The Effects of Oxygen Stress and Soil Aeration Management on Susceptibility of Oaks to Phytophthora Root Rot

Karel Jacobs, James MacDonald, Larry Costello, Alison Berry and Terrence Berger.

Oak trees planted or incorporated into urban landscapes are often subjected to environmental stresses, with poor root aeration being one of the most common and potentially damaging. Roots require a continual supply of oxygen, which moves from the above-ground atmosphere to the below-ground atmosphere through soil pores. But two factors which occur commonly in landscaped settings can interfere with this process. One is high soil moisture, resulting from frequent or heavy irrigations and/or poor soil drainage. This causes soil pores to remain water-filled, and inhibits air movement into the root zone. A second factor is soil compaction, which restricts oxygen supply to roots by collapsing the large-diameter pores and channels so critical to efficient air entry.

Near the surface of a porous, well-structured soil, oxygen may comprise as much as 21 percent of the soil atmosphere. Root vigor is believed to decline in many tree species if the oxygen concentration in the soil atmosphere drops to levels between 5 and 10 percent. Trees with roots exposed to chronic, mild levels of oxygen stress may exhibit symptoms such as early fall color and leaf drop. Trees exposed to more severe stresses may exhibit symptoms of progressive canopy thinning, limb dieback, and eventual death.

While chronic oxygen stress causes mild to severe root injury by itself, in some plants it also can lead indirectly to damage by increasing root susceptibility to pathogens such as *Phytophthora* spp. and *Armillaria* spp.. Those effects can occur even when stress is brief and not directly injurious to roots.

Several methods are commonly used in efforts to improve root aeration in landscape soils. They include fracturing or perforating compacted soils with high-pressure air or water injections, or augering holes which then may be filled with porous materials. In cases where air entry is inhibited by surface barriers such as overlying fill soils or asphalt or concrete pavement, porous paving blocks may be substituted or underground pipe systems may be installed to provide ventilation. Those practices have been used for many years with widely varying reports of success. Whether any of them significantly improve soil aeration problems that lead to direct root injury or indirect injury through enhanced activity of pathogens has not been critically studied.

A cork oak *(Quercus suber)* tree killed by *Phytophthora cinnamomi.* The fungus recovered from this tree was used in the inoculation experiments described in this report.

The objectives of our research have been to determine at what concentrations oxygen limits the growth of oak roots and increases their susceptibility to Phytophthora root rot. We have also sought to evaluate the effectiveness of some soil management practices in maintaining oxygen levels above those which injure roots. For our studies we selected three species of oak, *Quercus lobata* (valley oak), *Quercus suber* (cork oak) and *Quercus douglasii* (blue oak), since these species are believed to differ in their tolerance to flooding and low soil oxygen, with valley oak considered most tolerant and blue oak least tolerant.

Controlled Atmosphere Experiments

To determine the effects of various oxygen concentrations on root growth, germinated acorns with 10-centimeter-long radicles were transplanted to root viewing boxes

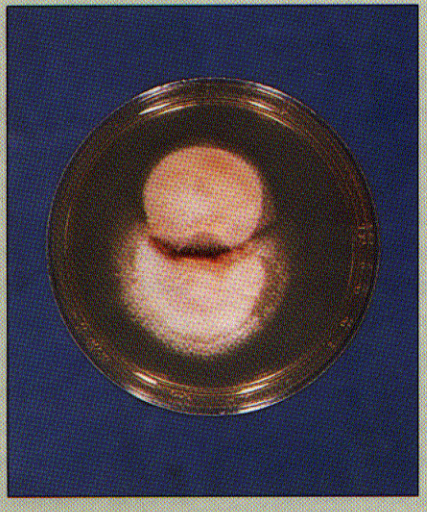
An oak tree with mushrooms of the oak root fungus *Armillaria mellea* developing during the cool, wet weather of late fall.

Armillaria Root Rot

Karel Jacobs, James MacDonald, Fields Cobb and Kenneth Wells

Armillaria root rot is a widespread disease affecting many perennial plants in California. The disease was first described from Sonoma County vineyards in 1881, and was quickly recognized as a problem associated with cleared oak habitats. The pathogen, identified as *Armillaria mellea* (Vahl ex Fr.) Kummer, is commonly referred to as "oak root fungus" or "honey mushroom."

