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Chapter 3

The physiological basis of ozone injury assessment attributes in Sierran conifers

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Abstract

The generalized effects of ozone (O_3) exposure on plants are well known, but translating responses known under controlled conditions to effective tools for assessments under field conditions can be complex. There are two methods generally used for assessing yellow pine (ponderosa and Jeffrey) response to oxidant exposure in the Sierra Nevada: the Ozone Injury Index (OII) and the Forest Pest Management (FPM) assessment. For both methods, chlorotic mottle and needle retention form the basis of the assessment. The response of these two key attributes to cumulative O_3 exposure and uptake, nitrogen deposition (either associated with O_3 deposition as a correlative environmental factor or as an experimental manipulation), and drought stress are described using field examples from both ponderosa and Jeffrey pine. Specific suggestions are offered for future, repeated field assessments of O_3 injury for consistency, and to help interpret historical data sets.

1. Introduction

The generalized effects of ozone (O_3) exposure on plants are well known, and have been reviewed from several viewpoints over the last decade (De Kok and Tausz, 2001; Heath and Taylor, 1997; Pell et al., 1997; Schraudner et al., 1997; Matyssek et al., 1995; Bytnerowicz and Grulke, 1992). In general, oxidant exposure results in lower photosynthetic capacity, higher respiration, lower stomatal conductance, lower nutritional content of tissues, as well as elemental imbalances. Oxidant exposure alters within plant priorities for resources: less carbon is allocated to roots, and less foliar biomass is retained.

The specific effects of O_3 exposure on conifers are well described (Darrall, 1989; Reich, 1987). Most of the research on O_3 effects has been conducted on herbaceous species (i.e., crops). Several important differences between herbaceous and coniferous species affect the total foliar uptake of the pollutant, the timing of the uptake, and the effect of multiple stressors. However, the underlying mechanisms of O_3 injury response in conifers and herbaceous species are assumed to be similar.

One of the most important differences between herbaceous and conifer species relevant to oxidant exposure is lower stomatal conductance. Stomatal conductance in conifers is roughly half that of herbaceous species, so that at the same O_3 exposure level, O_3 uptake in conifers is proportionately reduced. However, conifer foliage is generally longer-lived, and active over a greater portion of the year. A longer period of physiological activity increases the total O_3 uptake over the course of the year, and greater foliar longevity increases total O_3 exposure over the lifetime of the leaf. Conifers are physiologically active in early spring and late fall, during times of lower oxidant concentrations. These periods can contribute significantly to a net positive carbon balance over the course of the year. Patterson and Rundel (1995) reported that Jeffrey pine had significant stomatal opening (one third that of a summer day) in mid winter with 2 m of snow on the ground. At least pole-sized and larger trees can mitigate reductions in carbon acquisition due to oxidant exposure in the summer with carbon assimilation on favorable days in the winter.

Conifers have thicker cuticles relative to herbaceous species. Although cuticular uptake of O_3 is believed to be negligible (Kerstiens and Lendzian, 1989), other associated pollutants (e.g., HNO_3) in the southern Sierra Nevada and in the Transverse Range may compromise cuticular integrity (Bytnerowicz et al., 1999) and contribute to the total stress experienced. Needle longevity may be an advantage from the point of view of nutrient storage important in reparation responses to pollutants. However, cuticular lesions may be more common in older needles and trans-cuticular loss of water is unlinked to plant carbon gain. The potential for “leaky” cuticles to contribute to whole canopy pollutant uptake in mature trees (not to be confused with pollutant deposition to foliar surfaces) has yet to be quantified under field conditions.

The longevity of the whole tree also holds advantages and disadvantages for response to pollutants. Greater longevity of conifers translates into greater environmental volume from which to scavenge resources, a greater proportion of resources carried over from year to year and the potential to buffer the individual from environmental stressors and allocate more resources to plant defense (antioxidants). Conversely, longevity can also work against the tree in the form of multiple, cumulative stressors. A series of drought years can reduce O_3 uptake, but can also reduce carbon and nutrient acquisition, reducing resource allocation to defenses (antioxidants, resins against insect infestation), rendering the tree more susceptible to O_3 injury in a subsequent year of greater precipitation, greater stomatal conductance, greater O_3 uptake, and injury. These differences in resource acquisition and allocation all contribute to altering carbon and nitrogen dynamics within the tree.

Nitrogen deposition modifies the effects of oxidant exposure. Nitrogen deposition, in wet or dry particulate form, ultimately increases site fertility. This can occur to excess ("N saturation") as has been shown by Fenn et al. (1996). Increased nitrogen availability decreases carbon allocation to roots, further exacerbating the effects of oxidant exposure on roots. Increased nitrogen availability also exacerbates O_3 exposure effects on foliage turnover: fewer needle age classes are retained (Gower et al., 1993). Nitrogen amendments counteract the effect of oxidant exposure on photosynthesis by increasing nitrogen available for photosynthetic pigments and enzymes, increasing stomatal conductance, but deleteriously resulting in increased O_3 uptake. On the other hand, nitrogen amendments may mitigate the degree of foliar injury from oxidant pollution via higher nitrogen available for reparation of photosynthetic pigments. Nitrogen amendments also modify the antioxidant defense system in complex ways (Polle, 1998). In additional to these effects, estimated stomatal uptake of gaseous nitrogen oxides may be significant (Bytnerowicz et al., 1996) and phytotoxic (Tausz, 2001).

Over much of the Sierra Nevada, nitrogen deposition is not believed to be significant in the mid elevation forests. In the south central Sierra Nevada, estimates of deposition are in the range of 2–4 $\mathrm{kg\,ha^{-1}\,yr^{-1}}$ (Bytnerowicz et al., 2002; Chorover et al., 1994). However, during prescribed or wildfire events, local NO_x concentrations can become quite high (Bytnerowicz et al., 2002). With increasing population, fossil fuel use, continued excessive fertilizer use in the San Francisco Bay area and San Joaquin Valley, and increased frequency of anthropogenically-caused fires, we can expect nitrogen deposition to Sierra Nevada ecosystems to become a chronic problem. In areas experiencing moderately high O_3 and above (e.g., > 280 ppm h based on 24 h exposure over the 6 month growing season) such as the southern Sierra Nevada, nitrogen deposition may also be a significant co-stressor. In Sequoia National Forest near Mountain Home, high nitrogen deposition is suspected to cause forest tree responses similar to those found in the Transverse Range in southern California (Fenn et al., Chapter 5, this volume).

The focus of this chapter is western conifer response to oxidant exposure. Most of the examples are based on two yellow pine species, ponderosa and Jeffrey (*Pinus ponderosa* Dougl. Ex Laws. and *Pinus jeffreyi* Grev. & Balf.) responses because these two pines are present on nearly all of the forest plots surveyed for O_3 injury in the Sierra Nevada. Ozone exposure in the Sierra Nevada is greatest at lower elevations, and decreases with increasing elevation and distance from source (Bytnerowicz et al., 2002). Consequently, coniferous species that form lower treeline, such as Coulter (*Pinus coulteri* D. Don) and ponderosa pine, may have greater exposure and injury than species at upper treeline, such as lodgepole pine (*Pinus contorta* Dougl. ex. Loud.), red fir (*Abies magnifica* A. Murr.), and Jeffrey pine. The mid-elevation, Sierran mixed conifer zone is dominated by white fir (*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.), ponderosa and Jeffrey pine, incense cedar (*Libocedrus decurrens* Torr.), and relatively small amounts of sugar pine (*Pinus lambertiana* Dougl.). Unique to the Sierra Nevada are also stands of giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchholz) locally dominant in "groves." Of these species, ponderosa and Jeffrey pine are the most sensitive conifers to O_3 exposure (Miller et al., 1983), and as such, are the "bell weathers" for forest health in the Sierran mixed conifer zone.

