimating that 5 branchlets were needed. Their methods involved application of a sequential likelihood ratio test (SLRT). Strict application of a SLRT will generally underestimate the required sample size for a fixed sample scheme. However, they used several SLRT's and a conservative value of N was chosen that exceeds the N from individual tests. This application of SLRT techniques probably avoids underestimation. Thus their suggested sample size is likely adequate to estimate the true number needed.

Summary

Sufficient information exists to design a simple robust sampling design for long–term visible injury monitoring. Past studies and expert knowledge are available for critical sampling decisions. More complex designs are possible, but they increasingly become more dependent on assumptions and knowledge that are not presently available. More refined issues include spatial autocorrelation effects, optimal stratification criteria, optimal sample size determinations and long–term divergence of plots from surrounding populations. Future studies are needed to examine the impact of these factors on the design recommendations.

Design of observational studies involves approaches that compensate for the inability to experimentally manipulate trees and stands. Because of this limitation sample sizes should be estimated conservatively. In this design comparisons between sites at the same time, and between time periods for the same sites represent our "quasi–controls" and "quasi–treatments." If some sites are placed near air monitoring stations the link visible damage to pollutant exposure can be better quantified.

Reporting multiple measures is important to reduce the reliance of conclusions on assumptions that are approximate. Changes in forest species and stand structure, landscape features, and exposure may effect the assumptions used for a single index in unpredictable ways.

We also recommend additional surveys that do not require establishing permanent plots to complement monitoring efforts. Permanent plots may become less representative of surrounding forest conditions through time, either by different management applications, or by stochastic processes associated with local site conditions. Periodic examination of the represenativeness of plots is needed to insure that remeasured plots continue to reflect surrounding forest conditions.

Quality Assurance/ Quality Control

General Considerations

Ideally a work plan/quality assurance plan should be completed at the beginning of the project (Cline and Burkman 1989). An ideal quality assurance (QA) plan is designed to support an overall program, rather than just the methods used in collecting information. The QA project plan is a comprehensive description of research procedures and methods and associated internal QA/ quality control (QC) activities (Zedaker and Nicholas 1990). The QA plan is written by the principal investigators of the project and includes project objectives; experimental design, sampling procedures, and statistical methods and analysis; project management and personnel; research facilities and equipment; measurement and analytical procedures; data quality objectives and data quality assessment procedures; sample and data custody and archive procedures; data validation and analysis; and mechanisms for implementing changes in research or QA.

Quality Assurance at the Measurement Level

Within any project an important QA question is: what is the measurement error associated with each recorded variable? Determining the cause and reducing the amount of measurement error is the traditional emphasis of QA programs. QA for the measurement process includes operations and procedures in which the data produced are of the specified quality within a stated level of uncertainty. Quality is acceptable when data are consistent and have a small uncertainty when compared to the stated requirements. Precision and bias are the traditional quantitative indicators of the quality of measurement data. Typically, precision is estimated by repeated measures of reference materials or actual samples (duplicates). Estimation of measurement bias is best done by systematic use of reference materials. Estimates are not as feasible for certain biological data since reference materials change with time.

Quality Assurance Recommendations

The following specific procedures are recommended for a suitable quality assurance program:

- Provide an annual training session for workers gathering plot/tree data.
- Include the collection and analysis of quality control data in the overall statistical design.
- Collect and track remeasurement data throughout the measurement period.
- Include both internal and external components in the QA program.
- Implement a systemic approach to data handling and database management.
- Document QA and survey methods throughout the program.

Establishment of Monitoring Plots and Evaluation of Trees Injured By Ozone

Daniel Duriscoe, Kenneth Stolte, and John Pronos[1]

By establishing long–term monitoring plots, it is possible to record environmental and biological conditions of the plot and individual trees, evaluate the condition of crowns of trees in the plot, and determine the extent of ozone injury to western conifers. This chapter recommends various methods for recording data and selecting plots, and provides information for improved efficiency and safety of field work in studies on ozone injury to ponderosa pine (*Pinus ponderosa*) and Jeffrey pine (*P. jeffreyi*).

Data Recording

To record field data for most site and tree variables, data sheets may be used (*figs. 1* and *2*) or a field data recorder (FDR), such as the Corvallis MC–V™ with Mbase™ using appropriate custom programs[2] (*appendix A*). Tree, branch, and whorl–level data recorded on data sheets (*fig. 2*) can be entered electronically on a PC computer using customized programs in dBase IV™ or other database management software to facilitate efficiency (*appendix B*). However, some factors should be considered before using an FDR. For instance, the level of computer training and familiarity with the hardware and software of the field personnel should be sufficient to recognize and solve problems. At least two persons should conduct the field measurements (one to examine the branches and call out data and another to type it in). When working alone the extra task of managing the FDR is too cumbersome and time–consuming. In addition, paper data sheets should be carried to the field to provide a back–up in case a problem develops with the use of the FDR. And finally, data files should be downloaded daily to a PC from the FDR to avoid the possibility of losing large amounts of data by accidentally writing over an existing file, or if the battery in the FDR fails.

Plot Location and Number

The placement and number of plots for any given study will depend mainly on two factors: objectives of the study and availability of resources to complete the effort. Design should not be compromised so that differences of biological significance cannot be detected, even if resources are limited. If resources are not available to describe the entire population of the species of interest with sufficient confidence, we recommend that the level of effort be reduced by defining a sub–population of particular interest and adequately sampling that sub–population. An important step in the sampling design is to define the target population. This may be accomplished by stratifying the entire geographic extent of the species of interest, and progressively eliminating strata that meet rejection criteria. These criteria may be defined by the intersection of map themes. For example, an investigator may wish to eliminate slopes greater than 60 percent, closed–canopy forest stands, and Jeffrey pine type from the sample. The overlay of these themes and the area defined by their union would constitute the area to be eliminated from potential sampling. The area remaining would constitute the target population. This process may be quickly accomplished with a geographic information system (GIS), if it contains the appropriate data on all the themes of interest.

[1] Biological Technician, Resources Management, Sequoia and Kings Canyon National Parks, USDI National Park Service, Three Rivers, CA 93271; Deputy Director, Forest Health Monitoring, Southern Experiment Station, USDA Forest Service, 3041 Cornwallis Rd., Research Triangle Park, NC 27709; Plant Pathologist, Forest Pest Management, Stanislaus National Forest, USDA Forest Service, 19777 Greenley Rd., Sonora, CA 95370

[2] Trade names and commercial products or enterprise are mentioned solely for information. No endorsement by the U.S. Department of Agriculture is implied.

MANAGEMENT UNIT/SITE:__________ PLOT NAME/NUMBER:__________ CREW:__________

TOPO MAP:__________ LANDUSE:__________ DATE:__________

PLOT LOCATOR TREE (SPECIES, DBH, CHARACTERISTICS):__________

ELEVATION:__________ LATITUDE:__________ LONGITUDE:__________

COMPASS BEARING AT STARTING POINT:__________ %SLOPE:__________ ASPECT:__________

FOREST TYPE/COMMUNITY (3 MAIN TREE SPECIES):__________ SITE INDEX__________

UNDERSTORY VEGETATION:__________

SLOPE POSITION: summit shoulder backslope footslope terrace bottom

LANDFORM: ridgetop spur-ridge noseslope headslope sideslope cove draw

SKETCH IN LOCATION OF EACH TREE (WITH TREE NUMBER), AS ACCURATELY AS POSSIBLE.

SKETCH IN SECTION OF ROAD, TRAIL, OR PROMINENT GEOGRAPHIC FEATURE TO FACILITATE RELOCATING PLOT IN FUTURE.

ATTACH APPROACH LOGS AND PHOTOS OF PLOTS IF AVAILABLE

Figure 1—Site data sheet.

FOREST: | SITE: | PLOT: | TAG:

CREW: | COMMUNITY:

ASPECT: | SLOPE: | ELEVATION:

DATE: | LANDFORM: | SLOPE POSITION:

SP	#	FOL LEN	# (WHORL)	CM	FR	NL	BIOTIC	ABIOTIC	COMMENT
			1						
AGE			2						
			3						
SNDX	1		4						
			5						
			6						
POS			7						
			8						
HT			1						
			2						
			3						
DBH	2		4						
			5						
MREL			6						
			7						
			8						
ROCK			1						
			2						
MIST			3						
	3		4						
			5						
CONK			6						
			7						
BEET			8						
			1						
			2						
FIRE			3						
	4		4						
MECH			5						
			6						
			7						
LIGT			8						
			1						
BROM			2						
			3						
	5		4						
%LC			5						
			6						
LCR			7						
			8						

UNITS USED:
DBH ______
HT ______
FOL LEN ______
NL ______

FR = FAS RET (WHOLE WHORL)
0 = NONE
1 = 1 - 33%
2 = 34 - 66%
3 = 67 - 100%

CHL. MOTTLE
0 = NONE
1 = 1 - 6%
2 = 7 - 25%
3 = 26 - 50%
4 = 51 - 75%
5 = 76 - 100%

ABIOTIC INJURY
0 = NONE
1 = < 25%
2 = > 25%

BIOTIC INJURY
0 = NONE
1 = < 25%
2 = > 25%

MREL
PL = PLANAR
CC = CONCAVE
CX = CONVEX

BOLE INJ
0 = ABSENT
1 = PRESENT
2 = SEVERE

MIST: 0 - 6

%LC = % LIVE CROWN

LCR = LIVE CROWN RATIO (FILL-IN)

ROCK = % ROCK IN 20X TREE DIA AREA

SNDX = SITE INDEX

Figure 2—Tree and whorl data sheet.

The main objective of a study may be *estimation* of the true population parameters or *testing* hypotheses relating to spatial variation, temporal variation, or relationships between air pollution effects and other environmental factors, such as air pollution exposure. Long–term monitoring plots can be used for both purposes. Plots can be distributed throughout the range of the species (Duriscoe and Stolte 1989), or plots can be co–located with an ambient ozone monitor to test ozone exposure–response. No single recommended sampling design can ideally satisfy both objectives. In any case, results should always be presented with confidence intervals and confidence coefficients of estimates, or the probability and alpha–values for tests.

Plot Selection Criteria

For long–term monitoring studies in which the temporal trends in crown condition are of primary interest, the target population is restricted to trees that will provide a long–term data base; can be readily assessed for ozone injury to the lower crown; are not located in unusual microhabitats; are not infested with foliar insects and diseases that would confound the evaluation of air pollution injury; and are accessible and relocatable so that they have a reasonable chance of being re–visited on a regular basis. The spatial distribution of the species population should be defined by using the best available vegetation maps. Such maps are usually not available at the individual species level. If practical, an initial reconnaissance of the area may be made to verify the extent of the species of interest.

To minimize the within–plot variance, confounding factors should be stratified. For instance, the entire extent of the plot should be within the same general landform type (*fig. 3*). Transects (direction of the long dimension of the plot) should generally run along the elevational contour, and in a straight line for mapping convenience. Plot length should be 250 m and 80 m should be allowed for plot width. This effectively eliminates severely dissected landform types less than 250 m in size. Some sophisticated programming may be required to define these areas on a GIS. Selecting a uniform landform may sometimes be difficult in the field, but some degree of minor violation may be tolerated for practical reasons.

Another method to eliminate within–plot variance is to select a stand density that contains at least 50 trees from a 1 ha plot. Trees with crown positions including codominant, dominant or open–grown are those of primary interest. At least 30 trees should be as tall as the general forest canopy.

In addition, severe needle cast fungus infections (e.g. *Elytroderma deformans*) or dwarf mistletoe infections should be avoided. Large groups of bark beetle–killed trees should also be avoided as beetles may spread to nearby living trees the following year (recent aerial photographs may reveal these areas). Sites with high levels of bark beetle–caused mortality may also indicate that soil moisture holding capacity is low or a root disease center is present, thus making nearby trees susceptible to bark beetle attack during subsequent drought years.

