Black Stain Root Disease in Ozone-Stressed Ponderosa Pine

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ABSTRACT

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In order to determine the effects of ozone exposure on black stain root disease, caused by *Leptographium wageneri* var. *ponderosum*, 2-yr-old ponderosa pine seedlings (*Pinus ponderosa*) were fumigated with ozone in open-top chambers during daylight hours for 11 wk. The ozone exposure profile was based on ozone exposures measured during August 1987 at Giant Forest in Sequoia National Park, California. The ozone concentration gradually increased during the morning and early afternoon, reached a peak in the afternoon, and then decreased until sundown. The peak concentrations were 0.1, 0.2, and 0.3 ppm in three ozone treatments. The cumulative ozone exposures in charcoal-filtered air and in the three ozone treatments were 0.26, 0.95, 1.90, and 2.84 ppm-hr/day. Injury on the previous year's needles increased ($R^2 = 0.94$) and stem growth decreased ($R^2 = 0.61$) in inoculated and noninoculated seedlings as the ozone concentration increased. Needle injury was greater and stem growth was less in seedlings inoculated with *L. wageneri* var. *ponderosum* than in noninoculated seedlings. The length of the black stain in roots of inoculated seedlings increased as the ozone exposure increased ($R^2 = 0.40$); 43% of the seedlings exposed to charcoal-filtered air (with an ozone concentration of 0.26 ppm-hr/day) had visible black staining, whereas 79% of those exposed to ozone at 2.84 ppm-hr/day were stained. From these studies, we suggest that in areas where black stain root disease occurs on ponderosa pine, exposure to elevated levels of ozone is likely to result in increased losses due to the disease.

In recent years much research has focused on the effects of ozone on agricultural crops and forest trees (11,15). In order to understand the overall impact of elevated ozone concentrations on forests, however, information is required regarding how ozone affects the biotic components of the forest ecosystem. Few studies have investigated the effects of ozone on forest pathogens and root-disease components (10,12,13).

The effect of air pollution on plant disease development depends on factors such as the sensitivity of the host and the pathogen to the pollutants, the timing and concentration of pollutants, the stage of the pathogen present at the time of exposure, and the relative sensitivity of the pathogen and other plant-associated microbes to the pollutants. Ozone and sulfur dioxide have been shown to increase, decrease, or cause no change in disease severity (9,10,14,21). Plant diseases caused by obligate parasites, such as rust fungi, have been observed to decrease under air pollution stress, whereas diseases caused by facultative pathogens that infect senescent or necrotic tissue often increase under those conditions (9,10,14,21). The effect of a particular pollutant or pollutant mixture on the development of a given disease, however, cannot be accurately predicted solely from trophic characteristics of the pathogen.

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Root diseases of conifers caused by species of *Leptographium* occur in North America, Europe, South Africa, and New Zealand (23). Black stain root disease of conifers, caused by *L. wageneri* (Kendrick) Wingfield, occurs only in western North America (4). The principal hosts of the hard pine variant, *L. wageneri* var. *ponderosum* (Harrington & Cobb) Harrington & Cobb, are ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson), Jeffrey pine (*P. jeffreyi* Grev. & Balf.), and lodgepole pine (*P. contorta* Douglas ex Loudon). Another variant, *L. wageneri* var. *pseudotsugae* Harrington & Cobb, is isolated mainly from Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco). A third variant, *L. wageneri* var. *wageneri*, is isolated mainly from pinyon (*Pinus edulis* Engelm. and *P. monophylla* Torrey & Frémont) (4,7).

Stress factors, such as unfavorable soil moisture levels, insect infestations, or site disturbances, increase the incidence of root diseases caused by *L. procerum* (Kendrick) Wingfield and *L. wageneri* (1,4,6,17,23). In a survey of eastern white pine (*Pinus strobus* L.), *L. procerum* was isolated from 24% of the trees that were sensitive to oxidant air pollution, but it was not isolated from pollution-tolerant trees (13). This suggests that ozone stress predisposed the trees to insect vectors, disease caused by *L. procerum*, or both.