A. mellea is a complex species which has recently been separated into several, reproductively isolated "biological species." Those biological species appear to be very similar but can differ in important characteristics such as geographical distribution, host preference and pathogenicity. They can be distinguished only through "compatibility tests" performed in the laboratory. The tests involve growing unknown isolates in culture plates in the presence of known tester strains and observing the appearance of the cultures as they grow together and come into contact.



An incompatible reaction between an unknown isolate of *Armillaria* and one of the nine tester strains. The dark brown pigment at the interface between the two colonies shows the incompatibility reaction obtained when different species are paired in culture.

There have been nine biological species of *Armillaria* reported to occur in North America, but there is no information describing species or species distribution in California. We obtained mushrooms of *Armillaria* from eleven counties in northern and coastal parts of California, collecting them from around valley and coast live oak and other hosts in urban, rural and forested sites. Spores from the mushrooms were transferred to agar media to allow cultures to develop. Of 26 separate cultures, 22 were confirmed as *A. mellea*. Of the four others, all of which originated from Mendocino county, one was identified as *A. bulbosa* (also known as *A. gallica*), and three were compatible with tester strain NABS IX, which is an undefined "morphospecies." Thus, *A. mellea* is the dominate species associated with native oaks, but it is not the only species.

Karel Jacobs is a former graduate student, James MacDonald is Professor, Department of Plant Pathology, and Kenneth Wells is Professor Emeritus of Botany, University of California, Davis; Fields Cobb is Professor of Plant Pathology, University of California, Berkeley.

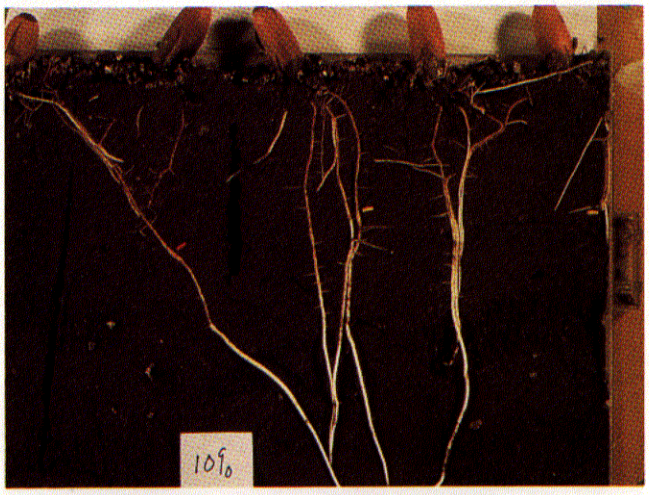
(rhizotrons) having removable plexiglass faces. The rhizotrons were placed in controlled environment growth chambers and slanted at 70 to 80 degrees to promote root growth along the plexiglass plate. After 10 days the plate was removed and transparent acetate sheets were laid over the exposed soil face to enable tracing of exposed roots with a colored marking pen. After initial root growth was traced, the plexiglass plate was reinstalled on each rhizotron.

Plants then were transferred to several small sealed containers, each ventilated with gas mixtures of known oxygen concentrations, ranging from 0 to 21 percent. After 5 days exposure to the various oxygen treatments, rhizotrons were removed from the sealed containers and reopened to allow root examination. Root tracings made prior to the oxygen treatments were placed back over the exposed roots and revealed the amount of root growth during treatment. We used a different color marking pen to trace new growth, measured the amount of pre- and post-treatment root growth from the root tracings, and employed those values to determine a "root growth value" for each plant. A root growth value of 50 percent indicated that half of the total exposed root length at harvest developed during the incubation period.

In the controlled atmosphere experiments, we found that root growth was unaffected as oxygen concentration decreased from 21 to 10 percent. However, at lower oxygen concentrations, growth was reduced. At 4 percent, root growth in all three oak species was approximately one half the amount of growth at 21 percent. In the first several experiments, blue oak appeared more sensitive to low oxygen than the other species, exhibiting a 50 percent reduction in root growth at slightly higher oxygen concentrations than valley or cork oak. But later experiments showed that there was relatively little difference between the species under our experimental conditions.