Trees growing in serpentine soils, near quarries or mine tailings, or in disturbed soils (roadcuts or old roadbeds) should be avoided unless there is a specific purpose to study how these variables may influence response to ozone exposure. However, stands of open–grown Jeffrey pine growing on bare rock

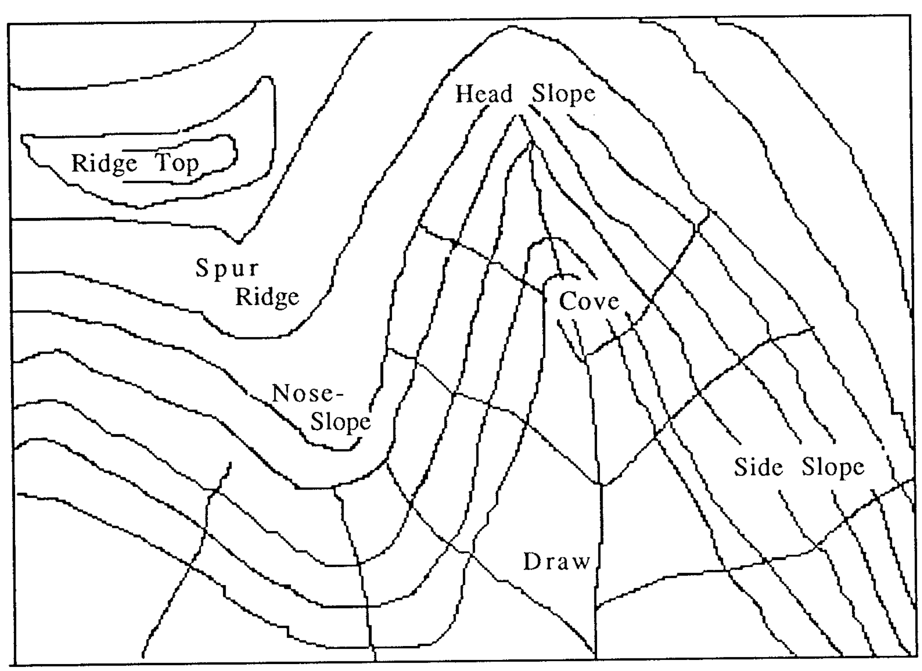


Figure 3—Seven types of landforms: ridgetop, spur–ridge, noseslope, headslope, sideslope, cove, and draw.

are common in the Sierra Nevada, and should not be eliminated from the target population. If dose–response relationships are part of the investigation, these environments should be stratified from other more mesic areas with little or no exposed rock, if correlative or predictive models are developed.

After the target population is defined, it may be further stratified into smaller sampling units as a means of increasing sampling efficiency. A simple areal and/or elevational stratification is recommended. The first level of areal stratification is usually defined by major drainage basins. Further strata may be defined systematically using a square grid (Duriscoe and Stolte 1989) or a design that produces equal areas of the target population only (Duriscoe and Graban 1992). In areas with large topographic relief, elevational stratification (into 300 m belts) may be appropriate. In any case, smaller sampling units will almost always contain less within–unit variability in crown condition than larger units. Other factors which may be considered for defining strata are species (e.g., ponderosa or Jeffrey pine) or variety (e.g., *scopulorum* or *arizonica* varieties of ponderosa pine in the southwest), site class, landform type, and soil moisture holding capacity (which may be modeled from an estimate of soil depth, slope position, slope angle, and/or aspect). Within each sampling unit, simple random sampling should be employed to locate potential plot sites.

A system of plot rejection criteria (required stand density, pathogen infection levels) and a procedure for selecting an alternate site if the sample point fails (another random point in the unit, a random offset from the failed point, or simply the nearest point to the failed point where a viable plot can be established) should be determined before venturing into all potential sampling points in the field. In general, plots should be rejected if the trees show obvious stresses from natural or human–caused factors that would mask any observable effect from ozone pollution.

Establishment of Plots

A belt transect 80 m wide and 250 m long (2 hectare) is the recommended size and shape for relatively dry and open western pine forests. For species that sometimes grow in small isolated stands, such as Jeffrey pine, western white pine (*Pinus monticola*) or coulter pine (*P. coulteri*), the entire stand may be sampled rather than using a belt transect (see below).

Certain methods for plot establishment are recommended following repeated trials in the field (*fig. 4*). First, a compass direction must be selected that will include a suitable number of sample trees. On a slope the compass direction is usually parallel with the contour of the slope. On flat areas it is possible to select a random compass direction. This becomes the long axis of the plot and also represents the center line. The long axis of the plot should be within the same landform type. Use a steel or fiberglass tape to measure distances and if the slope in either the long or the short dimensions of the plot exceeds 10 percent, add or subtract distance using the actual slope (percent) and a reference table.

The plot should be identified for re–location with a locator tree (a large conspicuous, or uniquely–shaped tree) within or near the plot. The distance and compass direction from the plot locator tree to the beginning point of the long axis of the plot should be measured and recorded. The diameter–at–breast–height (DBH) of the locator tree should be measured and a small aluminum plate should be nailed at breast height on the uphill side of the tree. Plot information should be etched on the plate, including plot name, agency, and date established.

The locator tree should be photographed from a known point (roadside turnout, mile marker, prominent ridgetop located on topo map, etc.) to facilitate

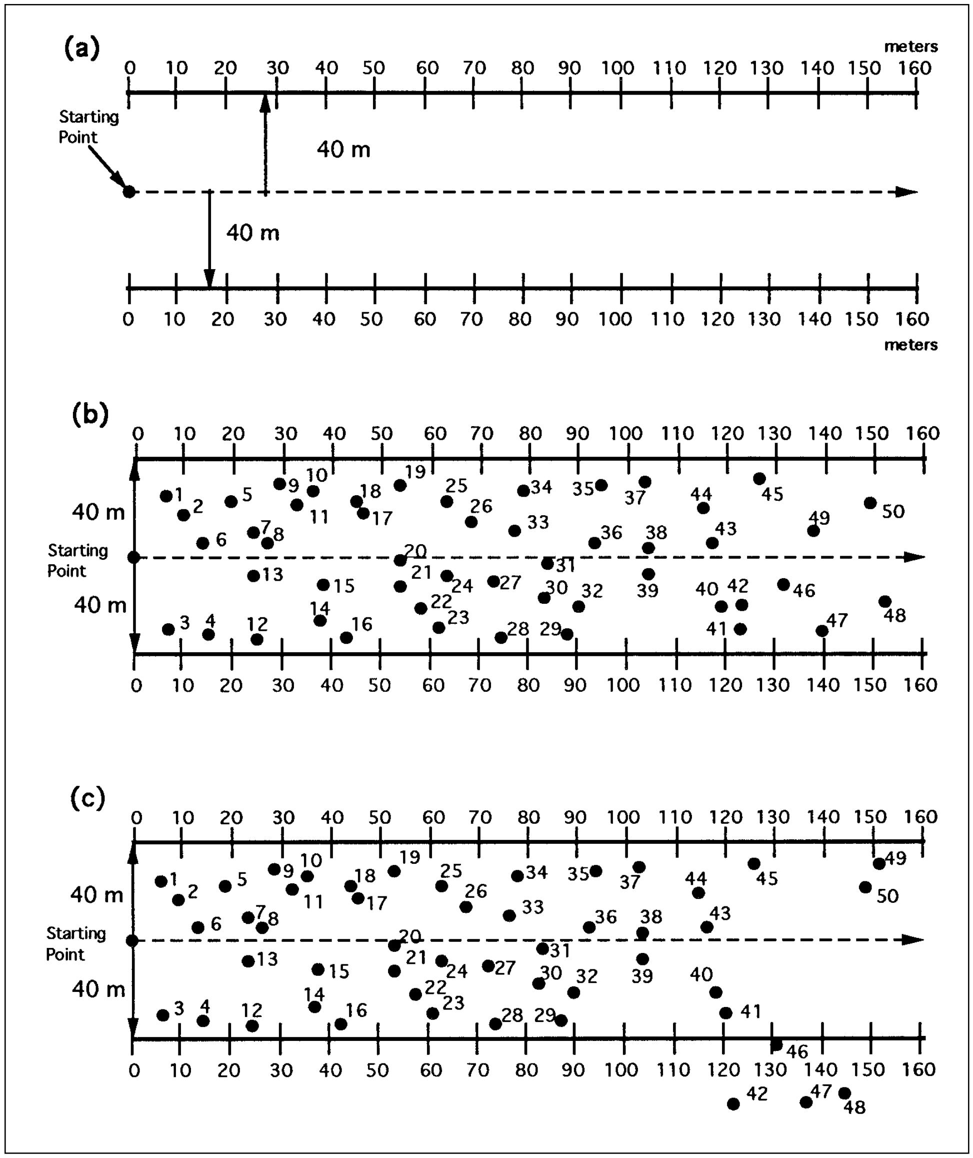


Figure 4—Establishment of a plot containing 50 trees. (a) Plots are 80 meters wide (40 meters on each side of the transect line). Length of the transect line is indeterminate; it is extended until 50 trees meeting specifications are found. (b) Mapping 50 trees within plot bounds and relevant to plot outline. (c) Mapping 50 trees in the plot; 46 are within the plot perimeter, with 4 trees mapped outside the plot perimeter.

relocation. We also recommend that if bedrock outcrops are evident in the vicinity, a stainless steel or brass tag should be imbedded in the rock with a concrete nail and epoxy, and the position of this tag geo–referenced with a global positioning system (GPS) to within 5 meters. If all plot features are then mapped from this reference point accurately, the location of the plot and its originally selected trees may be reconstructed for many future decades regardless of changes in the appearance of the vegetation. A descriptive log of how to find the locator tree or rock monument should also be included.

Trees for monitoring should be surveyed and selected by recording and mapping all trees of the species of interest with DBH > 10 cm (*fig. 5*) within 40 m (map distance) of each side of the center line (total width of transect = 80 meters). Trees should be tallied and classified by crown position (dominant, codominant, intermediate, and suppressed) and DBH (estimate or caliper)

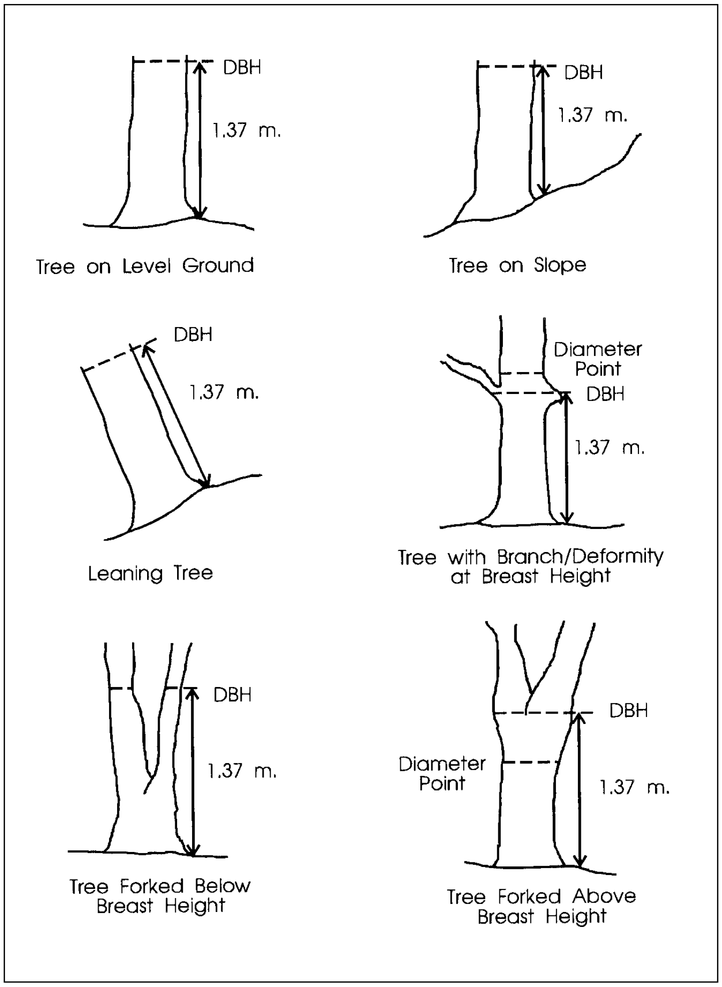


Figure 5—Proper method for measurement of diameter–at–breast–height (DBH) of the tree bole.

recorded on a Tally Sheet (*table 1*). If time allows, tree stems should be mapped by measuring distance out from start point along the transect center line and distance left or right of the center line. Whether the tree is suitable for long–term monitoring should also be noted. We recommend screening out trees for long–term monitoring with the following conditions: severe mechanical wounds, unpruneable crown (lowest branches > 10 m above ground), crown too small to stand repeated pruning, root disease, excessive lean, bark beetles, severe dwarf mistletoe, severe needle cast disease. This process should continue along transect from origin point until one of the following conditions is met: 50 dominant or codominant pines suitable for long term monitoring are obtained; a different landform type is encountered; the transect extends out of the pine vegetation type; or the transect is extended to 250 m from the origin point. If at least 30 dominant and codominant trees of the species of interest are not found, the transect could be extended beyond 250 m if the landform type does not change. If this is not possible the placement of the plot upslope or downslope could be adjusted. As a last resort, widening the transect may be necessary. In any case, the plot should be no wider than 80 meters. If an open– grown discrete stand is encountered that is irregular in shape with well–defined boundaries, is less than about 2 hectares in size, and contains less than about 100 canopy trees, it may be more efficient to enumerate the entire stand and map the stand boundaries rather than to try to fit a rectangle to nature. The objective is to

Table 1—*Tree selection work sheet for tallying trees to be labeled with tags. (See* **fig. 8** *for crown class descriptions.)* [1]

DBH		Crown Class				
inch	cm	Open Grown	Dominant	Codominant	Intermediate	Suppressed
4	10					
6	15					
8	20					
10	25					
12	30					
14	35					
16	40					
18	45					
20	50					
22	55					
24	60					
26	65					
28	70					
30	75					
32	80					
34	85					
36	90					
38	95					
40	100					
42	105					
44	110					
46	115					
48	120					
50	125					
50+	(Specify)					
Totals						
Sum		Canopy (Open Grown, Dom. CoDom.)			Subcanopy (Int., Sup.)	