The objective of this study was to determine if ozone stress in ponderosa pine affects the severity of black stain root disease, caused by *L. wageneri* var. *ponderosum*. If root disease severity or incidence increases in trees stressed by ozone, then forest damage in polluted regions may be greater than that expected due to ozone stress alone.

MATERIALS AND METHODS

Transplanting and inoculation of seedlings. In June 1987 1-yr-old ponderosa pine seedlings were transplanted into polyethylene Dee pots (Stuewe and Sons, Corvallis, OR). The pots were slightly conical and 24 cm high, with a top inside diameter of 6 cm. The seedlings were planted in nonsterilized UC mix (50% blow sand and 50% peat moss, plus 2.2 kg of dolomite, 1.5 kg of superphosphate, 148 g of KNO_3, and 148 g of K_2SO_4 per cubic meter) (3). They were grown in an air-conditioned greenhouse receiving charcoal-filtered air until the initiation of fumigation treatments in open-top chambers on 8 February 1988.

Inoculum of *L. wageneri* var. *ponderosum* was prepared by boiling 3-cm-long sections of ponderosa pine twigs (7–8 mm in diameter) in 10% malt extract for 2 hr, placing the twig sections in flasks, and autoclaving for 1 hr. After cooling, the twig sections were inoculated with agar plugs from a culture of *L. wageneri* var. *ponderosum* and incubated in the dark at 18 C for 10 wk (8). Isolate Cap E of *L. wageneri* var. *ponderosum* from the collection of Fields Cobb, at the University of California, Berkeley, was used in this study; it was isolated from ponderosa pine at Gaddis Creek, Blodgett Forest, near Georgetown, California. On 2 February 1988 half of the seedlings were inoculated by removing the planting mix surrounding the upper taproot and placing an infested twig section alongside the taproot, with the upper end of the inoculum block approximately 2 cm below the soil line. The planting mix was then replaced and packed firmly.

Ozone fumigation in open-top chambers. Fumigations were carried out at the Forest Fire Laboratory in Riverside, California. A group of 12 inoculated and 12 noninoculated ponderosa pine seedlings were randomly assigned to each of 12 chambers. There were three replicate

chambers for each of the four fumigation treatments. On 5 February 1988 the potted seedlings were placed in the ground, and the air spaces surrounding the pots were filled with soil, so that the seedling pots were buried except for the top 5–8 cm. The open-top fumigation chambers were then placed over the seedlings, and ambient air was circulated in them for 3 days during daylight hours, to acclimate the plants to the chamber environment. Ozone exposure occurred in the chambers for 11 wk, from 8 February 1988 to 27 April 1988. During the course of the experiment, the plants were watered as necessary by overhead spraying.

The open-top chambers (2.1 m in diameter and 2.7 m high) were enclosed with clear polyvinylchloride film (18). The lower portion of the chamber consisted of a double plastic layer. Air blown through a plenum connected to the outer wall formed a bellows, from which air entered the chamber through evenly spaced holes (2.5 cm in diameter) in the inner wall. The chamber blowers were controlled by automatic timers, which turned the blowers on shortly before ozone fumigation began at sunup and off shortly after ozone fumigation ended at dusk. Exposure treatments were for 12 hr per day.

The fumigation treatments were charcoal-filtered air and three ozone treatments, with ozone at a baseline concentration (1×) and at double (2×) and triple (3×) that level. The ozone concentration gradually increased in the morning and early afternoon to a peak at 15:30 hr and then gradually decreased until sundown, when fumigation ceased until the next day; the peak concentrations in the three ozone treatments were 0.1, 0.2, and 0.3 ppm (Fig. 1). Daily ozone exposure was calculated by multiplying the ozone concentration in parts per million by the duration in hours (ppm-hr) of the exposure for each concentration of ozone. Parts per million-hours per day is the sum of the ppm-hr values obtained for each of the 13 concentration-time periods in 1 day. The cumulative daytime ozone exposure in the charcoal-filtered treatment was 0.26 ppm-hr/day. The exposures in the 1×, 2×, and 3× treatments were 0.95, 1.90, and 2.84 ppm-hr/day, respectively.

