Symptomology of Ozone Injury to Pine Foliage

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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).

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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

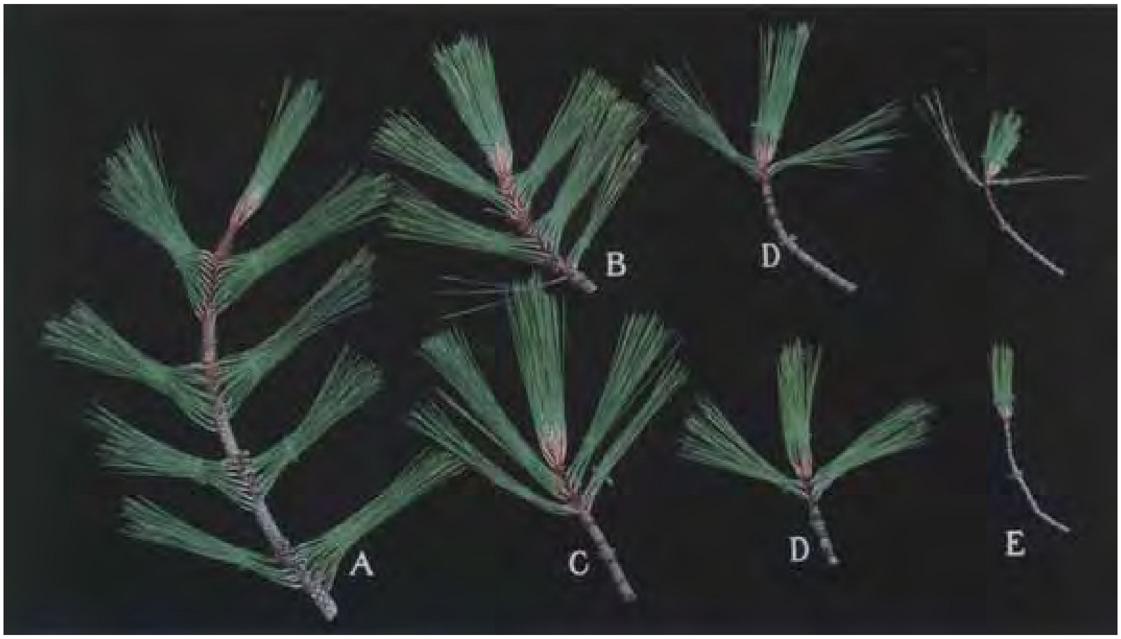

Figure 2—Progression of ozone injury on ponderosa pine branches sampled from different trees on the same site in 1994. The same progression could also occur on a single tree over time. A) A nearly healthy branch with five annual whorls of needles (numbered whorls 1-5 from youngest to oldest) with small 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.