[1]Note unpruneable trees with asterisk (*).

include at least 30 canopy trees > 10 cm DBH. Ideally, 50 dominant and codominant trees will be found in less than 250 m. If more than 29 but less than 50 can be included within the plot boundaries, the remainder (intermediate and suppressed) may be selected randomly from all those found within the plot, so that each tree has a probability of being selected. The remainder (between 0 and 20) should not exceed the total number of trees selected for monitoring (50). Selected trees should be identified with a numbered tag attached by an aluminum nail (#7 siding nail) at breast height on the uphill side of the tree (*fig. 5*). Tree diameter should be measured with a tape at a point just above the nail. Tree locations should be mapped as accurately as possible relative to each other, as well as the plot corners or the monument in rock. A hand–held compass and tape should be the minimum tools used. Information on forest dynamics may be obtained by mapping every tree stem of all species, if the resources are available to accomplish this task (Miller and others 1991).

Mortality of selected trees subsequent to plot establishment may be observed in long–term monitoring. The selection of replacement trees is not recommended, unless the number of surviving trees falls below 15 or 20. At that level, the useful life of the plot may be over, and a new set of 50 trees should be established, if necessary. Data from the old plot trees should *not* be directly compared to data from the newly established trees because the natural tree–to–tree variability in sensitivity to air pollution is high. The statistical power of long–term comparisons of *adjacent* groups of trees is very much less than a comparison of the *same* group of trees. And lastly, the site class for each plot should be calculated by selecting three open–grown or dominant trees. Trees should not have any obvious stress from competition, and should be measured for height and cored for a count of the number of radial rings. These measurements are then evaluated by the use of a site–class table (*appendix C*).

Collection of Site, Stand and Tree–Level Data

After locating, tagging, and mapping 50 acceptable trees, management data should be recorded on a site data sheet (*fig. 1*). Landform categories are illustrated in *fig. 3*.

Tree–level data should be collected by evaluating the crown and bole condition of each tree including whole crown, branch variables, and whorl variables. These evaluations are performed by visual estimates and by using simple instruments, both on the whole tree and on branch samples extracted from the lower crown. The approved methods for measuring DBH (*fig. 5*), selecting the category of slope position (*fig. 6*), and category of microrelief (*fig. 7*) should become familiar to the field crew (Zedaker and Nicholas 1990).

Whole tree and crown evaluations may be recorded on a data sheet (*fig. 2*), or in the FDR. Instructions are provided for judging the crown position (*fig. 8*), measuring tree height (*fig. 9*), and rating the severity of dwarf mistletoe infection (*fig. 10*). A visual estimate should be used to record further tree injury observations using these classes: 0=none; 1=present; 2=severe for the following: conks (identify species if possible); bark beetles (identify species if possible); witches brooms (with or without presence of dwarf mistletoe plants); fire scars (the rating refers to the percent of the circumference affected); mechanical injury (specify type); and lightning scars.

Collection of Branch and Whorl–Level Data

Branches must be cut from the lower crown of each tree so that foliage may be examined in hand. On steep slopes this may be a labor–intensive portion of the field work. It is important to obtain representative branches of the lower crown.

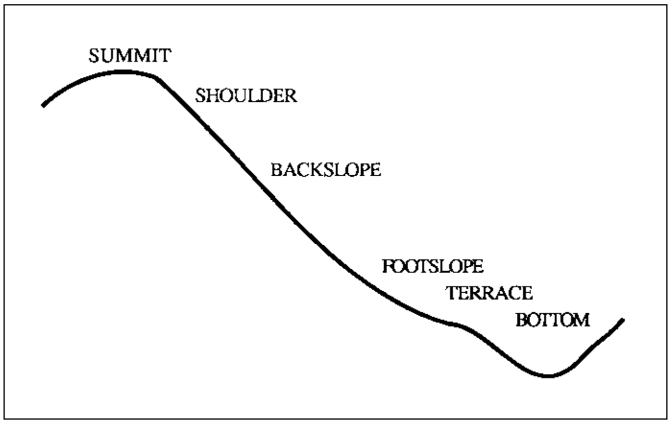


Figure 6—Slope positions that are available for establishment of pine monitoring plots. Six slope positions: summit, shoulder, backslope, footslope, terrace, and bottom (floodplain). Plots should fall within any slope position without overlap between slope positions.

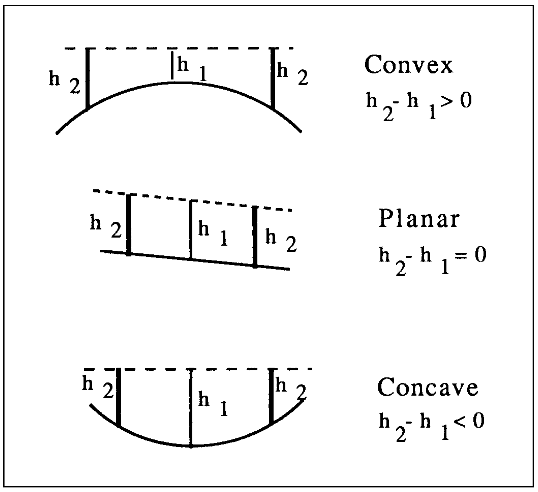


Figure 7—Microrelief types to be evaluated under the drip lines of each tree. Types are planar, concave, and convex.

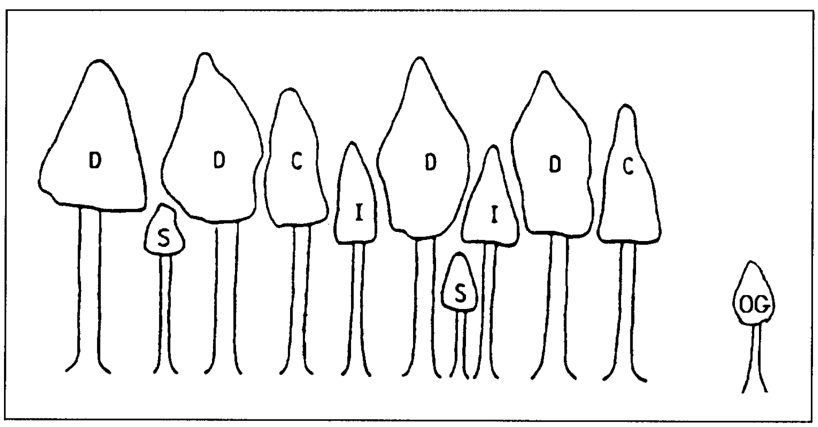
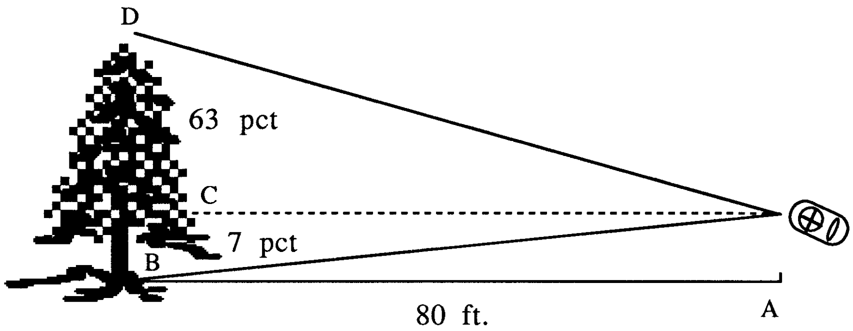


Figure 8—Crown Position Classes: D = dominant, C = codominant, I = intermediate, S = suppressed, OG = open–grown.

However, factors that may confound the evaluation of chlorotic mottle and needle retention that occur on specific branches should be avoided if they are not present on the majority of all branches in the lower crown. Branches with dead terminal buds, severe needle cast fungi, severe scale infestations, or that have obviously been severely chewed by insects or squirrels should therefore be rejected. Branches with cones or those which bifurcate (split) within the foliated length should be accepted, although their evaluation may be more time–consuming. "Branchlets" (the smallest side branch with a full complement of needle whorls) should be cut from near the crown edge (*fig. 11A*) without pruning the branch leader of major branches (*fig. 11B*); the middle of the lower



Using the percent scale, stand a proper baseline distance from the tree, in this example 80 ft. Sight the top of the tree (D) and read the percent scale (63 percent = C to D). Sight the bottom of the tree (B) and read the percent scale (C to B = 7 percent). Add the readings to get a total reading of 70 percent. Multiply the total reading by the baseline distance to get the tree height (70 percent × 80 ft. = 56 ft.). When the base of the tree is below eye level, the two readings are added. When the base of the tree is above eye level, the second reading is subtracted from the first.

Figure 9—Forest inventory method for determining tree heights.

crown should be sampled on all sides of the tree. The very lowest branch on mature trees should be avoided because it may soon die as the tree self–prunes with age; its overall vigor is probably less than the rest of the lower crown. Small trees should not be over–pruned.

Five branchlet measurements are required for adequate within–tree replication:

- Foliated length—The length of the woody stem should be measured from the base node of the oldest whorl with green foliage (even if younger whorls are missing) to the base of the terminal bud. This is a general measure of overall vigor of the tree and potential growth rate.
- Number of whorls—The whorls of needles retained should be counted. This is a measure of the age of the oldest foliage retained on the branch in years minus one. If a whorl is missing between older and younger foliage, it is tallied on the data sheet as a "0" (*fig. 12*). Whorls with short needles must also be included (*fig. 13*)
- Chlorotic mottle—The severity of chlorotic mottle resulting from ozone injury should be estimated as a visual estimate of the surface area of each whorl covered with mottle using the following classes (*fig. 14*):

  0 = no mottle observed

  1 = 1–6 pct

  2 = 7–25 pct

  3 = 26–50 pct

  4 = 51–75 pct

  5 = > 75 pct.
- Fascicle retention in whorls—The portion of the full complement of needle fascicles that remain (*fig. 12*), should be estimated by using the following classes:

  1 = 1–33 pct

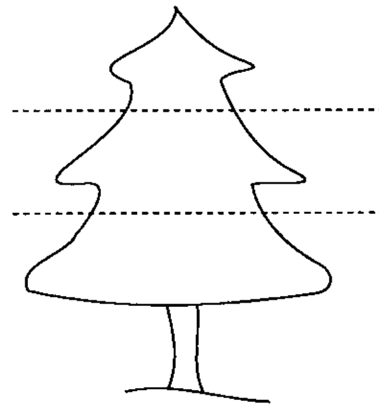
  2 = 34–66 pct

Step 1: Divide live crown into thirds

Step 2: Rate each third separately

Assign each third either 0, 1, or 2:
0 - No visible infection
1 - Light, less the 1/2 of branches have mistletoe plants
2 - Heavy, more than 1/2 of branches have mistletoe plants and/or brooms

Step 3: Add ratings of each third to obtain total tree rating



EEE

Total rating	Infection level
0	none
1 or 2	light
3 or 4	moderate
5 or 6	heavy

Example

This third has no visible infection 0

Less than half this third is infected 1

More than half this third is infected 2

Total rating for this tree 3

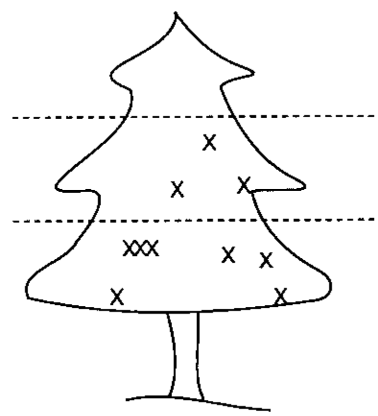


Figure 10—Description and example of Hawksworth Six–class method for evaluating dwarf mistletoe infection (Hawksworth 1977).