A programmable ozone controller (R. Wilborn, *unpublished report*) was programmed to duplicate an hourly average ambient ozone profile measured during August 1987 at Giant Forest, Sequoia National Park, California. This profile was chosen because it represented the diurnal ozone pattern common in the southern Sierra Nevada. The controller consisted of 12 pairs of adjustable voltage regulators and timers, which were activated in sequence. The voltage regulators controlled the ozone generator voltage, thereby controlling the ozone output. The timers determined the period when each voltage regulator was operative. The ozone concentrations in the 1×, 2×, and 3× treatments were ultimately controlled by means of a proportionally controlled gas flow manifold. Downtime during the fumigation study due to equipment malfunction accounted for approximately 1.6% of the total dispensing time.

The ozone concentration in the baseline reference chamber was monitored continually with a Dasibi ozone monitor (model 1003-AH, Dasibi Environmental Corporation, Glendale, CA). Every 2 min, one of the 12 chambers was sampled and analyzed for ozone concentration with a second Dasibi ozone monitor, so that each chamber was monitored once every 24 min.

Assessment of ozone damage and disease severity. Foliar injury symptoms due to ozone exposure were not easily distinguishable from those due to inoculation with *L. wageneri* var. *ponderosum*. Each seedling was evaluated for needle damage, apparently caused by ozone and black stain root disease, at the end of the 11-wk fumigation period, by means of a damage rating scale (Table 1). Only needles from the previous year were rated, because no visible injury was apparent on the current year's needles in any of the treatments. The seedlings were dormant when placed in the chambers on 8 February 1988, and spring growth had not yet begun. After the 11-wk fumigation period, the current year's stem growth was measured.

For assessment of black stain root disease severity after the fumigation period, the cambium was peeled away from the main taproot, and the length of the black stain along the root was measured. From a few selected seedlings the pathogen was reisolated from stained roots placed on potato-dextrose agar amended with 400 μg of cycloheximide,

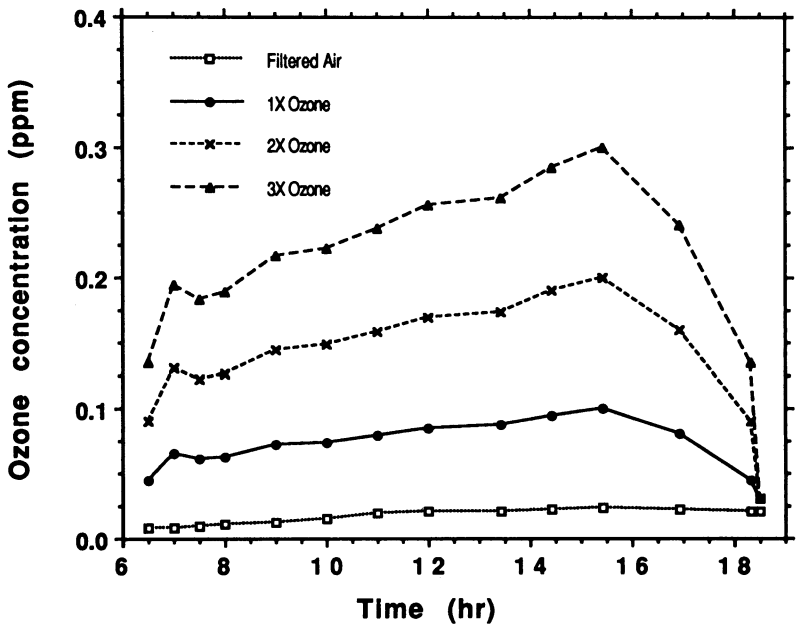

Fig. 1. Ozone exposure profile for 12 daylight hours in four treatments. The lowest profile is treatment with charcoal-filtered air. The two highest profiles are treatments with ozone in concentrations two and three times (2× and 3×) that of the baseline ozone level (1×). The fumigation profile was based on an hourly average ambient exposure measured during August 1987 at Giant Forest, Sequoia National Park, California.