The interaction of environmental factors, plant phenology (the timing of growth events and birth and mortality of plant parts), physiological status (nutritional or moisture status, and dormant or actively growing within the year), and tree age (interannual differences in resource acquisition and requirements for allocation) all contribute to the complexity of conifer response to O_3 exposure. Of relevance to this book, however, is the translation of the whole tree physiological response to O_3 exposure, in the collection of relatively few morphological (visible) attributes that are used to assess O_3 injury in the field.

The objective of this chapter is to describe seasonal variation in attributes used to assess O_3 injury, under a range of O_3 exposures and nitrogen deposition fluxes typical for the Sierra Nevada. The relationship between O_3 exposure and O_3 uptake, and their influence on the biological response attributes used to assess injury in the field will be discussed. Responses in different microsites (mesic vs. xeric) and in years of significantly different water availability (average or above and 20% below average) will be used to elucidate the effect of drought as an additional environmental stressor. Perhaps the most useful application of this information is to help determine in what month, and in which year to make consistent O_3 injury assessments based on what we know about the effect of environmental factors and temporal variation in attributes.

2. Attributes used in O_3 injury assessments of conifers

The attributes used in O_3 injury assessments include foliar chlorotic mottle and or necrosis, both branch (number of needle age classes retained) and within-whorl needle retention, needle length, and proportion of the live crown remaining. Trees used in assessment have already been selected for dominance or co-dominance, and to minimize the effect of pathogens (bark beetle, mistletoe, bud worm).

There are two commonly used assessment methods used in Sierra Nevadan forests: O_3 injury index (OII; Miller et al., 1996a) and Forest Pest Management (FPM; Pronos et al., 1978). In the OII assessment, five attributes are utilized for dominant or co-dominant yellow pine: chlorotic mottle, number of whorls retained, needle retention within whorls, needle length, and percent live crown.

The higher the OII score, the more oxidant injury. In the FPM assessment, the first needle age class that exhibits chlorotic mottle for more than one third of that foliage age class is recorded. In this assessment, the lower the score, the more oxidant injury. Arbaugh et al. (1998) has recently described these methods in more detail, as well as their development.

Additional attributes correlated with oxidant exposure have also been reviewed and statistically assessed for significant correlation with level of chlorotic mottle in Grulke and Lee (1997). In that paper, eight attributes were highly correlated with level of chlorotic mottle: foliar nitrogen or chlorophyll content (interchangeable in the multivariate analysis), number of whorls retained, a quantitative measure of within whorl retention, a measure of wood production (basal area increment or branch diameter), spring root carbohydrate content, and distance to the nearest conspecific neighbor as a measure of inferred competition. Foliar antioxidant concentrations and activities have also been clearly correlated with oxidant exposure levels and degree of foliar chlorotic mottle (Tausz et al., 2001). Tissue chemistry, and biochemical attributes (carbohydrates, pigments, antioxidants) are currently not a component of any field assessment of oxidant injury: they are costly and time consuming to assess. However, these as well as other attributes may be chosen for future assessments. Biochemical attributes may be particularly important in assessing response of species that are asymptomatic.

3. What is chlorotic mottle?

Of all the attributes used in O_3 assessment on the many different species in the Sierra Nevada, visible injury to the leaf or needle is the first step of O_3 injury assessment. In yellow pine, the visible injury is chlorotic mottle (Fig. 1; Miller et al., 1963, 1996a). The two most common assessments, FPM and OII survey yellow pine. The FPM method of O_3 assessment is wholly based on the first needle age class with significant chlorotic mottling, functionally greater than 10% on more than a third of the needles of that whorl. Forty percent of the OII score is based on chlorotic mottle of all needle age classes retained (up to 6 years).

Although no quantitative assessment techniques similar to that used for yellow pine have been developed for other species, foliar symptoms may also develop in response to O_3 exposure. In white fir, chlorotic mottling on the needle margins or at the tip or necrosis is apparent with O_3 exposure (Taylor et al., 1980). Seedlings of giant sequoia up to three years old also have chlorotic mottling of needles under current ambient pollutant levels (Miller et al., 1996b). Older giant sequoia trees at ambient or experimentally elevated O_3 levels do not have visible symptoms on foliage (Grulke et al., 1989, 1996). Incense cedar

Figure 1. Example of chlorotic mottle development on ponderosa pine on the left. The oldest foliage within a needle age class senesces first, as seen in the previous year whorl in photo on the right. This photo was taken at the end of July in a drought year (1994) at Strawberry Peak, in the San Bernardino Mountains.

is also asymptomatic at ambient O_3 levels. Other pines, such as lodgepole and sugar appear to be more tolerant to O_3 exposure than the yellow pines (Miller et al., 1983), but have similar symptoms: chlorotic mottle that begins near the tip. Most of the pines exhibit some foliar injury at current ambient O_3 levels (approximately 260 ppm h, cumulative O_3 exposure over the 6 month growing season; National Park Service ARD data base) in Sequoia National Park, the most polluted National Park in the country.

Because ponderosa pine is the most sensitive conifer to oxidant exposure, some individuals exhibit visible symptoms even at relatively clean sites in the southern Cascade Mountains in California (Grulke, 1999). There, O_3 concentrations are just slightly elevated over global background levels (38 to 42 ppb per hour averaged over the six month growing season; Grulke, 1999). At this O_3 exposure level (180 ppm h), 1% of the trees exhibited foliar injury symptoms.

Because O_3 concentrations are commonly moderate and above during the growing season in the western United States (Lee and Hogsett, 2001), and because of its high phytotoxicity, O_3 probably has the most negative effect of any air pollutant at ambient levels on tree vigor and growth (Reich, 1987). Once in the substomatal cavity, O_3 reacts with water and or cell membranes or

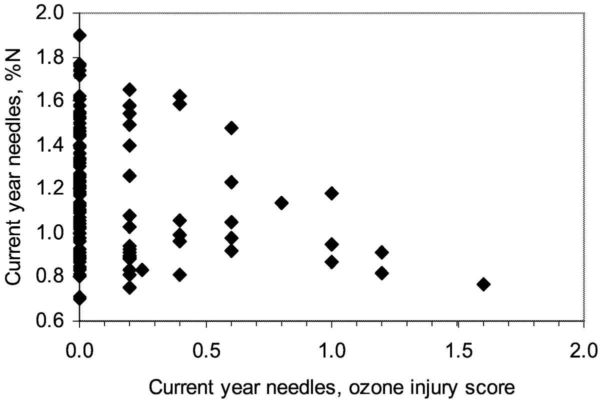

Figure 2. Relationship between chlorotic mottle index and leaf nitrogen content (% dry weight). Samples were taken in an above average precipitation year (1992), and current year foliage was collated for five branches per tree. The chlorotic mottle index corresponds to the following score: 0: no chlorotic mottle; 1: 1–6%; 2: 7–25%; 3: 26–50%; 4: 51–75%; 5: 76–100% chlorotic mottle.

walls to form reactive oxygen species (Polle, 1998). It is not clear if reactive oxygen species are caused by reaction of O_3 and water (Byvoet et al., 1995; Grimes et al., 1983), or by O_3 and constituents of the cell wall and membrane (Mehlhorn et al., 1990). Highly reactive oxygen species disrupt membrane integrity, pigment structure, and oxidize proteins (Mehlhorn et al., 1986) contributing to chlorotic mottle development and or cell death. Reactive oxygen species may also act as a signal to initiate or coordinate other processes such as ethylene production, which induces senescence (Pell et al., 1997; Schraudner et al., 1997). Reactive oxygen species are detoxified by antioxidant systems in the apoplasm, cytoplasm, and chloroplast (Alscher et al., 1997; Heath and Taylor, 1997; De Kok and Tausz, 2001).