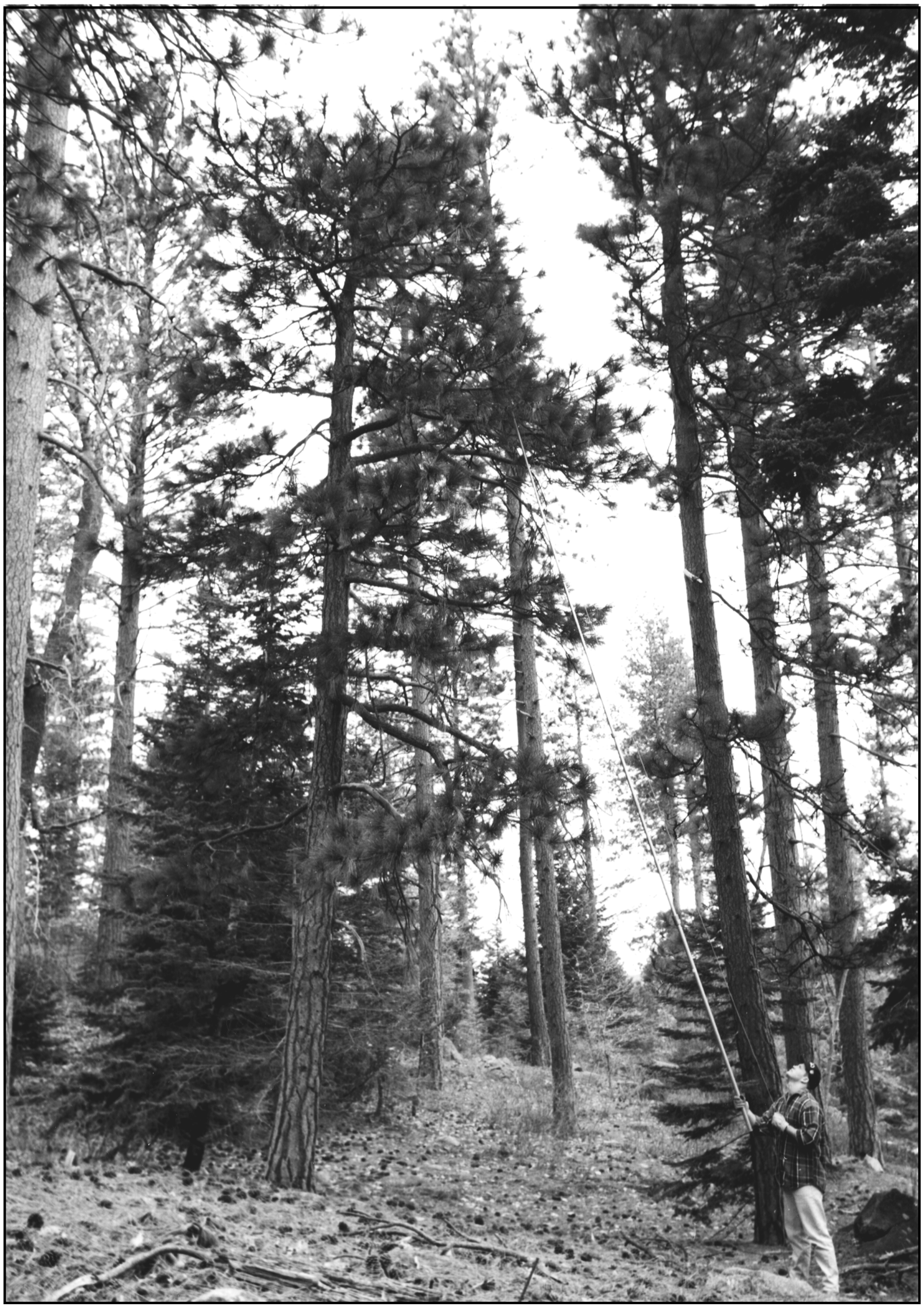


Figure 11A—Five sample branches should be cut around the tree at the mid to lower crown of each plot tree.

Figure 11B—Lateral branches should be selected for cutting, not the terminal portion of the branch.

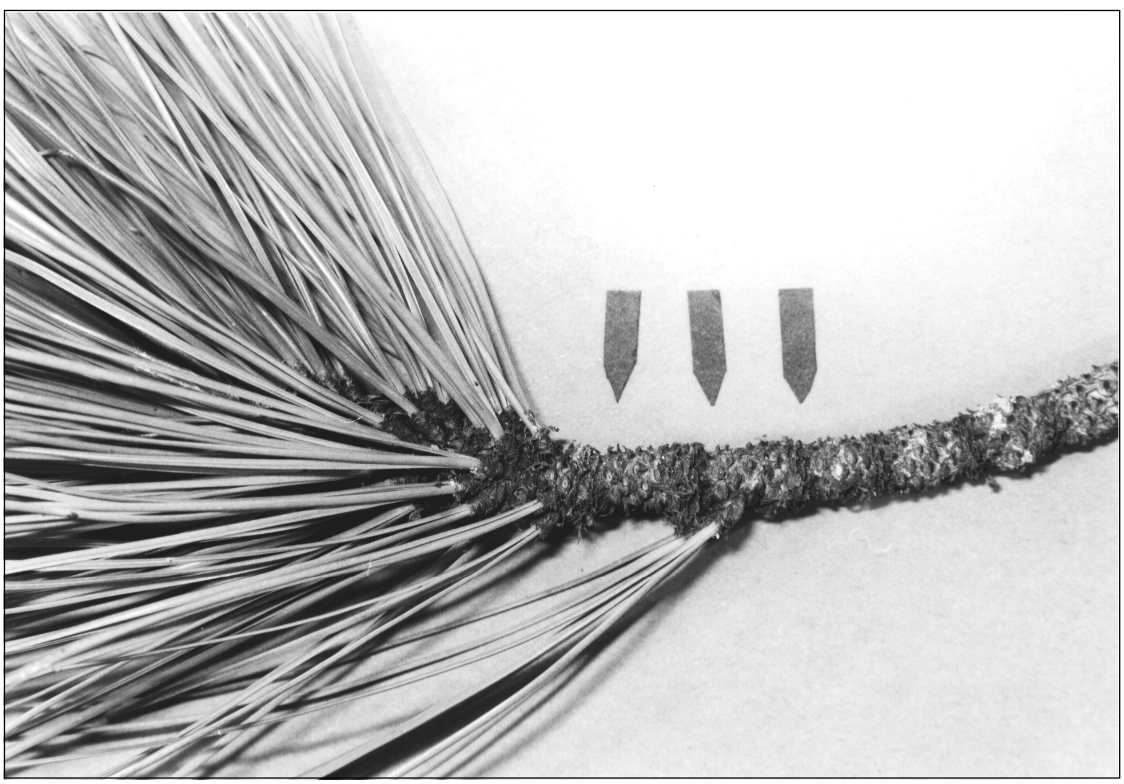


Figure 12—A branch with partially or completely missing whorls. Pointers mark the nodes that separate needle whorls. Nodes can be identified by residual bud scales that form a raised, darker ring around the stem. Scars where needles were once attached appear as small pits.

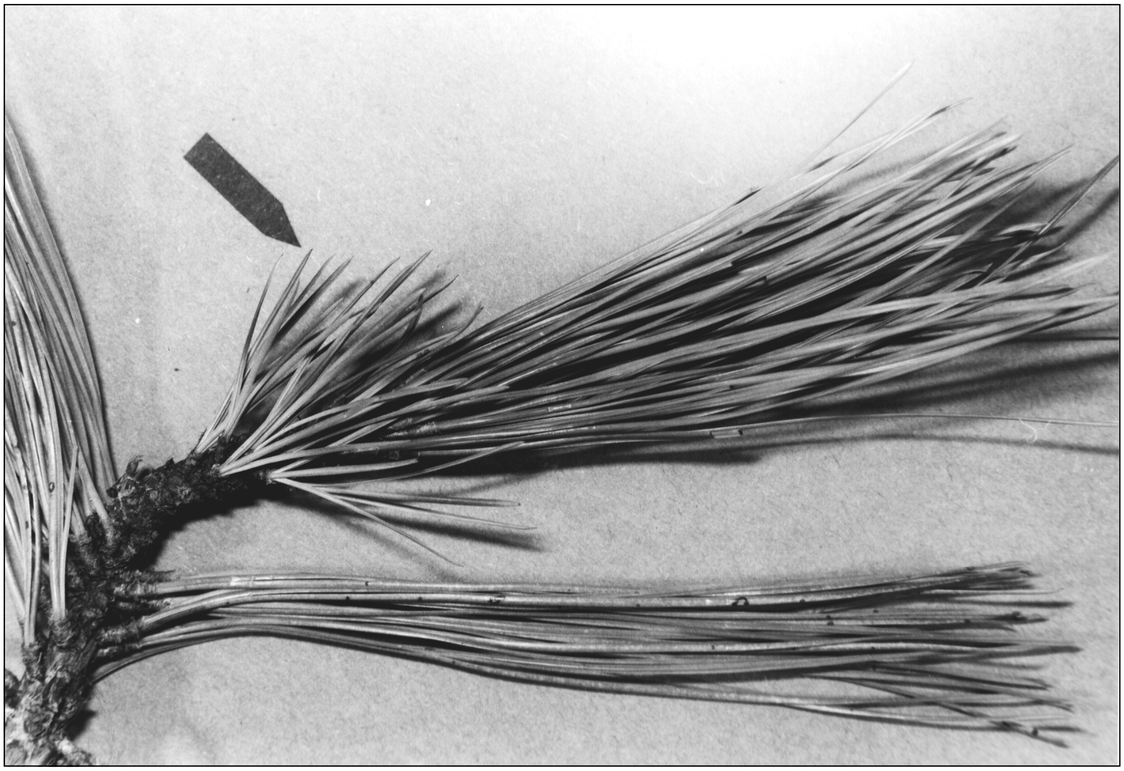


Figure 13—Needle whorl with extremely short needles formed during a drought year may be obscured by longer needles of adjacent whorls. Care must be taken to identify and include whorls with short needles.

3 = 67–100 pct

To estimate fascicle retention, abscission scars (small pits in the scaly branch surface) should be noted between the whorl nodes (*fig. 12*). Most often on younger needle whorls none will be present, and the retention class will be 3 (100 pct). It is not necessary to actually count all abscission scars and retained fascicles; a visual estimate is sufficient.

- Common needle length—The common needle length in each whorl should be measured with a ruler to the nearest centimeter in the center portion of the whorl (*fig. 15*). The needle tips should not be chewed off at the same point by insects, but if they have been, a "missing" data value should be recorded or another branch selected.

Biotic and Other Abiotic Injury to Needles

This category is a visual estimate of needle damage by biotic agents such as: CI = chewing insects, SI = sucking insects, FI = fungal injury, and OTH = other, (read as pct of needles affected), and other abiotic (WF = winter fleck, OTH = other) injuries observed on the needle surfaces (read as pct of needle surface affected) and should use the following classes:

0 = none

1 = 1–25 pct

2 = >25 pct

Procedural Tips

When possible, work should be done in pairs or a crew of three. An efficient crew includes a pruner, an observer, and a data recorder. Pruned branches

0

1

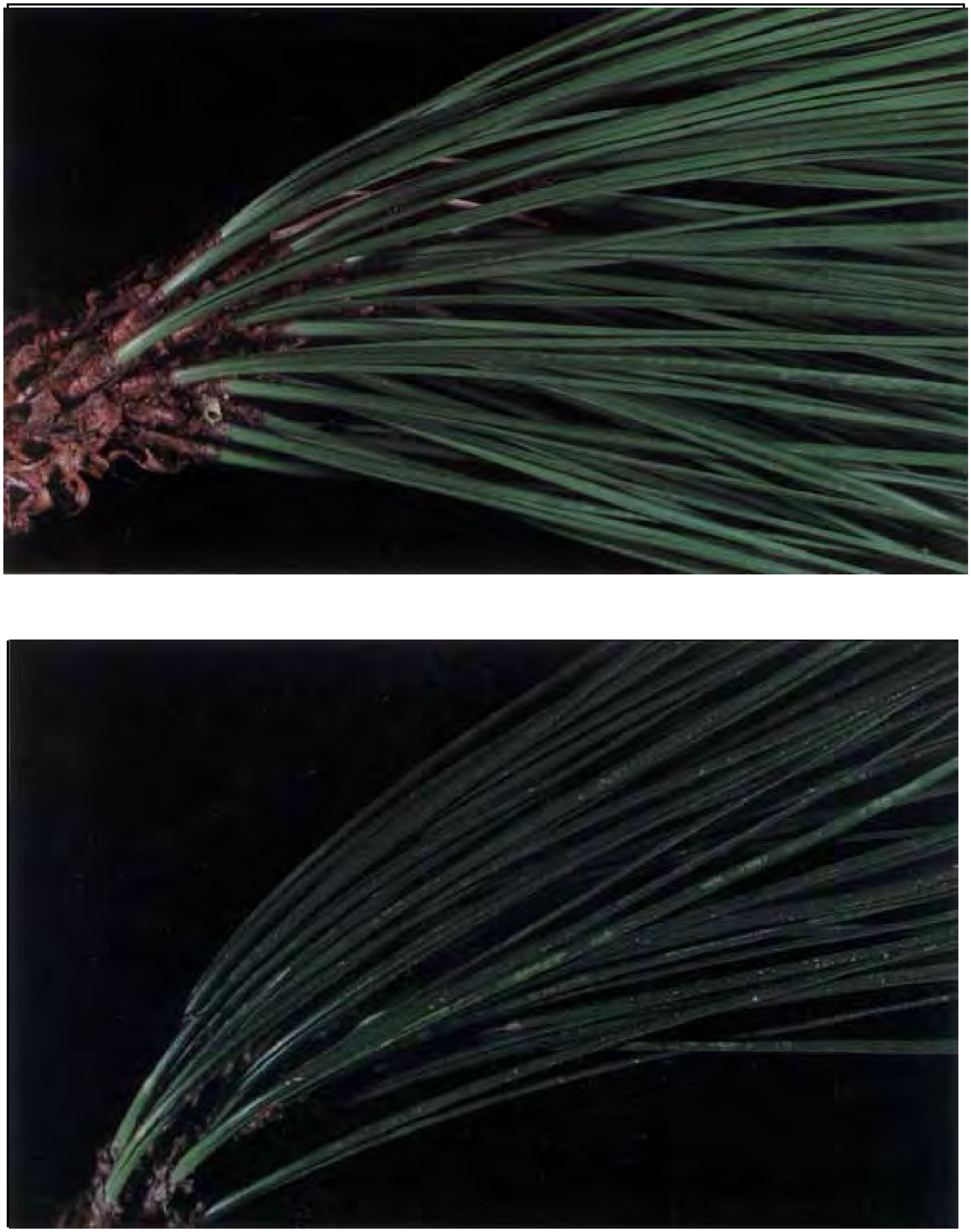
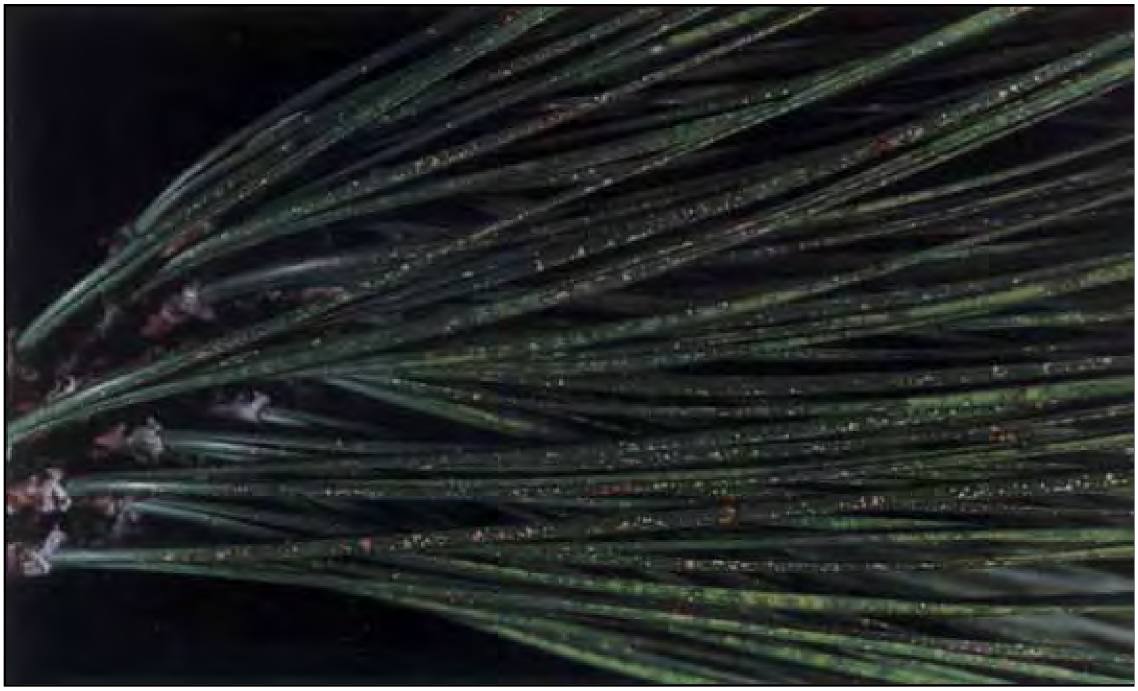