Table 1. Damage rating scale for the previous year's needles on inoculated and noninoculated ponderosa pine seedlings exposed to charcoal-filtered air or ozone fumigation treatments

Damage score	Symptom
0	No discernible needle damage
1	Faintly discernible mottling on a few needles
2	20% of the needles with slight mottling
3	30% of the needles with slight or moderate mottling
4	40% of the needles with slight or moderate mottling
5	Fairly strong mottling on most needles
6	Strong mottling on most needles
7	Extensive mottling throughout, with some severely chlorotic needles
8	Some necrotic or abscised needles, with severely chlorotic needles or very strong mottling throughout
9	Almost all needles necrotic
10	All needles necrotic or abscised

100 μg of streptomcyin sulfate, and 70 μg of penicillin-G per milliliter of medium (8).

Statistical analysis. Data were first analyzed as a split-plot design using ANOVA, with the chambers as whole plots and fungal inoculation as a subplot treatment. For needle damage, stain length, and stem growth, this analysis indicated significant effects associated with ozone treatments and fungal inoculation, but interaction effects were not significant. The nonsignificance of interactions indicates that the effects of ozone and fungal inoculation are additive. Examination of contrasts that approximated a linear response to ozone exposure (expressed as ozone concentration × time) were significant in all cases; contrasts approximating quadratic and cubic responses were nonsignificant. These facts led to the subsequent fitting of a regression model that used linear response to ozone exposure and parallel slopes for inoculated and noninoculated trees. The within-chamber Pearson correlations of residuals from ordinary least squares regression were nonsignificant for both needle damage and stem growth. In all regressions, lack of fit was tested to ensure that the postulated model reasonably approximated the functional form of the data.

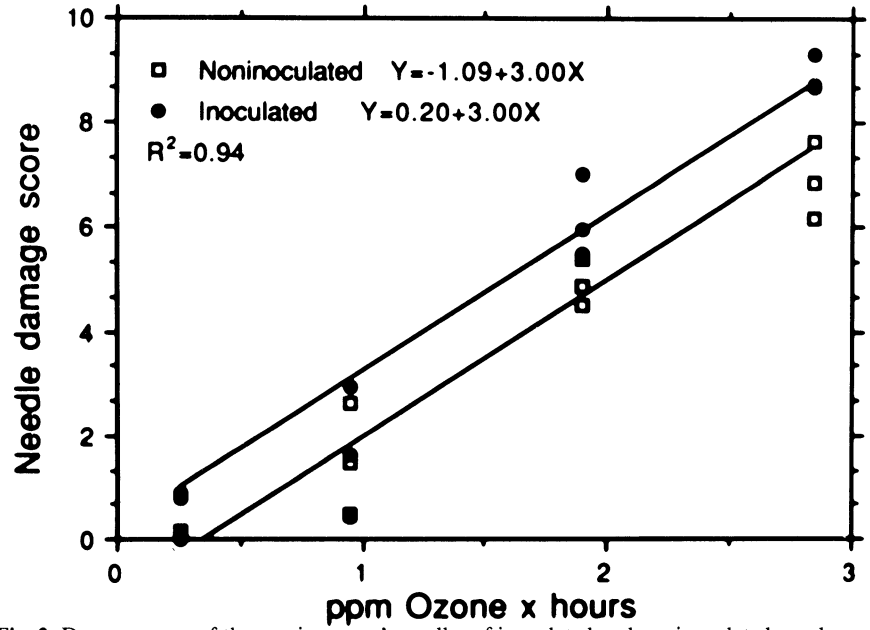

Fig. 2. Damage score of the previous year's needles of inoculated and noninoculated ponderosa pine seedlings exposed to four fumigation treatments (charcoal-filtered air and ozone in three concentrations) for 11 wk. See Table 1 for needle damage rating scale.