Photosynthesis is inhibited by O_3 exposure (Weber et al., 1993): both pigment structure and RUBP carboxylase activity are altered (Schweizer and Arndt, 1990). When the photosynthetic pigments have been damaged, the pigment must be fully broken down (and or new N and Mg must be taken up and transported to the leaf) for pigment to be regenerated (Bjorkman and Demmig-Adams, 1995). The relationship between chlorotic mottle estimates and chlorophyll content or leaf nitrogen content is not precise (Fig. 2). Chlorotic mottle is by definition patchy and on a whole needle basis, nitrogen content on a per leaf basis may not differ between symptomatic and asymptomatic needles. Needles exposed to both O_3 exposure and drought stress had higher nitrogen content than that of controls because significant foliar biomass was lost, and nitrogen may have been translocated out of senescing into remaining needles (Temple and Riechers, 1995). This explains in part for the within-growing season changes in chlorotic mottle estimates: older needles (generally more symptomatic) within the whorl may be lost through the growing season, and thus the chlorotic mottle estimate decreases for the whole whorl. Higher chlorotic mottle is significantly correlated to lower net assimilation in Jeffrey pine (Patterson and Rundel, 1995).

4. Factors influencing the expression of chlorotic mottle

Perhaps the simplest demonstration of the effect of environmental stressors on the expression of chlorotic mottle can be made by plotting the relationship between chlorotic mottle and cumulative O_3 exposure or uptake for three sites differing in pollutant exposure and between years with average or below-average total annual precipitation (Fig. 3). These sites have been described previously (O_3 exposure, Grulke, 1999; O_3 uptake, Grulke et al., 2002a), and range from roughly 60 (Barton Flats) to 70 (Strawberry Peak), to 80 ppb (Crestline) average O_3 concentration per hour (24 h per day, April 15 through October 15), and low (6–9 $\mathrm{kg\,ha^{-1}\,yr^{-1}}$) to high nitrogen deposition (30–40 $\mathrm{kg\,ha^{-1}\,yr^{-1}}$; Kiefer and Fenn, 1997).

In an average precipitation year, percent chlorotic mottle increased regularly with cumulative O_3 exposure, but the same level of cumulative O_3 exposure did not elicit the same level of chlorotic mottle between sites in that year. In the 20% below average precipitation year, chlorotic mottle developed more quickly at the moderate and moderately high pollution site: the peak expression occurred at the end of June. At both of these sites, there was a significant reduction in chlorotic mottle in the last month of the growing season at the two most polluted sites, and in the last two months of the growing season at the moderate pollution site. Drought stress decreased chlorotic mottle of the whole whorl because the older, more damaged needles within the whorl were lost (Miller et al., 1996b). When cumulative O_3 uptake (calculated by a physiologically based model, or empirically) is used instead of exposure in the relationship, there was no improvement in the relationship with percent chlorotic mottle. These results strongly suggest that interannual comparisons of ozone injury should not be made between years that significantly differ in soil moisture availability (e.g., average vs. below-average precipitation years).

The most polluted site based on cumulative O_3 exposure was also the site of the greatest O_3 uptake by the trees, whether it was a year of average or below-average precipitation. The converse was true for the moderate pollution site, Barton Flats (Grulke et al., 2002a). The maximum chlorotic mottle within the growing season roughly followed the rank order of cumulative O_3 exposure at the three sites (Fig. 4). For example, at the most polluted site, maximum chlorotic mottle was 35% at the most polluted site, 20% at the moderately

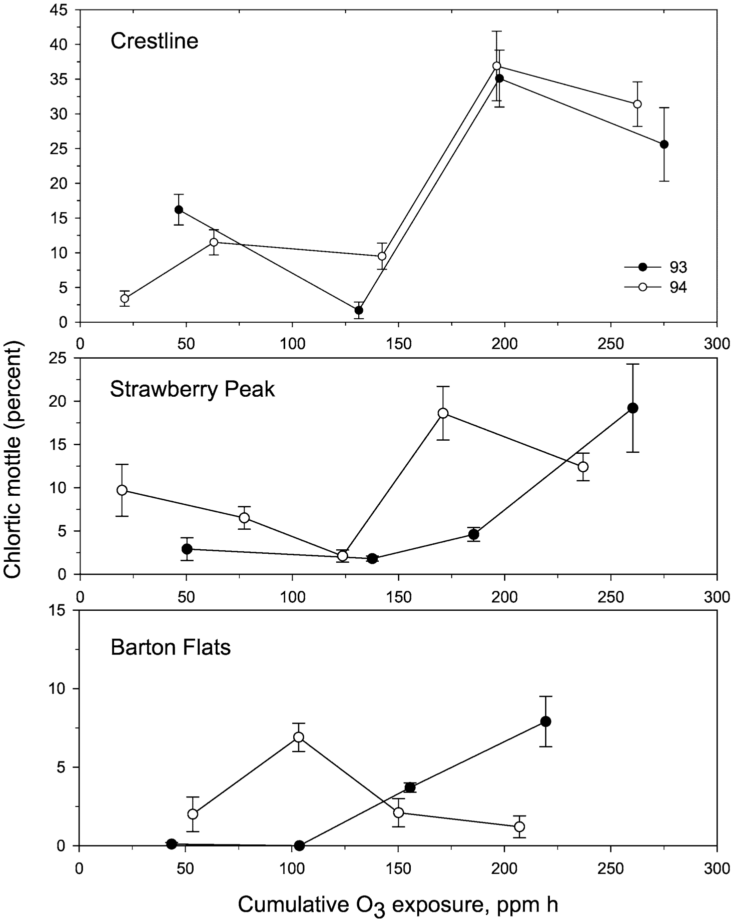

Figure 3. Relationship between chlorotic mottle and cumulative O_3 exposure at three sites in the San Bernardino Mountains (Crestline—highly polluted; Strawberry Peak—moderately high pollution; Barton Flats—moderate pollution) in a year of average (1993) and below-average total annual precipitation (1994). Chlorotic mottle of the previous year only has been plotted because this needle age class has the highest carbon gain in ponderosa pine. Chlorotic mottle was monitored monthly on five branches per tree on twelve trees per site.

high pollution site, and 8% at the moderate pollution site. In the drought year, the seasonal maximum occurred at much lower cumulative O_3 exposure. If the seasonal minimum and maximum percent foliar chlorotic mottle from all sites is plotted with respect to cumulative O_3 exposure, there appears to be a relationship (increase in chlorotic mottle with increase in O_3 exposure), but the relationship is not statistically significant (Adj. $r^2 = 0.31$; $P = 0.06$). A slightly