Figure 14—Examples of the different categories of chlorotic mottle used to estimate intensity of ozone injury to needles: 0 = No Mottle, 1 = 1–6 pct, 2 = 7–25 pct, 3 = 26–50 pct, 4 = 51–75 pct and 5 = > 75 pct.

2



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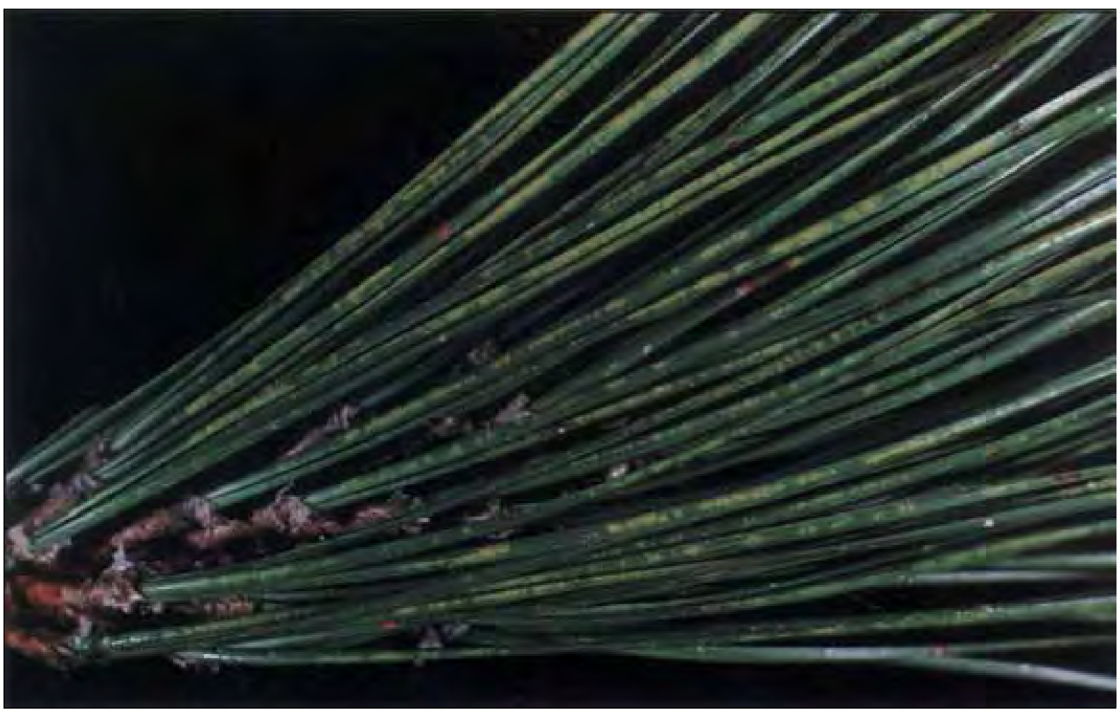
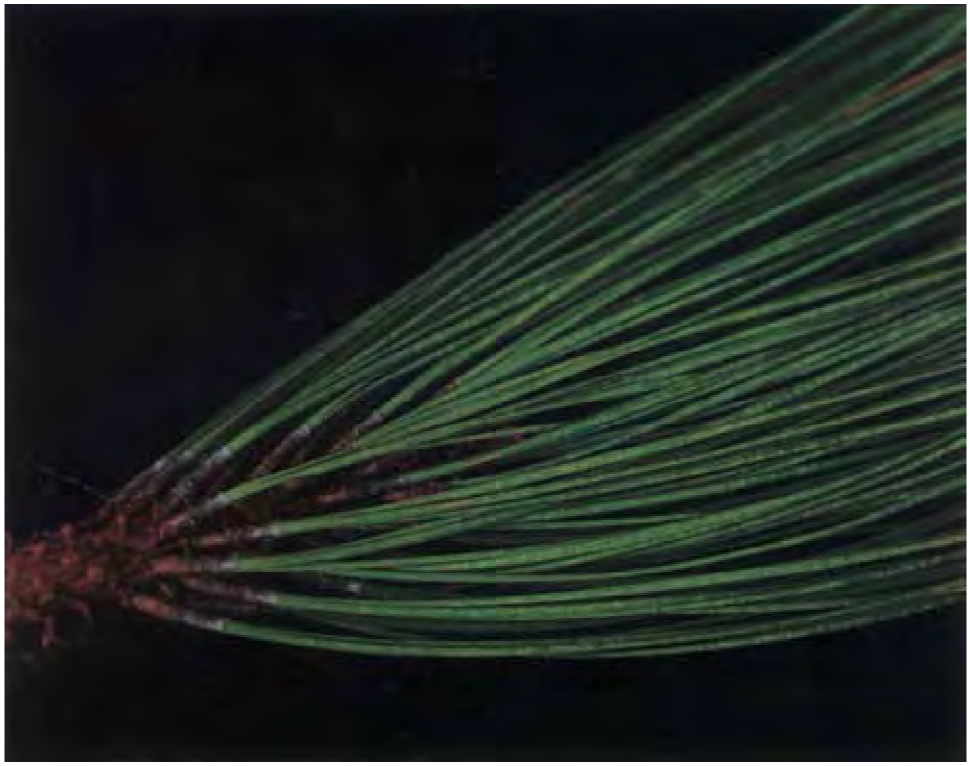


(Fig. 14, continued)

(Fig. 14, continued)

4



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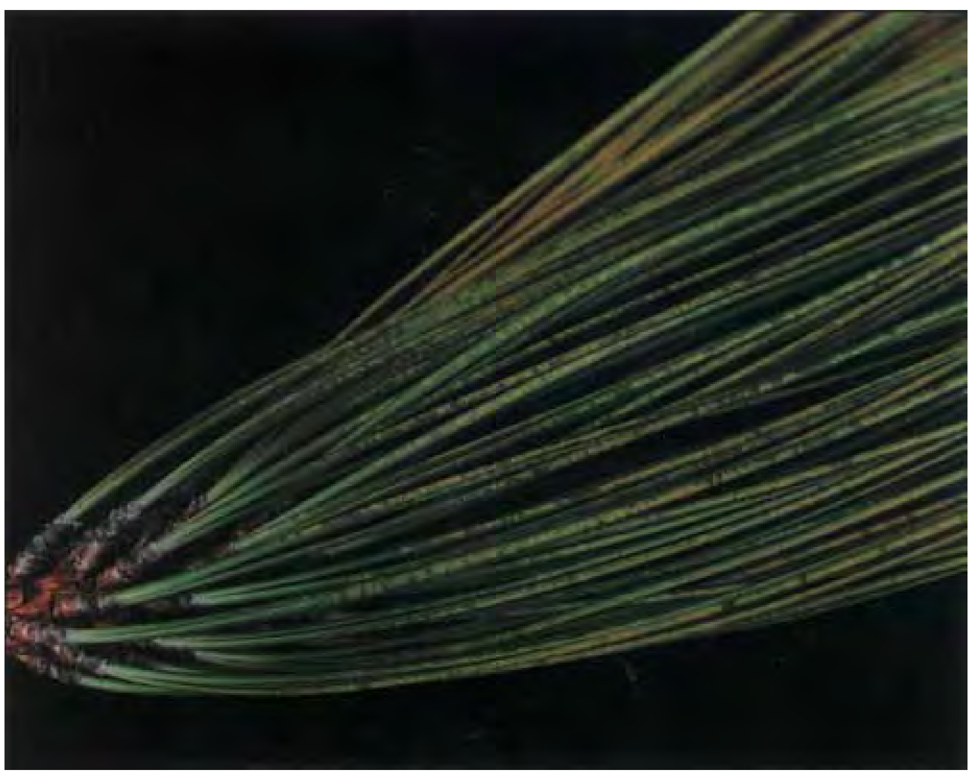


Figure 15—The method for measuring needle length of each whorl present.

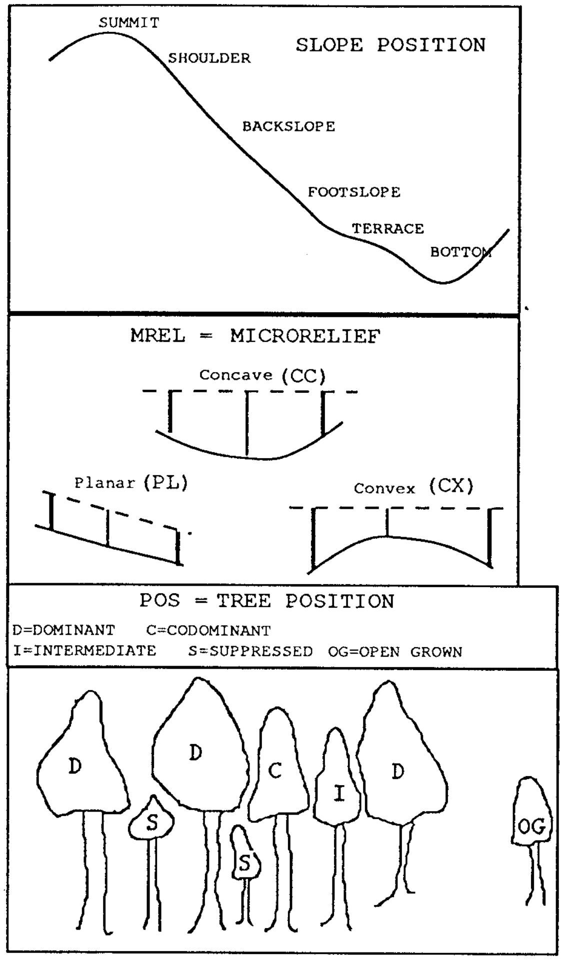
should be organized at the base of each tree in the shade. If branches cannot be evaluated within 1–2 hours they should be placed in labeled plastic bags and in a cooler or ice chest.

