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

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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

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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

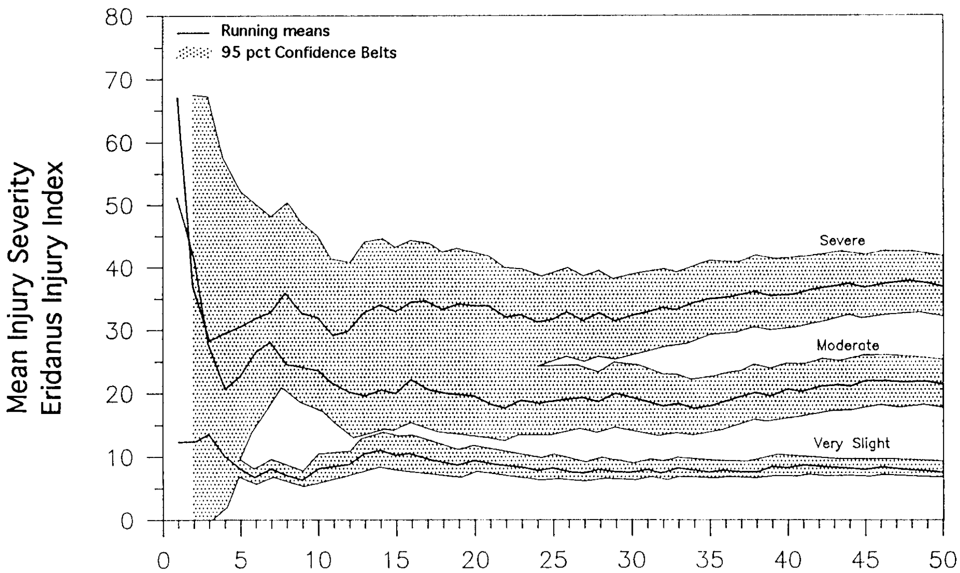

Figure 1—Variability in ozone injury between stands of ponderosa and Jeffrey pine in the Sierra Nevada (Duriscoe 1988). Line represents mean injury and shaded area represents the 95 percent confidence interval associated with the mean. (a) Severely injured stand with high variability in injury between 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–

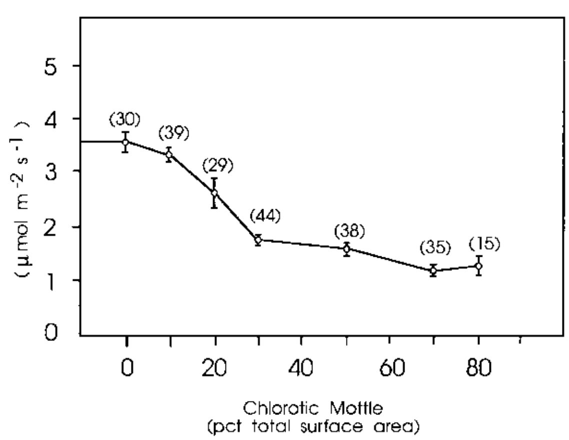

Figure 2—Relationship between severity of chlorotic mottle, represented by percent surface area with mottle, and net photosynthesis in Jeffrey growing under conditions of adequate soil moisture in early summer 1988, Sequoia National Park (Patterson and Rundel 1989).

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.

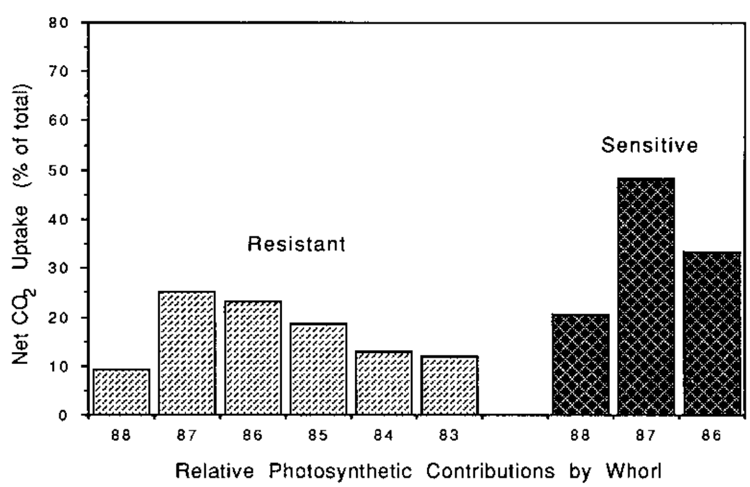

Figure 3—Relative photosynthetic contributions of different age needle whorls in sensitive (with ozone injury) and resistant (without ozone injury) genotypes of Jeffrey pine in 1988 in Sequoia National Park (Patterson and Rundel 1989).

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

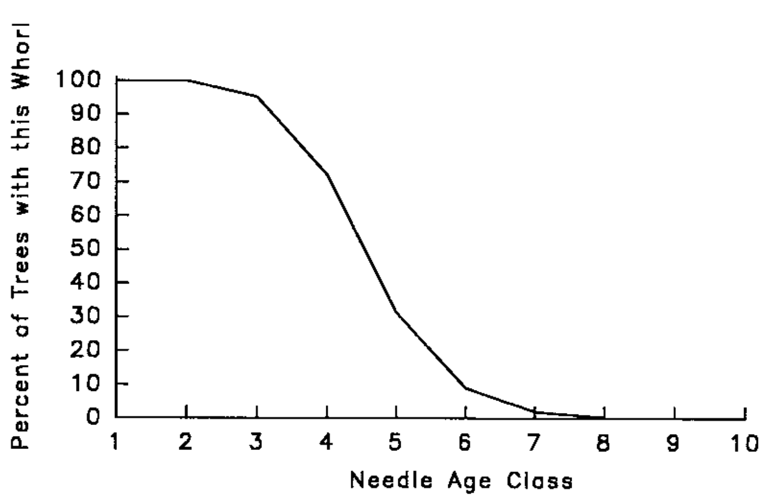

Figure 4—Weighting of the importance of needle whorls based on a curve showing the percent of 1,700 trees from the Sierra Nevada and San Bernardino Mountains with complete whorls of different ages.

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.