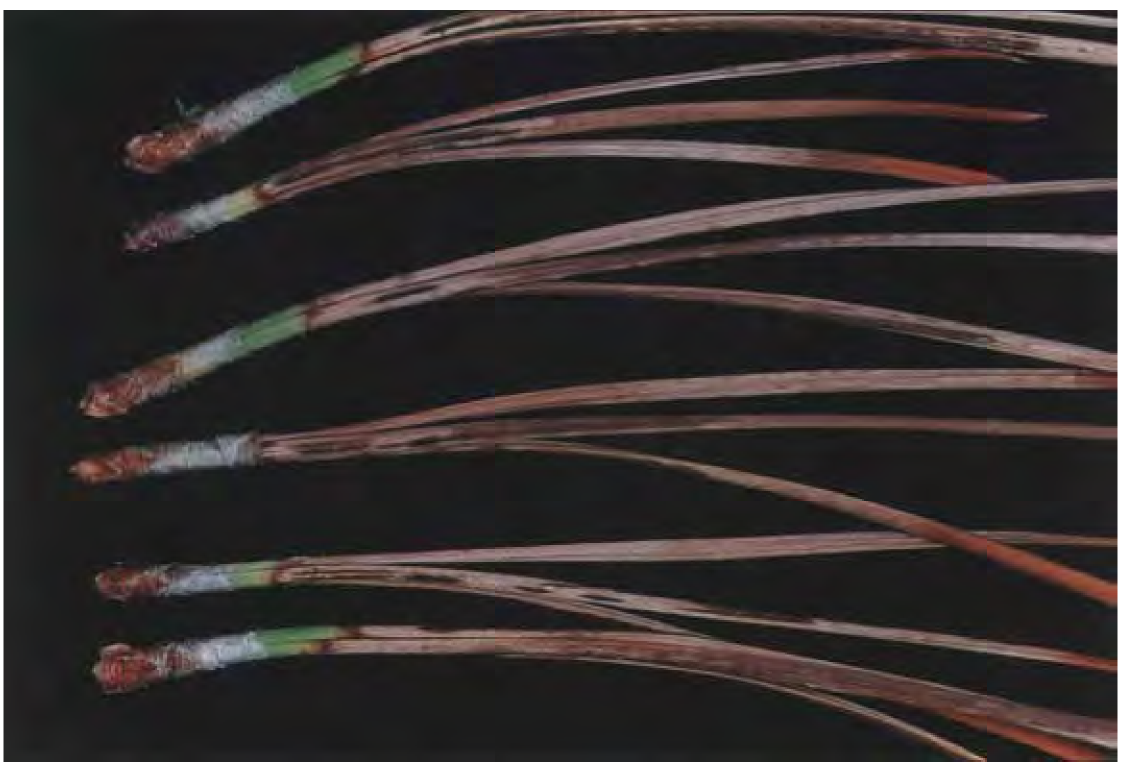

Figure 3—Elytroderma needle disease may be identified by the elongated dark fruiting bodies of the causal fungus.