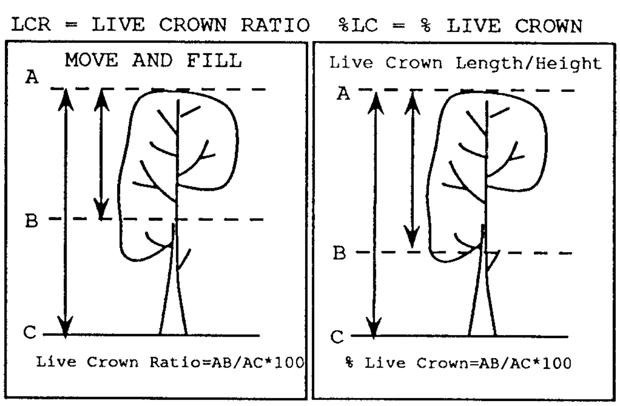
A ball point pen or permanent fine–tipped marker (Sharpie extra fine) should be used to write the number of each branch on the cut surface or, if resin is too heavy, a 1 to 2 cm strip of bark should be sliced off with a pocket knife and the branch number (1 through 5) written on the surface of the wood. This is important for quality assurance checks and for re–checking foliage before leaving the field. A copy of the code sheet and illustrations should be used for quick reference in the field (*fig. 16*).

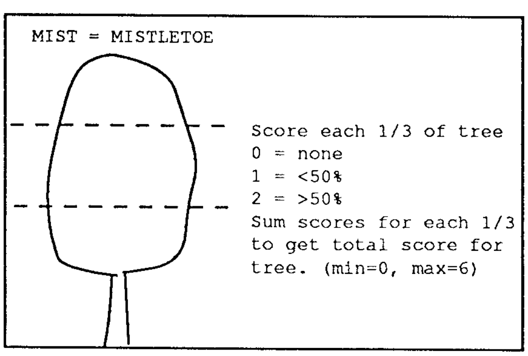
It is usually faster to observe all whorl–level variables in a sequence (e.g. mottle, biotic, abiotic, retention, length)—beginners find it easier to concentrate on one variable and complete the necessary estimates or measurements on all whorls. Because this requires repeated separation or isolation of whorls it is not efficient in terms of hand motions required. As soon as the observer is confident about the estimation or measurement of individual variables, we recommend that all variables are measured one whorl at a time. Visual estimates do not require prolonged scrutiny: experience has shown that the first impression is usually the most accurate. If working alone, the observer can use an audio tape recorder to read data into and keep both hands on the branch and ruler. Data can be entered into the computer later.

Safety

The primary safety concerns are those typical of work in the lower elevations of western mountains (climate, topography, poisonous flora and fauna). In







CODE	DEFINITION	POSSIBLE VALUES			
		ABSENT	PRESENT	SEVERE	
CONK	Conk	0	1	2	
BEET	Bark beetles	0	1	2	
FIRE	Fire scar	0	1	2	
LIGH	Lightning	0	1	2	
BROM	Witches broom	0	1	2	
MECH	Mechanical inj to lower bole	0	1	2	
MREL	Microrelief	PLANAR (PL)	CONCAVE (CC)		CONVEX (CX)
POS	Tree position	Dominant	D		
		Co-dominant	C		
		Intermediate	I		
		Suppressed	S		
		Open grown	OG		
LCR	Live Crown Ratio (move and fill) est to nearest 5%				
%LC	Percent Live Crown (% of bole with live branches) est to nearest 5%				
ROCK	% rock cover in 20Xtree dia. area				
FR	% Fascicle Retention	0. No fascicles retained			
		1 (1-33)			
		2 (34-66)			
		3 (67-100)			
CM	CHL Mottle	0 (0)	1 (1-6)	2 (7-25)	
		3 (26-50)	4 (51-75)	5 (76-100)	
BIOTIC INJURY		0 (none)	1 (< 25%)	2 (> 25%)	
SC	Scale				
SI	Sucking Insects				
CI	Chewing Insects				
W	Weevils				
FI	Fungus(needle cast)				
ABIOTIC INJURY		0 (none)	1 (< 25%)	2 (> 25%)	
WF	Weather Fleck				

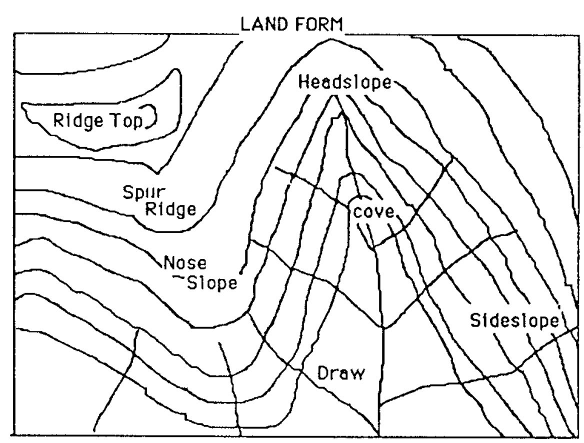


Figure 16—Quick reference sheet of site, tree and branch variables to be taken to the field.

addition, the use of a 30–ft telescoping pole–pruner can be hazardous under adverse conditions of slope, inclement weather (lightning), and fatigue. Power lines should be avoided when using a pole pruner. The pruner should be stable if it is leaning against a tree in an extended position. Back injury can result from hoisting the pole pruner from ground to vertical when fully extended. When extended or collapsed in a vertical position, pole segments in some designs can collapse rapidly causing injury to fingers and hands if they are between locking ferrules. Wearing leather gloves, such as baseball batting gloves, improves grip and may help prevent injury.

A hard hat should be worn because dead branch stubs, cones or the pruned branches themselves constantly fall from the tree. Safety glasses are also advised to prevent direct injury from a heavy falling object and also to protect eyes from the accumulation of fine debris that is dislodged from bark and needles.

Steep slopes are more hazardous because the pruner's attention is less focused on immediate surroundings while trying to get positioned to prune a branch. The field crew should be alert to poison oak and rattlesnakes. They should avoid working alone and if possible carry a hand–held radio or cellular phone so that assistance can be requested if needed.

Data Management and Analysis of Ozone Injury to Pines

Susan Schilling and Dan Duriscoe[1]

This section outlines the procedures for data management and analysis developed by the data archiving group located at the USDA Forest Service's Pacific Southwest Research Station, Riverside, California. The field data were gathered annually at approximately 33 FOREST plot locations from 1991 to 1994.

Data Entry

Tree– and whorl–level data—either from written data sheets or entered into a field data recorder (FDR)—can be merged into a master database, and the ozone injury index (OII) can then be calculated for each tree. Information from data sheets can be entered on a PC using customized software available from the Data Archiving Group (*appendix B*). After proofreading, copies of the electronic data files as well as the original data sheets should be archived. Site data should then be proofread, reformatted, and entered into the FOREST database.

Database Description

The FOREST relational database contains several files related by a key field for each site (*table 1*). The key field is composed of an abbreviation of the site name, the plot number and the tree tag number. Every site has one "plot" file containing one record for each tree tagged. Other fields in the plot file contain data such as whether the tree is currently alive, and other information that will not change, such as elevation. A tree file—one for each year data is collected—has one record for each live tree in the plot and contains data relating to crown and bole condition. The whorl file—one for each year data is collected—contains

Table 1—*Variables included in the FOREST relational database.*

Plot	Tree	Whorl	Index
ID	**ID**	**ID**	**ID**
Forest	Date	Branch #	EII
Site	Position	Whorl #	VI
Plot #	Species	Chlorotic Mottle	RET
Tag	DBH	Retention	LGT
Crew	Height	Needle Length	CD
Aspect	Pct Live Crown	Biotic Injury	height
Slope	Live Crown Ratio	Abiotic Injury	DBH
Elevation	Rock	Comment	Foliated Length
Landform	Mistletoe		Needle Length
Slope Position	Conk		
Community	Bark Beetle		
Year	Fire Scar		
Alive	Lightning Scar		
Year dead	Broom		
	Microrelief		
	Bole comment		
	Foliated Length Branch 1–5		

[1]Computer Programmer, Pacific Southwest Research Station, USDA Forest Service, 4955 Canyon Crest Drive, Riverside, CA 92507; Biological Technician, Sequoia and Kings Canyon National Parks, USDI National Park Service, Three Rivers, CA 93271

all the data collected from the whorls and has one record for each whorl. A yearly OII index file is created from data in the tree and whorl files by the dBase program, “Index.prg,” and contains the calculated OII for each live tree.

Development and Application of the Ozone Injury Index (formerly the Eridanus Injury Index)

Index Components

The ozone injury index (OII) is a modified version of the additive Eridanus injury index (EII) proposed by Duriscoe (1988), and is comprised of the primary effects of ozone air pollution on pines:

- Production of chlorotic mottle symptoms;
- Accelerated senescence of needles and subsequent reduced whorl retention from ozone stress that also reduces the amount of carbon fixation;
- Reduced percent live crown as lower branches die first in ozone declining trees;
- Reduced needle length in newly initiated needles as carbon reserves become limiting (Miller and others 1963).

Whorl retention is weighted 40 out of 100; mottle is weighted 40 out of 100; needle length is weighted 10 out of 100; and live crown ratio is rated 10 out of 100. The OII incorporates the degree of pollution injury at the branch level to give a tree level score. The maximum injury score is 100 and indicates a tree that has only one whorl of needles remaining, greater than 40 percent chlorotic mottle injury on that whorl, short needles (1 centimeter or less), and a percent live crown of less than 10 percent. The minimum index score is zero and indicates a tree with no chlorotic mottle symptoms on any of the foliage (an asymptomatic tree): if no chlorotic mottle is found on any needles, i.e., if the chlorotic mottle factor is zero, then reductions in whorl retention, live crown ratio, and needle length cannot be attributed to ozone air pollution. Therefore, these factors are not computed and the composite OII is set to zero for asymptomatic trees regardless of condition of the crown.

The index weights whorl retention (40 percent) and chlorotic mottle (40 percent) greater than the other index components—percent live crown (10 percent), average needle length (10 percent)—and is therefore not strictly monotonic (if an index is not strictly monotonic there should be a valid reason for increased emphasis on any one factor) (Muir and McCune 1987). The increased emphasis on whorl retention and mottle is based on physiological studies by Patterson and Rundel (1989). Their work also indicates that all whorls are significant contributors of fixed carbon. Thus, the index fulfills the requirements elucidated by Muir and McCune (1987) for assessing pollution injury on vascular plants.

Caveats

Chlorotic mottle has a relatively linear effect in reducing net photosynthesis of Jeffrey and ponderosa pine to about 40 percent of needle surface. After 40 percent of the surface area is affected, increasing levels of mottle on photosynthesis is negligible (Patterson and Rundel 1989). Therefore, when the visible injury ratings of the whorls are averaged for a branch, all ratings above three are set to three. If no chlorotic mottle is present on any whorl, the injury index is zero. Mottle injury is also weighted by the whorl on which it occurs. If only two whorls are present on a branch the mottle rating from each is weighted equally. If there are three whorls the mottle is weighted, .35, .35, .30 for the first, second, and third whorls, respectively. If the branch has four or more whorls, the mottle is weighted .28, .28, .24, .20 for the first to fourth whorls. Mottle appearing on a fifth or older whorl does not contribute to the index and is used only to delineate a symptomatic/asymptomatic branch. The maximum mottle score is 40, the minimum score is zero (a mottle injury score of zero implies an overall OII of zero).

Whorl retention evaluation assumes that retention of five annual whorls indicates a healthy ponderosa pine (Munz and Keck 1973). Although Jeffrey pines can be expected to retain eight or more whorls, the use of five as a threshold for ozone effects is reasonable since retention of more than five whorls indicates a relatively healthy pine tree. If a pine has chlorotic mottle on whorls five or older the mottle factor of the injury index will indicate the tree is asymptomatic, and the whorl retention factor of the index will record no injury. A pine is not likely to be even moderately impacted if it retains more than five whorls of needles. Whorl retention, and percentage of fascicles retained per whorl (1 = 1–33 percent; 2 = 34–66 percent; 3 = 67–100 percent), are scored on the data sheet to indicate missing whorls or portions of missing whorls because of insect predation, fungi, or other stresses (non–air pollution causal agents). The index computes whorl retention as fractions of whole whorls (i.e., 1, .66, .33), except for less than one whorl retention. Branches with less than one whorl are considered to have a full whorl in the index, since retention of 1 whorl or less produces the maximum injury score (40) for whorl retention (see example below). Maximum injury score for whorl retention is 40 (only one whorl of needles), minimum score is 0 (five or more full whorls of needles).

Modal needle length in centimeters of representative needles is measured in the middle of each year's whorl of needles. This assumes that maximum expansion of the needle has occurred and that the needle length of a very healthy ponderosa is 21 centimeters and Jeffrey pine needle is 19 centimeters. Needle lengths shorter than one centimeter are computed as one centimeter. Maximum injury score for needle length is 10 (needles 1 centimeter or less), minimum score is 0 (needles 21 or 19 centimeters or greater).

Percent live crown is the proportion of the total crown that has any live branches with any number of live needles. This assumes a crown retention of 10 percent as a minimum value. Maximum injury score for percent live crown is 10 (10 percent or less of the bole with a live branches), minimum score is 0 (80 percent or more of bole with live branches).

Equation for Calculating the Ozone Injury Index (OII)

Definitions and Weightings

Each of the following four variables require individual calculation before summation. These calculations provide averaging and introduce the specified weightings.