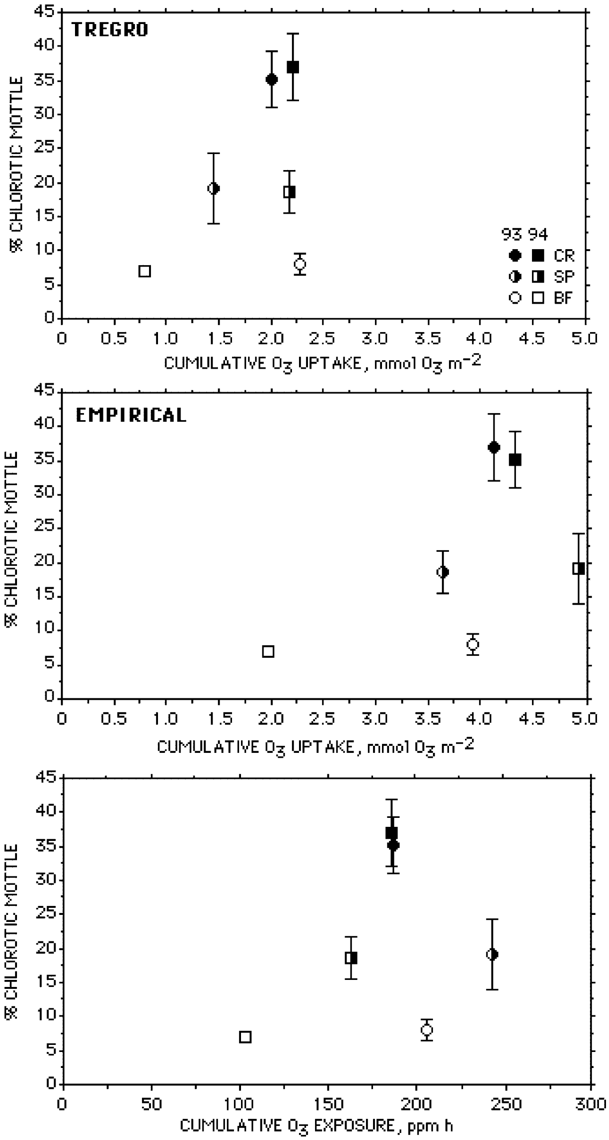

Figure 4. The seasonal maximum chlorotic mottle (from Fig. 3) is plotted for three sites across a pollution gradient with respect to O_3 exposure (lower graph), O_3 uptake (middle graph, as estimated from an empirical model (Grulke et al., 2002a); and upper graph, as estimated from a physiological model, TREGRO; courtesy of C.C. Fan and W.A. Retzlaff). Data in a year of average precipitation (1993) and a drought year (1994) is presented based on a 120 yr record maintained by the San Bernardino County Water District. The percent chlorotic mottle is not well correlated to cumulative O_3 exposure. Cumulative O_3 uptake, whether empirically or physiologically modeled, was a better predictor of response.

better fit was obtained with cumulative O_3 uptake (Adj. $r^2 = 0.35$; $P = 0.04$). However, the cumulative O_3 exposure at the time of maximum seasonal expression was not significantly correlated to the maximum percent chlorotic mottle. Cumulative O_3 uptake as calculated by a physiological model, TREGRO, had the same predictive capability as that of statistically calculated O_3 uptake (Adj. $r^2 = 0.34$; $P = 0.04$; CC Fan and WA Retzlaff, unpubl. simulation for ponderosa pine using the same data set). Site O_3 exposure alone does not wholly explain the physiological response and may interact with environmental factors in unexpected ways. However, a significant correlation coefficient was obtained between a chlorotic mottle score and several O_3 exposure indices (7 and 24 h SUM00, and 7 and 24 h SUM06; Salardino and Carroll, 1998) of forest stands distributed along the Sierra Nevada.

Foliar oxidant injury usually accelerates only after the upper soil horizons have dried out (Grulke and Balduman, 1999). Although foliar N and chlorophyll at high nitrogen deposition and O_3 exposure sites is high early in the summer, foliar N and chlorophyll content decreases with increasing O_3 exposure after mid summer (Grulke, 1999). Cumulative O_3 exposure, high radiation, and drying of the upper soil horizons (insufficient uptake of N to replenish damaged pigments) perhaps all contribute to the expression of chlorotic mottle. Cumulative O_3 exposure early in the summer is significant (Grulke et al., 2002a), but upper soil horizons are rich in nitrogen and fine root mass is high until they dry out, even in a year of average precipitation, in mid to late July. When fine roots (and associated mycorrhizae and microbial communities) die off in near-surface soil horizons, trees rely on deep roots for water (Hubbert et al., 2001). Even at sites where the ground water is also rich in nitrogen (Fenn and Poth, 1999), chlorotic mottle still develops. Either deep roots (and associated mycorrhizae) are not as effective at nitrogen uptake, or oxidant damage is too great for reparation activities to keep up with N uptake from the pedosphere. In the autumn with favorable soil temperatures and moisture permitting, fine roots and mycorrhizae grow in the near surface soil horizons, and chlorotic mottle can significantly decrease (Fig. 5).

Because OII integrates foliar response of up to six needle age classes, theoretically it should not be correlated to cumulative O_3 exposure in any one year of assessment unless site cumulative O_3 exposure is fairly consistent from year to year. In order to generate a meaningful value for site O_3 exposure, O_3 exposure should be accumulated over the average number of years that needles are retained on the branches, as described in Arbaugh et al. (1998) and tested in Salardino and Carroll (1998). The OII scoring system works well to rank significant differences in site to site injury level. The FPM is also a robust scoring system that works well to coarsely rank stand injury under a wide range of environmental co-stressors. Integrating cumulative O_3 exposure for this score is even more problematic than for OII because chlorotic mottle is not integrated

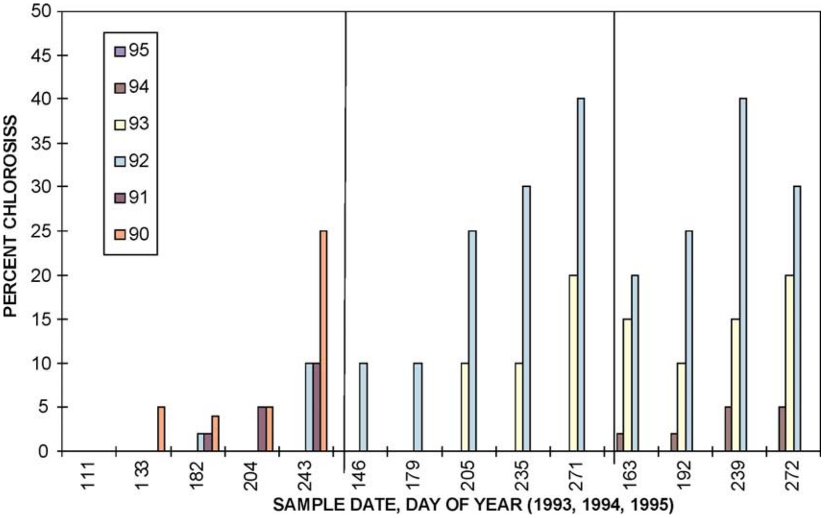

Figure 5. Typical phenology of chlorotic mottle in each needle age class, given for one branch, over the course of three years, in the San Bernardino Mountains (Grulke, unpubl. data).

over all needle age classes, but is based on the needle age where chlorotic mottle is first signficantly expressed. In both scoring systems, chlorotic mottling is modified by the degree of overwinter reparation of damaged pigments (Fig. 5).

Perhaps the most effective use of the FPM and OII score would be to conduct canopy assessments in average or above-average precipitation years, preceded by at least one average or above precipitation year to best rank injury in widely ranging geographic sites. Assessment in a below-average precipitation year, or of stands with a disproportionate number of trees in xeric microsites could significantly decrease the OII score, or increase the FPM score (Fig. 6) if assessments were made on the same day of year. Both scores reflect a microsite difference in O_3 uptake, based on both leaf-level and canopy-level measurements (Grulke et al., in press). Trees in mesic sites had 20% greater seasonal O_3 uptake than Jeffrey pine trees in xeric sites. Choosing trees in consistently mesic microsites should increase the chance of a relationship between site O_3 exposure and foliar injury development. Assessing canopy injury at the same phenological stage (such as near or at the end of needle elongation, correlated with upper soil horizons drying to $< 4\%$ soil moisture; Grulke et al., 1998, 2001) would significantly aid in interannual comparisons of foliar injury.