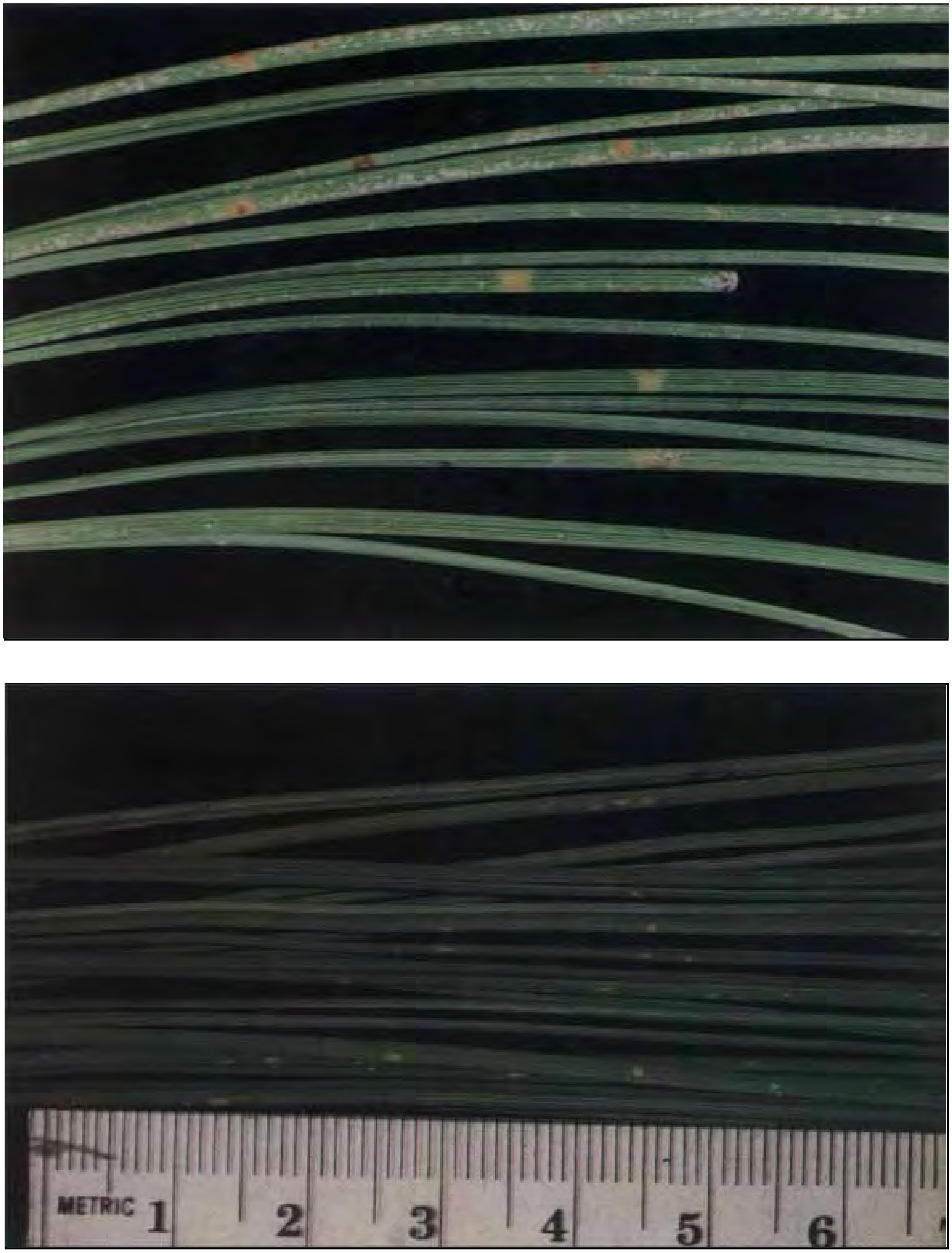

Figure 4—Yellow bands (upper photo) or yellow spots (lower photo) may reveal a distinct necrotic point in the center believed to be the mark left by the piercing mouthparts of sucking insects.