OII = VI + RET + LGT + CD

in which:

VI = Visible injury (chlorotic mottle)

RET = Number of needle whorls present and fascicle retention

LGT = Modal length of needles (average all whorls)

CD = Percent live crown

Visible injury (VI) (chlorotic mottle)

VI includes weighted average of up to the first four whorls:

5+ Whorls: VI=1+{40*((CM1*.28)+(CM2*.28)+(CM3*.24)+(CM4*.20))/3}

4 Whorls: VI=40*((CM1*.28)+(CM2*.28)+(CM3*.24)+(CM4*.20))/3

3 Whorls: VI=40*((CM1*.35)+(CM2*.35)+(CM3*.30))/3

2 Whorls: VI=40*((CM1*.50)+(CM2*.50))/3

1 Whorl : VI=40*(CM1)/3

in which:

CM1 (chlorotic mottle) is the average mottle on all current year whorls (except if mottle rating of a whorl is > 3 it is set to 3 before average is calculated). CM2 is the average mottle on all one–year–old whorls, and so on.

Retention (RET)

If oldest whorl on any branch is 1:

FWHORL=WR1*.33

If oldest whorl on any branch is 2–6:

FWHORL=(oldest whorl–2)+(sum fascicle retention 2 oldest whorls*.33)

If oldest whorl on any branch is > 6:

FWHORL = oldest whorl – 1

Then:

RET = 40*[100.37/(1+e^(1.72*(FWHORL–4.532)))+.6304]/100

in which:

e=2.718

WR1=Average fascicle retention of all current year whorls on all 5 branches.

WR2=Average fascicle retention of all 1–year–old whorls on all 5 branches.

WR3=Average fascicle retention of all 2–year–old whorls on all 5 branches.

Needle length (LGT)

If species is Jeffrey pine:

LGT=(18–Len)*10/18

If species is ponderosa pine:

LGT=(21–Len)*10/21

in which:

Len is the average needle length of all whorls.

Percent live crown (CD)

CD=(80–percent live crown)*10/70

OII Calculation

Sample Data Set for One Tree

BRANCH	WHORL	CM	RET	NL
1	1	0	3	20.5
1	2	0	3	17.5
1	3	0	3	21.0
1	4	1	1	13.0
2	1	0	3	17.0
2	2	0	3	18.5
2	3	0	3	20.0
2	4	5	3	14.0
3	1	0	3	16.5
3	2	0	3	17.5
3	3	0	3	21.0
3	4	1	2	11.5
4	1	0	3	21.0
4	2	0	3	20.0
4	3	0	3	20.0
4	4	1	2	15.0
4	5	0	0	0 (This whorl is missing)
4	6	2	1	15.0
5	1	0	3	18.0
5	2	0	3	17.0
5	3	1	3	19.0
5	4	1	1	13.0
5	5	0	0	0 (This whorl is missing)
5	6	0	1	13.0

Weighting of Components and Computation of the OII

Chlorotic mottle

Oldest whorl is 6

CM1=(0+0+0+0+0)/5 = 0

CM2=(0+0+0+0+0)/5 = 0

CM3=(0+0+0+0+1)/5 = .2

CM4=(1+3+1+1+1)/5 = 1.4

VI=1+{40*[(0*.28)+(0*.28)+(.2*.24)+(1.4*.20)]/3}=5.37

Retention

Oldest whorl is 6

Fascicle retention of two oldest whorls is:

WR5 = 0

WR6 = (0+0+0+1+1)/5 = .4

FWHORL = (6–2)+((0+.4)*.33)]} = 4.132

RET = 40*[100.37/(1+2.718^(1.720*(4.132-4.532)))+.6304]/100

= 26.97

Needle length

LEN=17.2, Species is ponderosa

LGT=(21–17.2)*10/70 = 1.81

Percent live crown

Percent live crown = 66

CD=(80–66)*10/70 = 2

Result

OII=VI+RET+LGT+CD = 5.37 + 26.97 + 1.81 + 2.0 = 36.15

Quality Assurance, Training, and Certification in Ozone Air Pollution Studies

Susan Schilling, Paul Miller, and Brent Takemoto[1]

Uniform, or standard, measurement methods of data are critical to projects monitoring change to forest systems. Standardized methods, with known or estimable errors, contribute greatly to the confidence associated with decisions on the basis of field data collections (Zedaker and Nicholas 1990). Quality assurance (QA) for the measurement process includes operations and procedures so that the data are of the specified quality within a stated level of uncertainty. Quality is acceptable when data are consistent, lack uncertainty, and meet users' needs. Some of the elements of a QA plan for ozone air pollution studies are proposed for use in the interagency project, Forest Ozone REsponse STudy (FOREST).

Data Quality Objectives and Quality Assurance

Site Data

Site data variables should be obtained by at least a crew of two independent observers. A third observation can be obtained by an individual who has acted as a trainer. Each measured variable should be determined to ± 10 percent (Zedaker and Nicholas 1990), and evaluations of categorical elements (forest type/community) should be determined after experienced observers agree on the following variables:

Estimated or measured	*Categorical definitions*
topo map location	landform type
azimuth	slope position
latitude	microrelief
longitude	aspect
elevation	forest type (3 main species)
percent slope	land use
site class	understory vegetation
	description, plot locator tree

To ensure that data collection meets the acceptable data quality objectives, the FOREST project cooperators should perform or delegate the remeasurement of plot variables on one of three plots established around each ozone monitor. The percent frequency of misclassification or greater than 10 percent difference in measured variables should be recorded. An independent remeasurement by the QA crew should also be done by expert crews.

Tree Data

To ensure that data collection meets the acceptable data quality objectives, the FOREST project cooperators should perform or delegate the remeasurement of tree variables on 10 percent of the trees in each plot. The percent frequency of misclassification or greater than 10 percent difference in measured variables should be recorded. An independent remeasurement by the QA crew should also be done. The tree data collection should include the following variables and their acceptable limits:

[1]Computer Programmer, Pacific Southwest Research Station, USDA Forest Service, 4955 Canyon Crest Drive, Riverside, CA, 92507; Plant Pathologist, Pacific Southwest Research Station, USDA Forest Service, 4955 Canyon Crest Drive, CA, 92507; Air Pollution Specialist, California Air Resources Board, Research Division, 2020 L Street, Sacramento, CA 95814

Species	agreement by at least two observers
Crown position	agreement by at least two observers
Percent bare rock	± 10 percent
Tree height	± 1.0 m
Percent live crown	± 5 percent
DBH	± 0.2 cm
Mistletoe	± one category (out of six total)
Conks	agreement by at least two observers of ± one category
Beetles	agreement by at least two observers of ± one category
Witches brooms	agreement by at least two observers of ± one category
Fire scars	agreement by at least two observers of ± one category
Mechanical injury	agreement by at least two observers of ± one category
Lightning scars	agreement by at least two observers of ± one category

Branch and Whorl Data

Each field season the following branch and whorl variables should be remeasured by a trained QA monitor, chosen by the cooperators. The QA monitor must be a Certified Data Collector, but should not be a member of the crew that did the original measurements.

Foliated length	± 1.5 cm
Number of whorls	± 1
Chlorotic Mottle	± 1 class
Retention	± 1 class
Needle length	± 1.5 cm
Biotic injury	± 1 category
Abiotic injury	± 1 category

Procedures require that the crew has numbered each branch and returned them to the shady side of the tree from which they were cut. The following steps should be followed on each of the three plots to assure consistent quality data collection:

- At the end of each field sampling day, two trees should be randomly selected from the total scored that day, until five QA trees have been identified for each plot.
- From the two trees selected that day, two branches should be randomly selected.
- Each branch should be tagged with plot and tree number. The branch number should already be written on the cut end of the branch, and it should still be legible.
- The branches should be bagged in plastic and kept refrigerated until all five QA trees have been scored for one plot.
- The 10 branches should be boxed and insulated with paper or equivalent material.
- Boxes of branches should be mailed to the QA monitor. Include data printouts or copies of data sheets for those branches.

The independent QA monitor reports the results of the remeasurements to the individual cooperators. Results for all cooperators/plots should be summarized in a report. This information should be used to either accept or reject data sets collected in a given year. It should be reviewed at an annual debriefing meeting in which representatives from field crews should determine their needs for additional training.

Training and Certification

At least one member of each data collection crew will be a Certified Data Collector. A Certified Data Collector will have successfully completed at least one full session of training (2 days classroom, 1 field) and returned for the current year's field training day (second day of the full session). Only a Certified Data Collector may score branches.

The yearly training session should be scheduled no more than 1 month before the first crew is to begin collecting data, which has been specified as no earlier than the first week of August. The training session will consist of 1 full day in the classroom and 1 full day in the field. New trainees will attend both days; returning crews will be required to attend only the second (field) day. The classroom day will begin with presentations of the purpose of project FOREST and overviews of the data collection methods. In the afternoon, sub–groups will rotate between trainers to receive hands–on training in scoring branch and whorl variables.

The field day will begin with training on tree variables to be collected that year, demonstrations of cutting branches, and a review of QA procedures. Each sub–group will be assigned two trees from which they will cut two branches. Each member of the crew will score the branches. The groups will rotate to the next two trees (branches precut by the previous group will be left at the base of the tree). After most of the day has been occupied with practice and questions, the students will evaluate different trees for DBH, height, percent live crown, etc. Branches already measured and evaluated for ozone injury will be presented to students as a test. The trainee's data sheets will be corrected against the instructor's results using the data quality standards. Any trainee who is not within acceptable QA limits will be retrained in the problem area and retested.

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Glossary

Abiotic	A non–living physical agent, e.g. temperature, wind, or chemicals, that causes injury to plant tissue.
Abscission	The normal or abnormal separation of leaves from a plant; leaf–fall.
AQRV	Air Quality Related Values, a term used in the Clean Air Act to describe ecosystem components that are vulnerable to air pollution, e.g. plants, soil, surface waters, visibility, and odor.
Bioindicator	A plant species, selection, or clone used to monitor air pollution injury because of its specific sensitivity to particular air pollutants.
Biotic	A living agent, e.g. a fungus, bacterium, or insect, causing injury or disease in plant tissue.
Bole	The main stem or trunk of a tree.
Branch	A limb, offshoot, or ramification of any main stem of a tree.
Canopy	The cover formed by the leafy upper branches of trees in a forest.
Chlorotic	Abnormally yellow color of leaf tissues resulting from loss of chlorophyll after injury by air pollutants or other abiotic agents.
Chronic	Injury continuing for a long time or recurring frequently due to relatively low concentration exposures to air pollutants.
Complement	A pre–determined amount or quantity of pine needle fascicles that constitutes a complete needle whorl of a particular age.
Damage	Injury that reduces value or the money equivalent for injury.
DBH	Diameter–at–breast–height of the bole or stem of a tree as measured at 1.4 m above the ground on the uphill side.
Fascicle	A small bundle of three or five pine needles (depending on species), sometimes called a dwarf shoot, that together form a needle whorl or complement.
Genotype	The genetic makeup of an organism with reference to a single trait, e.g., sensitivity to an air pollutant.
Gradient	The rate of change with distance of a variable quantity, e.g. the change of ozone concentration in the downwind diffusion path from a source area.
Index	A numerical expression serving to describe the state of an individual based on an additive combination of separate attributes, e.g. whorl retention, chlorotic mottle, needle length, percent live crown.
Injury	A particular form or instance of harm that is usually visible and causes an impairment of the normal function of a leaf–tree.
Locator tree	A prominent tree with evident promise of longevity that is selected and labeled permanently as a means of locating a nearby population of sample trees (in a plot).

Mesophyll	The cells of a leaf with a large quantity of chloroplasts forming the tissue of the same name that is the primary site of photosynthesis.
Monitor	The process of using plants (bioindicators), other devices, and systems to detect and record chronic injury from air pollution.
Mottle	Marked with spots or blotches of different color, e.g. yellow islands of tissue against the normal green is the appearance of chlorotic mottle on all needle surfaces caused by ozone injury.
Necrosis	Death of a circumscribed portion of a plant, e.g., death of needle tips or bands at any position on the needle.
Needle	A small, slender, rod–like leaf usually in bundles of three or five called fascicles; number of needles per fascicle depends on pine species.
OII	Ozone injury index, a numerical index comprised of four variables, namely, whorl retention, chlorotic mottle, needle length, and percent live crown. OII is scaled from 0–no injury, to 100–maximum injury.
Ozone	A form of oxygen with three atoms and oxidizing properties that are harmful to plants and irritating to humans; formed from the photochemical reaction of unsaturated hydrocarbons and nitrogen dioxide.
Plot	A small parcel of land sufficiently large to contain an adequate sample of trees for repeated observation of crown condition (expressed as the OII); usually 80 m wide and sufficiently long to contain at least 50 sample trees on the same exposure, slope and landform.
Population	The assemblage of organisms (trees) living in a given area (plot).
QA	Quality Assurance, a process whereby data quality is assured by standardizing procedures, setting data quality objectives, and requiring remeasurement to ascertain the quality of measurements and the suitability of the entire data set.
QC	Quality Control, the application of measures to improve data quality on the basis of knowledge gained from QA procedures.
Retention	The state of all possible needle fascicles retained within a single annual whorl, or of the entire tree retaining a variable number of whorls depending on its history of injury from biotic and abiotic agents.
Sampling	The act or process of selecting a sample for testing. Usually a process called stratified random sampling is used in which sample areas are randomly selected from among large units (entire forests), intermediate units (individual watersheds), and focused units (vegetation types).
Stratification	The hierarchal division of an area to be sampled into distinct strata (see sampling).