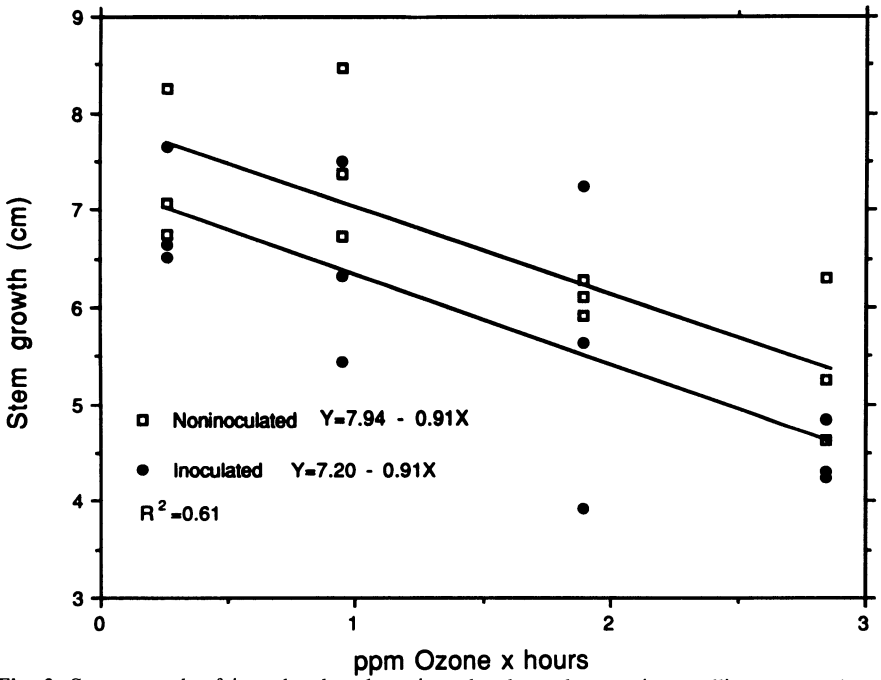

Fig. 3. Stem growth of inoculated and noninoculated ponderosa pine seedlings exposed to four fumigation treatments (charcoal-filtered air and ozone in three concentrations) for 11 wk.

RESULTS AND DISCUSSION

Symptoms of injury increased in needles from the previous year and stem growth decreased in inoculated and noninoculated trees as the ozone exposure increased (Figs. 2 and 3). The slopes of regression lines for needle injury (R^2 = 0.94) and stem growth ($R^2 = 0.61$) versus cumulative ozone exposure were not significantly different for inoculated and noninoculated plants. Needle damage was greater and stem growth was less in seedlings inoculated with *L. wageneri* var. *ponderosum* than in noninoculated seedlings.

Seedlings with roots damaged by the black stain organism exhibit foliar symptoms, such as chlorosis, shortened needles, fewer needles, reduced terminal growth, and necrosis, similar to symptoms associated with other root diseases (4,5). The black stain organism colonizes the xylem tracheids (4) and probably interferes with conductive functions of the xylem. In the present study, foliar damage and reduced stem growth over and above that caused by ozone alone in inoculated plants may be the result of the combined stresses of ozone and root impairment due to infection with *L. wageneri* var. *ponderosum*.

The length of root staining in seedlings increased as the ozone exposure increased (R^2 = 0.40; Fig. 4). The length of root staining in symptomatic plants was underestimated, as the data also included asymptomatic seedlings (0-cm stain). The percentage of seedlings with black stain also increased with ozone exposure. Of the seedlings exposed to charcoal-filtered air (with an ozone concentration of 0.26 ppm-hr/day), 43% had black stain, whereas 64, 62, and 79% of the seedlings exposed to ozone in concentrations of 0.95, 1.90, and 2.84 ppm-hr/day were stained.

The predisposition of ponderosa pine seedlings to black stain root disease by ozone stress corroborates previous observations regarding tree stress and increased susceptibility to insects and fungal pathogens (6,12,17). Earlier field studies demonstrated that stress factors such as unfavorable soil moisture, insect infestation, soil compaction, topsoil displacement, root disturbance, and precommercial tree thinning increase the incidence of root disease caused by *L. wageneri* and L. *procerum* (1,4,6,17,23). Environmental stresses can affect host susceptibility to insect vectors (6,17) as well as disease development following infection (2,19,20).