The level of chlorotic mottle could differ between sites with the same cumulative O_3 exposures because of associated nitrogen deposition at these sites. Greater nitrogen availability could aid repair of damaged pigments, but it could also increase stomatal conductance (via stimulated photosynthe-

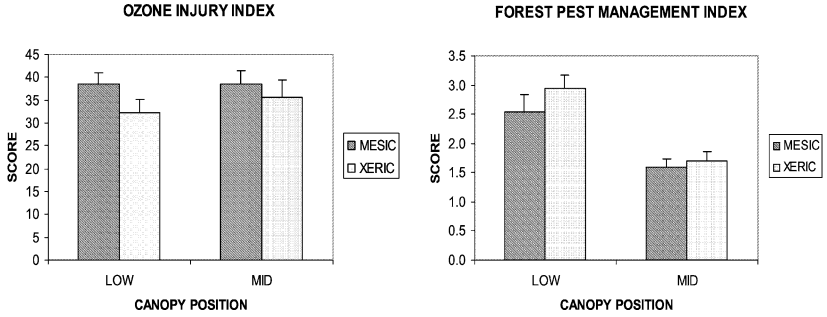

Figure 6. OII and FPM scoring for Jeffrey pine trees in mesic and xeric microsites, at lower and mid canopy. The OII score is much less sensitive to canopy position than FPM. Mesic trees had poorer health as indicated from both the OII (higher score, more injury) and the FPM (lower score, more injury) assessment techniques. Leaf-level and canopy-level measures of gas exchange indicate that trees in mesic sites had 20% greater seasonal O_3 uptake than trees in xeric sites (Grulke et al., in press) given the same site O_3 exposure.

sis), and thus O_3 uptake. From a biochemical point of view, nitrogen-limited trees had greater antioxidant concentration than that of controls (Polle, 1998). Conversely, excess nitrogen in fertilizer manipulations increased secondary metabolites with antioxidizing capacity (Pell et al., 1995).

Experimental applications of nitrogen to trees in consistently mesic or xeric microsites on the same slope may help determine the direction of change expected with concurrent environmental stressors (O_3 exposure, drought) and enhancers (nitrogen deposition). After three years of experimental amendments of nitrogen availability ($+50$ kg ha^{-1} yr^{-1}), mid canopy chlorotic mottle decreased, foliar nitrogen increased slightly, foliar carbon decreased, and foliar C : N decreased relative to control trees in mesic microsites for mature Jeffrey pine in Sequoia National Park (Fig. 7). The opposite trends occurred in midcanopy foliage of trees in xeric microsites. In a similar experiment, N-fertilized ($+50$ kg ha^{-1} yr^{-1}) mature yellow pine at a dry site had lower bole growth than that of controls (Fenn, unpubl. data). The two experiments had similar background O_3 exposures, approximately 260 ppm h accumulated over the growing season. At a high pollution site (approximately 320 ppm h seasonal O_3 exposure, 30–40 kg ha^{-1} yr^{-1} background N deposition) with more moisture availability, additional N amendments ($+50$ kg ha^{-1} yr^{-1}) increased bole growth. In dry sites, perhaps N amendments decrease fine root mass and pedosphere N uptake. In mesic sites, fine root (+ mycorrhizae) may be able to take up and transfer more N to the foliage. Perhaps foliar carbon content of fertilized trees is lower in mesic sites because of within-tree allocation to bole growth (or other tissue), N-stimulated foliar growth reduced specific leaf weight, or increased respiration due to greater O_3 exposure in this site.

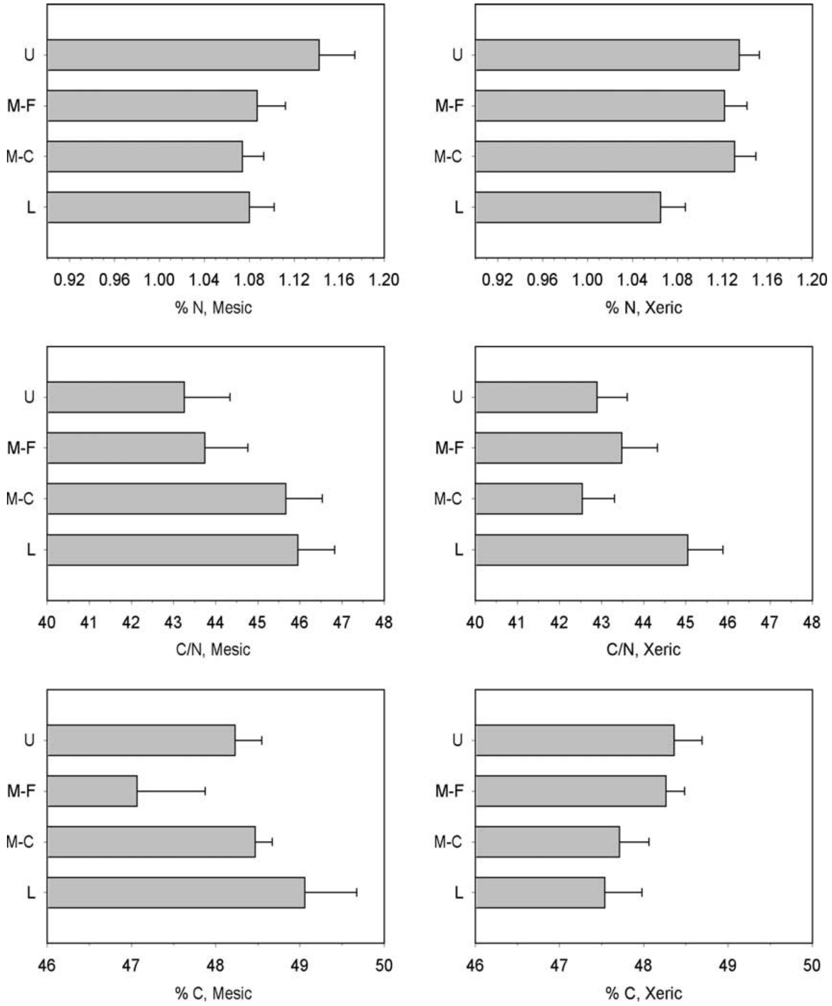

Figure 7. Within canopy variability in carbon, nitrogen, and C : N ratio in Jeffrey pine, and effect of 3 years of nitrogen amendments on mid canopy tissue chemistry. Each bar represents the average and ±1 S.D. of foliage from 32 trees. Within canopy foliar chemistry differed between trees in mesic and xeric microsites. Nitrogen amendments increased foliar nitrogen, decreased carbon, and decreased C : N of mid canopy foliage on mesic trees. It had the opposite effect on tissue chemistry of xeric trees. Trees were assigned to different microsites based on topographic position, proximity to perennial surface water, and ability of mesic site trees to increase bole growth in average and above precipitation years (Grulke et al., in press).