Survey A sampling, or partial collection, of facts and data taken and used to indicate what a complete collection and analysis might reveal, e.g., sampling along a transect.

Transect A sample area established by cutting across an area of interest (vegetation type) in a path of indeterminate length, where specific (not all) information is gathered.

Tree ring A tree ring or annual ring is a yearly formation of new wood in stems, observable as a ring on the cross–section of a tree trunk. Stress reduces ring– width.

Whorl A circular arrangement of like parts (pine needle fascicles) around a section of an axis (branch).

Winter fleck Necrotic spots and blotches found only on the upper surface of pine needles after exposure to at least one winter. The histological symptoms are distinct from ozone, but the exact cause(s) are uncertain.

Appendix A—Field Data Recorder Operation Instructions

Project FOREST Field Data Recorder Manual (6/94)

Do not use these programs unless you have a PC with database software that can use .DBF (i.e., dBase, Foxpro) files *and* a Corvallis Microtechnology MC–V in good condition with MBASE and a MC–COMKIT (includes the cable to connect the PC and MC–V and Corvallis Microtechnology Kermit software). This package contains a disk with a data entry program that will be uploaded to the field data recorder (MC–V).

INITIAL LOADING OF PROGRAM AND FILES TO MC–V

On the PC:

Put Kermit disk in drive A (if drive B then substitute B for A in following commands).

Type the following commands (you type the **BOLD**), <enter> after each.

C:\> **MKDIR CMTCOM**

C:\> **CD CMTCOM**

C:\CMTCOM> **COPY A:*.* /V**

C:\CMTCOM> **CD**

Put the disk containing the field data recorder data entry program in drive A (if drive B then substitute B for A in following commands).

Type the following commands, <enter> after each.

C:\> **MKDIR FDRPROG**

C:\> **CD FDRPROG**

C:\FDRPROG> **COPY A:*.* /V**

C:\FDRPROG> **CD**

C:\> **MKDIR FDRDATA**

(This is the directory where you will store the data)

UPLOAD Program files to the MC–V

On the MC–V, CD to the drive marked FILES. Connect the cable to the COM1: port on the MC–V and the serial port, COM1:, on the PC.

On the PC type:

C:\CMTCOMM> **mode com1:96,n,8,1<enter>**

C:\CMTCOMM> **KERMIT SEND C:\FDRPROG*.* <enter>**

On the MC–V:

KERMIT R <enter>

STARTING THE INPUT PROGRAM

To enter data “current year” whorl first:

MBASE CURRENT

to enter data “oldest” whorl first:

MBASE OLDEST

GENERAL INPUT INSTRUCTIONS AND CAUTIONS

Make sure KEYBEEP is on (KEYBEEP ON) before the program is started.

To enter data, type the data into the highlighted field and press ENTER.

Data can only be edited while it is on the screen. You cannot go back a screen but you will be given a chance to re–do the data. Once you go to the next screen the data can only be edited on the MC–V by exiting the program and using the commands available in MBASE (EDIT, BROWSE).

If you find another whorl when entering oldest first, enter them in the right sequence but change the whorl number so you will have two whorl 3's for example; include an edit note in the comment field for later correction.

If a mistake is made on one whorl and you want to re–enter the data, you do not need to re–enter all the whorls on that branch. Enter the correct whorl number and re–enter that whorl (leave a note in the comment field to delete the other incorrect data).

You can use arrow keys to move around the current screen and edit data before final "ENTER Y TO CONT."

Avoid using backspace to edit data in a field; type–over works fine.

Some fields have default values; to accept data already entered in a field just hit ENTER.

Be careful to type–over all the existing incorrect data in a character field, but for a numeric field just type the number and ENTER (if decimal is 0 it does not need to be entered).

Typing zero over some other number in a "1" wide numeric field causes the field to go blank; but the field is NOT blank—the zero was entered, it is simply not shown.

When the screen says "ENTER Y TO CONT" the highlighted box should contain an F. If the data is okay and you want to continue to the next input screen press Y. When the screen asks for a Y or N, there should already be a T or F in the box.

Y is the same as T; N is the same as F.

To answer yes, Y and ENTER. To answer no, N and ENTER. No other input will be accepted. Just ENTER to accept T or F already displayed.

Some numeric fields will only accept data in a preset range. If you enter a value outside the range (i.e., a 9 if the range is 0–5), the cursor will not move until it has been corrected.

DAILY DOWNLOAD OF COLLECTED DATA:

Connect the cable to the COM1: port on the MC–V and the serial port, COM1:, on the PC.

on the PC

```
C:\> CD CMTCOMM
C:\CMTCOMM> mode com1:96,n,8,1<enter>
```

on MC–V

```
KERMIT SEND *.DBF<enter>
```

on PC

```
C:\CMTCOMM\> KERMIT R <enter>
```

PREVIOUSLY DOWNLOADED FILES WILL BE OVERWRITTEN BY THIS. BE CAREFUL TO RENAME THE FILES ON THE PC AFTER THEY ARE DOWNLOADED!

on the PC:

```
C:\CMTCOMM> COPY WHORL.DBF C:\FDRDATA\SLW0821.DBF
```

(Site is Shaver Lake, Whorl data collected on August 21)

PRINT AND PROOFREAD

Keep a copy of all the files on floppy.

To print the .DBF files on the PC you can use any software that can use dBase III+ DBF files (dBase, Foxpro and many other database programs can handle dbf files).

Print and proofread the new data after *every* field day. Each downloaded file will also contain the previous day's data but you only need to print the new data. When you are finished at a Site (or Plot) you may want to delete each day's files and keep only the last file with all the data.

On the MC–V, the files will eventually get too big for the storage area. When starting data collection at a new Site (or Plot) clear the WHORL file of data. DON'T DO THIS UNTIL YOU ARE SURE ALL THE DATA IS SAVED ON THE PC AND BACKED UP ON A FLOPPY.

On the MC–V type these commands (<enter> after each):

```
MBASE
.USE WHORL
.ZAP
Y
.QUIT
```

SENDING IN THE DATA

If possible, make any necessay corrections to the dbf files before you send then. But if you are not familiar with dBase, just send the files on a floppy and the printouts with the corrections written on them.

Send the floppy and printouts to:

Dr. Paul Miller
Pacific Southwest Research Station
USDA Forest Service
4955 Canyon Crest Drive
Riverside, CA 92507\

Appendix B—Instructions for Data Entry from Field Sheet to PC

Project FOREST Data Entry from Field Data Sheets

This package contains three data entry programs for the transfer of FOREST data from completed field data sheets to computer files. The data entry programs display on–screen prompts for the data, create an ASCII/TEXT file, and append the data to it. The programs will work on any IBM PC, XT, AT, laptop, or compatible computer with a mouse. A color monitor is not required but a printer is necessary to print the output data files for proofreading.

Installing:

Copy the files on the floppy to the hard drive of a PC.

`C:\>COPY A:*.*` to copy from a floppy in drive A to current directory

Data entry notes:

Data is entered on three separate screens.

A data entry screen is started by typing PLOT, TREE, or WHORL depending on which type of data is to be entered. Plot data, the data at the top of the data sheet, is entered on the Plot screen. Tree data, the data on the left side of the data sheet, is entered on the Tree screen. Whorl data is entered on the Whorl screen.

Enter the TREE data from all the field data sheets first, then go back and enter all the WHORL data.

On a screen, after each prompt, type the data and press `<ENTER>`.

A help message for each field will appear in the lower left corner.

Use ARROW keys to move around the screen or just click on a field with the mouse.

To edit a field use BACKSPACE or type over with correct data.

ENTER to accept data already displayed.

Missing Data: Character fields — leave blank; Numeric fields — enter –9 if possible, otherwise enter 0.

To continue with the next tree/whorl, move to “Continue?” press Y and ENTER. The data will be saved and the cursor will return to the first prompt. The data fields will still contain the old data, either replace or ENTER to accept unchanged.

To QUIT move to “Continue?” press N and ENTER.

Data entered will be saved in TREE.DAT, WHORL.DAT, or PLOT.DAT. These are TEXT files that can be edited with any ASCII/TEXT editor.

To print a .DAT file:

```
C:\>PRINT TREE.DAT <ENTER>
```

To view a .DAT file on screen:

```
C:\>TYPE TREE.DAT |MORE
```

Data Entry Screens:

To enter PLOT data type:

`C:\>PLOT<ENTER>`

(only new plots)

To enter TREE data (tree bole data and foliated lengths) type:

`C:\>TREE<ENTER>`

To enter WHORL data type:

`C:\>WHORL<ENTER>`

Sending the data in:

Please print and proofread the data. The files are ASCII/TEXT format and can be edited with any TEXT editor, like EDLIN. Many word processing packages can import TEXT files (Word Perfect), just remember to save the edited files in TEXT format. If you have no way of editing the files, send the printouts with the corrections marked on it with the floppy.

Copy all the .DAT files to a floppy:

`C:\>COPY *.DAT A:*.*` (copy all files with a .DAT from current directory to a floppy in drive A)

Mail the floppy and copies of the original data sheets to:

Paul Miller
Pacific Southwest Research Station
USDA Forest Service
4955 Canyon Crest Drive
Riverside CA 92507

Appendix C— Site Class

SITE CLASS

— Reflects ability of site to grow trees, a measure of quality or productivity.

— Is determined by tree *age* and *height*.

— Requirements of a site tree:

- Must be predominant or dominant and grown freely all its life.
- Must be at least 50 years old.
- Must be ponderosa, Jeffrey, or sugar pine, Douglas–fir, white or red fir.
- Must be suitable form class, not a cull or deformed tree.
- Must have its original top.

540 – **DETERMINING AGE OF LARGE TREES**

Use an increment borer at least 16 inches long. When determining the age of a tree that has a radius greater than the length of an increment borer, use the following procedure: Bore into tree as far as possible, extract core, and count the rings. Measure the diameter of the tree and divide by two, then subtract the bark thickness. This gives the radius of the wood part of the tree. Measure the length of the core and subtract from the radius of wood to determine how much longer the core would have to be to reach the pith. Count the number of rings in the last (innermost) inch and extrapolate to the center. Add this to the ring count on the extracted core and then add 10 years to account for the time required to grow 4–1/2 feet tall. Dunning recommends 10 years in all cases.

Calculation for determining age of large trees;

DBH / 2	=	______________	(inches)
Bark Thickness	=	______________	
Difference	=	______________	
Core length	=	______________	
Difference	=	______________	
No. of rings innermost inch	=	______________	
Sum = Age	=	______________	

Example

Determine the age of a Douglas–fir 60.0 inches DBH with bark thickness of 2.0 inches when a core 16.0 inches long has 100 rings and the innermost inch has 5 rings.

60.0 / 2 = 30.0 inches (radius of wood and bark)

30.0 – 2.0 = 28.0 inches (radius of wood)

28.0 – 16.0 = 12.0 inches (short of hitting center)

5 × 12.0 = 60 rings

100 + 60 + 10 = 170 years old

REGION FIVE SITE CLASSES

(Height by Age and Site Class Code)[1]

Site Class (Field 9)

Age	0	1	2	3	4	5
40	95	81	66	49	43	35
50	106	90	75	56	49	39
60	115	98	82	63	53	43
70	122	105	88	68	58	45
80	129	111	93	73	61	48
90	135	116	98	77	64	50
100	140	121	102	81	67	54
110	145	125	106	84	70	54
120	149	129	109	87	72	55
130	153	133	112	90	74	57
140	157	136	115	93	76	58
150	160	139	118	95	78	60
160	163	142	120	98	80	61
170	166	144	123	100	81	62
180	169	147	125	102	83	63
190	172	149	127	104	84	64
200	175	152	129	106	86	65
220	179	176	133	109	88	67
240	184	160	136	112	90	68
260	188	163	139	115	93	70
280	191	166	142	117	95	71
300	195	169	145	120	96	73
320	198	172	147	122	98	74
340	201	175	150	124	100	75
360	204	177	152	126	101	76
380	206	180	154	128	103	77
400	209	182	156	130	104	78

[1] Based on pondersoa pine, Jeffrey pine, sugar pine, Douglas-fir, red fir, and white fir. Age is in years. Total height is in feet of average dominant and predominant trees with tree age of at least 50 years. Adapted from Dunning's site index curves for height at 300 years (Dunning 1942). (Predominant and dominant defined in Field 21, Crown Position.)

United States
Department
of Agriculture

Forest Service

**Pacific Southwest
Research Station**

General Technical
Report PSW-GTR-155

Evaluating Ozone Air Pollution Effects on Pines in the Western United States