James et al (12) reported that oxidant air pollution injury to foliage of ponderosa and Jeffrey pines increases the susceptibility of roots to infection and colonization by *Heterobasidion annosum*

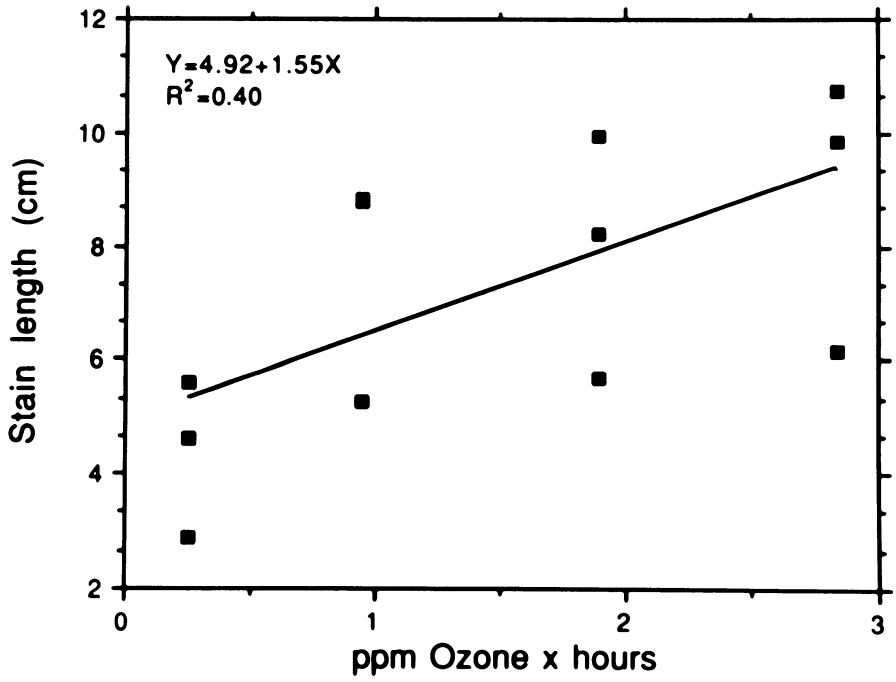

Fig. 4. Length of black stain in taproots of ponderosa pine seedlings inoculated with *Leptographium wageneri* var. *ponderosum* and exposed to four fumigation treatments (charcoal-filtered air and ozone in three concentrations) for 11 wk.

(Fr.) Bref. in fumigation chambers and in the San Bernardino Mountains of southern California. In the fumigation study reported herein, black stain root disease increased in 2-yr-old ponderosa pine seedlings exposed to ozone. Inasmuch as black stain root disease increased in ozone-stressed ponderosa pine seedlings in this study and in mature trees subjected to various stress factors (4,6,17), and because of the apparent relationship between infection of eastern white pine with *L. procerum* and oxidant air pollution (13), and since ozone stress increased the susceptibility of ponderosa and Jeffrey pines to annosus root rot, it seems likely that ozone stress of mature ponderosa pine would result in increased frequency and severity of black stain root disease.

Root and foliar symptoms of black stain root disease of ponderosa pine were more severe in seedlings exposed to any of the three ozone treatments for 11 wk than in those exposed to charcoal-filtered air for 11 wk. The peak ozone concentration in the 1× treatment (0.1 ppm) is frequently exceeded by 9-hr average concentrations in the San Bernardino Mountains (16). Daily peak concentrations commonly exceed 0.1 ppm during the summer months in the southern Sierra Nevada (22). Thus, the lowest ozone treatment in this study is a realistic ambient concentration. The peak concentration in the 2× treatment (0.2 ppm) and to a lesser extent that of the 3× treatment (0.3 ppm) are within the range of peak concentrations occurring in the western regions of the San Bernardino Mountains, where the maximum daily ozone concentration commonly ranges between 0.20 and 0.33 ppm during the summer (16,22).

Black stain root disease of ponderosa pine has not been found in polluted areas such as the southern Sierra Nevada or the San Bernardino Mountains. It does occur on pinyon in the San Bernardino Mountains, however (4). We suggest that in areas where the disease occurs on ponderosa pine, exposure to elevated concentrations of ozone would result in increased losses due to black stain, in addition to the direct impact of ozone on ponderosa pine.

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