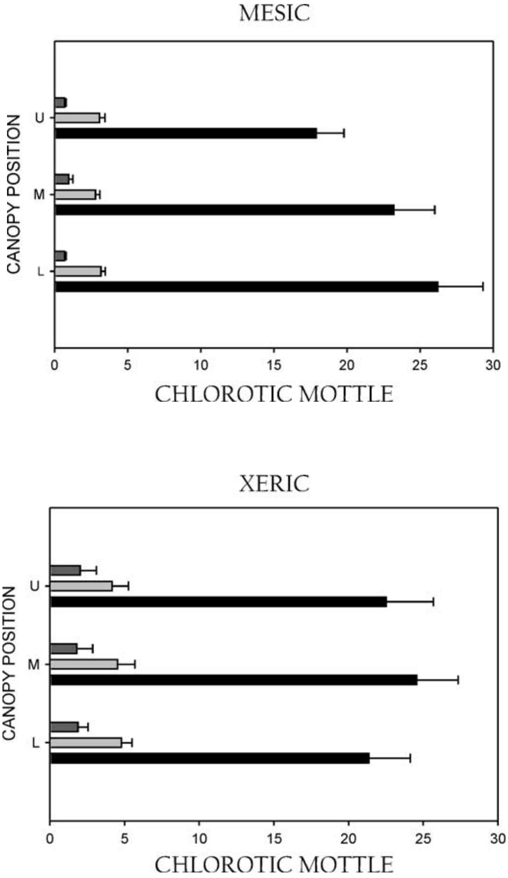

Figure 8. Within-canopy variability in foliar chlorotic mottle in Jeffrey pine, Sequoia National Park. Each bar at each canopy position (U = upper; M = middle; L = lower) represents chlorotic mottle of current (dark grey), previous year (light grey), and four year old needles (black). Bar length represents the average and ±1 S.D. of 32 trees in each microsite (same trees as presented in Fig. 7).

The level of chlorotic mottle could differ between sites with the same cumulative O_3 exposures because of differences in drought stress. Jeffrey pine in xeric microsites had 20% lower O_3 uptake than mesic microsite trees, but chlorotic mottle was greater at all canopy levels in current and previous year needles, and in four year old needles in the upper canopy (Fig. 8; Grulke et al., in press). This may be a field example of free radical production within the chloroplast due to drought stress, lower stomatal conductance, lower C_i, and high radiation loads (Tausz et al., 2001; Foyer et al., 1994). These trees had greater antioxidant concentrations relative to mesic microsites in June, prior to the onset of seasonal drought stress (Tausz et al., 2002). In late summer, drought stress and O_3 uptake combined to decrease carbon acquisition in ponderosa pine (Grulke et al., 2002b). In simulations with white fir, a 25% reduction in annual precipitation at moderate O_3 levels was protective against injury as assessed by tree biomass (Retzlaff et al., 2000). Drought stress imposed at greater levels in combination with moderate O_3 exposure was deleterious to carbon acquisition in white fir.

5. Factors influencing needle retention (branch-level; whorl-level)

There are two types of needle retention measurements of interest in O_3 injury assessments: the number of live needle age classes on the branch, and the within needle age class (whorl) retention. The former is represented with a simple count, and the latter is either estimated by thirds (Miller et al., 1996a) or measured ([length of the branch with live needles]/[branch elongation growth]) in a given year; Grulke and Balduman, 1999). In OII, the branch-level needle retention (the number of needle age classes or whorls retained on the branch), and the within-whorl needle retention is 40% of the O_3 injury score. Long-lived foliage acts as a storage location for nitrogen, but with a carbon cost for maintenance (Chapin, 1980). Ozone exposure accelerates needle senescence (Heath and Taylor, 1997; Pell and Dann, 1991), with the net result that N can be translocated out of senescing tissue to replace damaged photosynthetic pigments. Ozone exposure can also increase foliar respiration due to reparation: it may become too costly to maintain older needles with lower assimilation rates.

Nitrogen deposition, positively correlated with O_3 exposure, also accelerates needle loss (Gower et al., 1993), but via a different mechanism. Nitrogen amendments increase foliar nitrogen contents, and older leaves may have redundant nitrogen with too high carbon cost for maintenance. Trees with nitrogen amendments in Sequoia National Park had higher foliar nitrogen content, and fewer needle age classes relative to control trees in mesic microsites (Fig. 7; Grulke, unpubl. data).

The FPM score is described as the youngest needle age class with significant chlorotic mottle development. However, the whorl number and the level of chlorotic mottle may co-vary, as suggested by the ratio of chlorotic mottle and foliar retention as a new index (“CM/FR,” Salardino and Carroll, 1998). Ponderosa pine trees with known allelic and genotypic diversity at a site with both high O_3 and nitrogen deposition illustrate the interaction between chlorotic mottle and needle retention (Staszak et al., unpublished data; Prus-Glowacki et al., 1999). Ponderosa pine with a genetic signature indicating sensitivity to O_3 had two strategies: (1) many needle age classes retained, but high chlorotic mottle across all needle age classes (e.g., FPM score, 0); and (2) few needle age classes retained, but little chlorotic mottle (e.g., FPM score, 3). Also, ponderosa pine with a tolerant O_3 genetic signature less than 2 m from a conspecific tree had morphological and tissue chemistry attributes similar to a sensitive tree (lower foliar nitrogen, higher chlorotic mottle, fewer needle age classes) than typical for a “tolerant” tree.

Drought stress also reduces both the number of needle age classes retained, and retention within a needle age class. The response of both types of needle retention is illustrated in Fig. 9 for four sites differing in pollutant exposure. The number of needle age classes retained significantly declined with increasing pollutant exposure, as has been described previously by both Pronos et al. (1978) and Miller et al. (1996a). At the relatively clean atmosphere site, there was little difference in within-whorl needle retention between an average and below-average precipitation year. In the southern Californian sites within increasing pollutant exposure, there was significant loss of both whorls and within-whorl needle retention in a drought year. Drought stress acts synergistically with O_3 exposure to promote premature senescence of foliage. Both nitrogen (from reduced root mass and uptake) and carbon (increasingly lower net carbon balance with reduced stomatal aperture, lower assimilation, and constant respiratory losses) may be limiting in a drought year to facilitate this response.

The quantitative measure of within-whorl retention is a better measure of needle retention than the estimated needle retention score of Miller et al. (1996a) for ponderosa pine. Ponderosa pine loses needles regularly from the lower or oldest needles to the upper or younger needles within a whorl. However, Miller et al. (1996a) needle retention score is a better measure for Jeffrey pine because needles are lost erratically within the whorl.

Male cone scars can sometimes be mistaken for needle loss within the whorl: their scars would reduce estimated needle retention and increase the OII score (implying greater injury). Each male cone develops at the expense of fascicle production (for lodgepole pine, McDick et al., 1990), thus needle mass will necessarily be lower in whorls that bear them. Female cones develop from terminal buds, thus form at the expense of branch growth (for Monterey pine, *Pinus radiata* D. Don, Cremer, 1992). Both male and female cone production accounted for 5% of total aboveground production in this species, but this would not be detected as a reduction in crown mass in a field assessment.