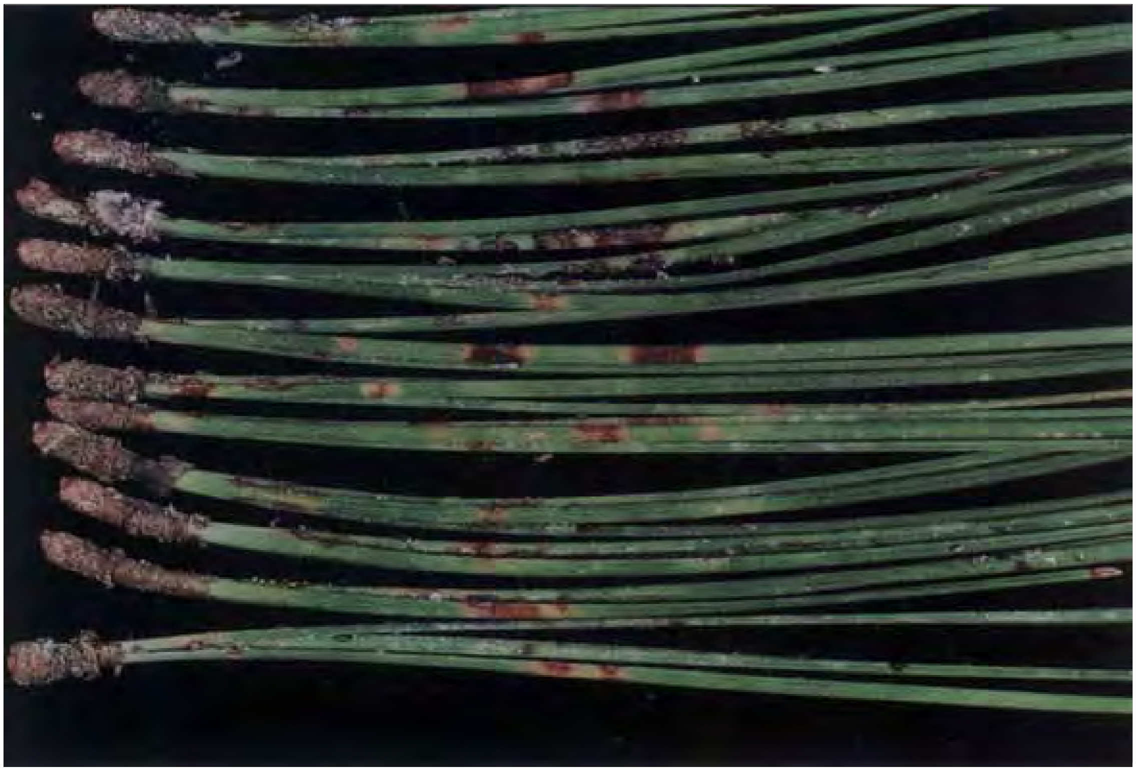

Figure 5—Yellow or brown bands or islands of needle tissue may be caused by the long–term residence of scale insects. In this photo the black scale insect still remains on the needle.

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.

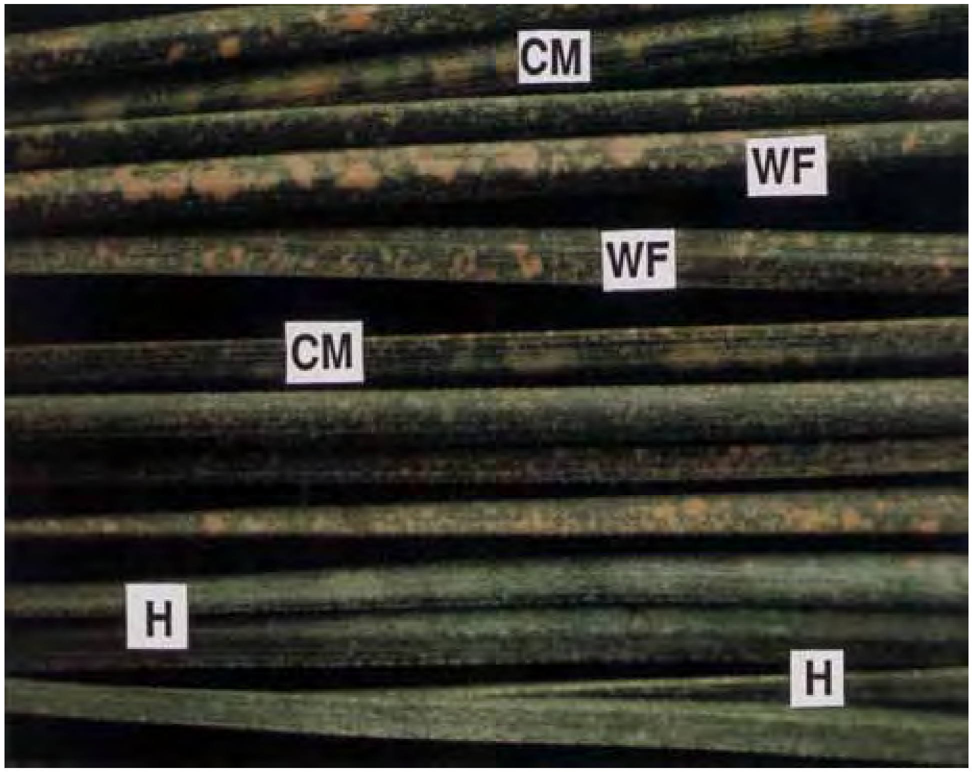

Figure 7—Three healthy (H) ponderosa pine needles (lower) are compared to those with a mixture of chlorotic mottle (CM) caused by ozone, best illustrated by the second needle from the top in which irregular chlorotic islands are visible. Weather fleck (WF) is displayed by the fourth and fifth needles 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.