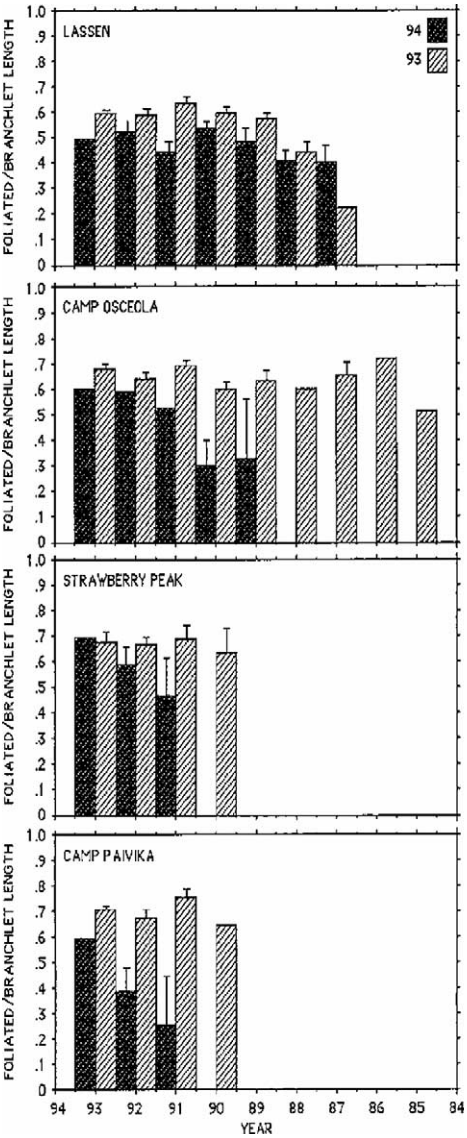

Figure 9. Example of both branch-level and whorl-level needle retention in a year of above-average precipitation (1993) and below average precipitation (1994) (from Grulke and Balduman, 1999). Within-whorl retention is a quantitative measure constructed from the [length of the branchlet with green needles retained]/[total branchlet length]. In this case, the foliated proportion of the branch is calculated for each branch age class, not the entire branch as in Miller et al. (1996a). Reprinted courtesy of Kluwer Academic Publishers B.V., Water, Air, and Soil Pollution, Volume 116, Figure 1, p. 242.

6. Within-canopy variability in O_3 injury expression

Both OII and FPM assessments are generally conducted in lower canopy due to ease of access for extensive surveys. To illustrate the difference in expression in O_3 injury within the canopy, morphological and tissue chemistry attributes in three canopy positions of 30 trees each in mesic and xeric microsites were measured. The following attributes were used in the multivariate analysis:

Table 1. Summary statistics for upper, mid, and lower canopy in mesic and xeric microsites. Attributes include number of whorls retained, chlorotic mottle (%), needle length (mm), branch length (mm), proportion of the branch foliated (%), branch diameter (mm), and foliar carbon (%) nitrogen (%), and C : N. Data is presented for previous year tissue only. A two-way nested ANOVA was used to test for significance between canopy position (CAN POS), microsite (SITE), and their interaction.

Attribute		Upper	Mid	Lower	Can Pos $P =$	Site $P =$	A × B $P =$
# WHRLS					0.050	0.233	0.680
	M	5.0 (0.2)	4.6 (0.2)	4.5 (0.2)			
	X	4.7 (0.2)	4.9 (0.2)	4.7 (0.1)			
% Chlor					0.836	0.007	0.874
	M	3.1 (0.4)	2.8 (0.3)	3.2 (0.3)			
	X	4.2 (0.4)	4.5 (1.2)	4.8 (0.7)			
N length					0.286	0.012	0.209
	M	15.6 (0.4)	15.7 (0.4)	15.7 (0.6)			
	X	15.5 (0.4)	14.8 (0.3)	14.0 (0.3)			
BR length					0.0001	0.0003	0.242
	M	65 (5)	41 (3)	34 (2)			
	X	49 (3)	35 (2)	27 (2)			
% Foliated					0.001	0.014	0.021
	M	75 (2)	68 (1)	64 (2)			
	X	73 (2)	73 (2)	71 (2)			
BR diam					0.0001	0.057	0.778
	M	14.1 (0.5)	11.6 (0.3)	10.4 (0.3)			
	X	14.4 (0.5)	12.4 (0.4)	11.2 (0.3)			
% C					0.817	0.032	0.165
	M	48.3 (0.3)	48.4 (0.2)	49.0 (0.6)			
	X	48.4 (0.3)	47.7 (0.4)	47.5 (0.4)			
% N					0.032	0.558	0.418
	M	1.13 (0.03)	1.08 (0.02)	1.08 (0.02)			
	X	1.14 (0.02)	1.13 (0.02)	1.06 (0.02)			
C : N					0.045	0.050	0.442
	M	43.6 (1.0)	45.5 (0.9)	45.9 (0.8)			
	X	42.9 (0.7)	42.6 (0.8)	45.0 (0.8)			

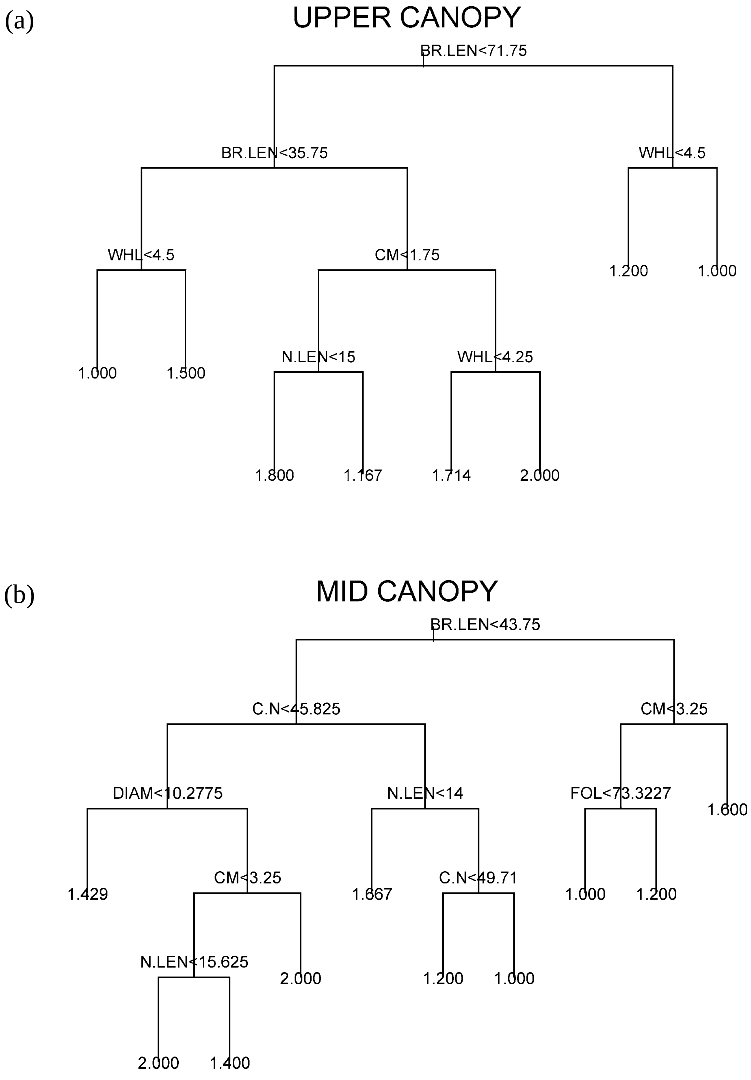

Figure 10. Tree models of morphological and tissue chemistry attributes that significantly contribute to distinguishing between trees in mesic and xeric microsites in the upper, mid, and lower third of the canopy. Numbers at the base of the dendritic line indicate the proportion of trees in mesic (1.0) or xeric (2.0) microsites that satisfy the series of criteria in higher level branches. Foliage or branch tissue of the previous year was measured in thirty Jeffrey pine trees in each microsite, in Sequoia National Park in mid August, 2000. BR LEN = branch length; CM = chlorotic mottle; C.N = C : N ratio in needles; DIAM = branch diameter; FOL = proportion of branchlet with needles retained (see Grulke and Balduman, 1999); N LEN = needle length; WHL = number of live needle age classes retained. (*Continued on next page*)

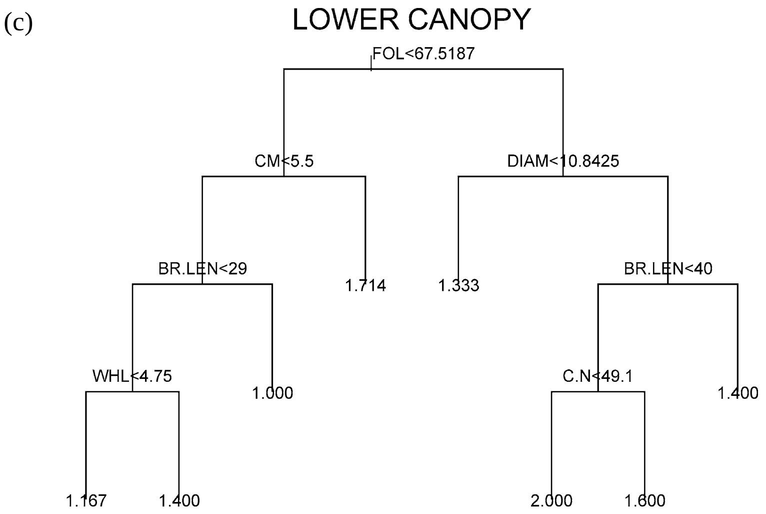

Figure 10. (*Continued*)

chlorotic mottle, % foliar nitrogen and carbon, foliar C : N, average number of needle age classes retained, proportion of the branch foliated, and branchlet diameter. These attributes were chosen based on the statistical analysis of ponderosa pine described in Grulke and Lee (1997), and subsequent application to Jeffrey pine (Grulke et al., in press). Table 1 provides summary statistics for these attributes for upper, mid, and lower canopy of mesic and xeric trees (prior to multivariate grouping).

In contrast to that reported by Muir and Armentano (1988) for ponderosa pine, needle retention (number of whorls retained on the branch and within-whorl foliar retention) in the lower canopy was significantly lower than that in the upper canopy of Jeffrey pine, regardless of microsite. Less foliage in mid-canopy in mesic microsites may be an indication of greater O_3 uptake in this microsite. The greatest portion of the canopy foliar mass is in the middle third of the tree, followed by the upper canopy, then the lower canopy in ponderosa pine (from whole trees harvested in Grulke and Balduman, 1999). Foliar loss in mid canopy due to oxidant injury, or oxidant and drought stress, is perhaps more critical to whole tree carbon and nitrogen balance than foliar loss in lower canopy. Except for the number of whorls retained on the branch and % foliar N, all other attributes significantly differed between microsites. Except for % chlorotic mottle, needle length, and % foliar carbon, all other attributes measured significantly differed with canopy position.

A tree model (Fig. 10) was used to illustrate which attributes, at what values differentiated the two groups of trees (1.0 = all mesic trees; 2.0 = all xeric trees; values between 1 and 2 indicate the relative proportion of trees in the two microsites) at each canopy level. For example, the average number of whorls retained was not used to differentiate groups in the mid canopy, but was significant in the upper canopy. Whorl number was marginally useful in lower canopy in differentiating groups. Some attributes (the proportion of the branch foliated; foliar N and C content) significantly assisted in differentiating between groups of trees at all canopy levels.

Divisive clustering (S-Plus 2000; figures not presented) was applied to sort trees into different groups according to the values of the same attributes (used in the tree model above) in each canopy position. This multivariate technique yielded the best separation of microsites, with the highest cluster coefficient. However, the groups were robust: other multivariate analyses yielded the same end groups. Many different combinations of attributes were used to optimize the resulting cluster coefficient. Although some of the attributes could have been excluded for one or more of the canopy positions, a consistent approach was used across all three canopy levels.

Clustering analysis applied to the three canopy layers differed in the number of groups identified (three for lower and upper canopy, and two for mid canopy measurements). The first group largely consisted of mesic site trees at all canopy levels, and the second group largely consisted of xeric site trees at all canopy levels suggesting whole tree differences in the expression of these attributes in the two microsites. The third group consisted of a relatively few number of trees (2 out of 3 trees were mesic in the third group of the upper canopy; 5 out of 6 trees were xeric in the third group of the lower canopy). In Group 1, these attributes misclassified 50% of the trees when measured in upper canopy, 15% of the trees when attributes were measured in mid canopy, and 20% of the trees when measured in the lower canopy. If the upper canopy acts as a sink for resources, attributes measured there would be less effective at indicating microsite location. In Group 2, these attributes misclassified 40% of the trees when measured in the upper canopy, 65% of the trees when measured in mid canopy, and 60% of the trees when measured in the lower canopy. Trees in mesic microsites were more consistent in expression of attributes correlated with O_3 injury in yellow pine.

7. Conclusions

The O_3 injury assessments that have been developed for yellow pine are robust and were designed for a coarse assignment of forest health. They do well at this and scores reflect O_3 exposure regimes averaged over many years. Of the many known physiological responses to O_3 exposure in conifers, only a few attributes are used to integrate the whole tree response to the stressor. The intent of this chapter was to review responses of foliar chlorotic mottle and needle retention, the key attributes relevant to field assessments of O_3 injury. Correlative and manipulative examples of foliar chlorotic mottle and needle retention response to (1) O_3 exposure and uptake, (2) nitrogen deposition frequently associated with O_3, and (3) drought stress in years of significantly below-average annual totals help to describe the range of field responses expected. Understanding sources of variation in the key O_3 injury assessment attributes may improve between-observer, and between-year assessments, and may help interpreting historical data sets.

Several recommendations are offered for future field assessments of O_3 injury. Because Jeffrey pine has lower stomatal conductance, greater antioxidant defense, and differing patterns of loss within a needle age class, scores should be reported separately for the two species of yellow pine at any one site. Interannual differences in the development (and loss) of chlorotic mottle are significant: the peak foliar injury may appear two months earlier in a drought year relative to a year of average precipitation, although the maximum level of foliar injury developed in a given site appears to roughly correlate with interannual, average site O_3 exposure. The differences reported in leaf- and canopy-level gas exchange and thus O_3 uptake for trees in mesic and xeric microsites, the manifestation of O_3 uptake in chlorotic mottle, tissue chemistry, and needle retention suggests strongly for choosing trees in consistently mesic microsites for consecutive, interannual comparisons, or for restricting repeated assessments to years of $\pm 10\%$ average precipitation years. Due to reduced foliar production within whorls with male cones, these branches should be avoided in field assessments. Although lower canopy is more easily accessible for large surveys, it is the least representative of the canopy. Future field surveys should be based in the mid canopy, allowing the capability of remote sensing to be linked to ground based measures of O_3 injury.

Ozone injury assessments have been carefully developed for yellow pine, and it is possible that this approach can be applied to both sugar and lodgepole pine because despite their relative tolerance to O_3, both exhibit chlorotic mottling on their needles. Ozone injury assessments will probably never be developed for giant sequoia, white fir, or incense cedar because visible foliar symptoms do not develop to any appreciable extent. Although subtle physiological responses to O_3 exposure can be demonstrated in white fir (Retzlaff et al., 2000) and giant sequoia (Grulke et al., 1989, 1996), the physiological response is not translated to a consistent set of morphological responses unique to O_3 exposure. Without the definitive chlorotic mottle that Miller et al. (1963) correlated to O_3 exposure, the changes in growth and needle retention observed cannot be conclusively assigned to the stressor in a field survey. Oxidative stress can, however, be supported on asymptomatic species using biochemical techniques.

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