Inoculation Experiments

Since it tolerated our experimental manipulations better than other species, our examination of oxygen effects on the susceptibility of roots to *Phytophthora* infection focused primarily on cork oak. That species can be seriously affected by this root-and-canker disease in landscaped areas, and we used an isolate of *Phytophthora cinnamomi* recovered from a severely diseased cork oak in all experiments.



A root viewing box (rhizotron) with cover removed to illustrate how root tracings were made to quantify root growth before and after oxygen treatment. This rhizotron was incubated at 10-percent oxygen, which did not inhibit root growth.

Oak seedlings were established in rhizotrons as described above. After the initial establishment period, when rhizotrons were opened for root tracing, some roots were inoculated at their tips with small numbers of zoospores or small amounts of mycelium. Markers were placed next to roots so we could later distinguish the inoculated roots from the noninoculated control roots. After the incubation period, roots were removed and surface-disinfected in 70-percent ethanol. They were then cut into serial segments and cultured onto a selective agar medium. The extent of root colonization was assessed by determining how far from the point of inoculation *P. cinnamomi* could be recovered from the tissues.

To determine the effects of oxygen stress on aspects of root resistance to pathogens, we performed histochemical analysis of aerated and oxygen-stressed root tissues. Healthy and inoculated roots were removed and cut into thin sections for microscopic examination. The tissue sections were treated with various chemical stains to identify the extent and location of root cells containing lignin and suberin. These substances serve to reinforce plant cells and tissues and can slow pathogen growth in roots.

The inoculation experiments showed that more roots were successfully infected, and the extent of colonization was greater, when they were exposed to oxygen levels below 4 percent. The low oxygen concentrations which caused the greatest increase in root susceptibility corresponded with those which caused the greatest root stress

A petri dish containing an agar medium onto which a root from a cork oak seedling has been cultured. The root was inoculated at the tip prior to oxygen treatment. Five days later, it was recovered and cut into serial 1-centimeter-long segments, which were laid on the agar medium in sequential order. The extent of fungal colonization was determined by the number of segments colonized behind the point of inoculation.

as evidenced by inhibited growth. We found that mycelium was more effective than zoospores in causing root infections, particularly at higher oxygen concentrations. We also found that the response of individual roots to infection was not uniform, with some roots appearing more susceptible than others at a given oxygen concentration. This variability was less evident when roots were incubated at very low oxygen levels (less than one percent).

Microscopic examination of stained root sections showed that roots exposed to low oxygen had a poorly-developed endodermis and reduced accumulation of lignin and other phenolics relative to nonstressed plants. There also appeared to be disorganization and reduced suberization of epidermal tissues in stressed roots relative to well-aerated roots. The lignified and suberized tissues in roots are thought to be important barriers to fungal penetration, and their reduced development and deposition in stressed roots may have allowed the extensive colonization of tissues revealed in the microscopic examinations.

Soil Aeration Status Near Healthy and Declining Oaks

In addition to these laboratory and growth chamber experiments, we measured soil aeration status at several landscaped sites where oak trees were either growing vigorously or undergoing decline. At each location, we installed tensiometers to measure soil moisture, gas sampling tubes to measure soil oxygen concentration, and platinum microelectrodes to measure the rate of oxygen diffusion within soil. Monitoring of soil moisture and oxygen status was conducted daily for 2 to 4 weeks. At the end of the monitoring period, undisturbed soil cores were collected from each site for structural analysis.

In field measurements of soil aeration status around healthy and declining trees, we found that oxygen concentration was not well correlated with tree condition. When we extracted gas samples from the soil and analyzed them, we found the oxygen levels around vigorous trees to range between 16 and 20 percent. But the oxygen concentration around declining trees was rarely below 14 percent and was sometimes as high as 18 percent. While those oxygen concentrations should not limit root growth, we found that the root mass in soil samples from around declining trees was less than one fourth that of vigorous trees. This suggested that even though oxygen was present in the soil, it was not able to move effectively to growing tree roots and, therefore, limited root development.

The oxygen diffusion rate (ODR) of a soil can be measured with specialized electronic equipment. Soil ODR is significantly influenced by soil moisture content and compaction, which both reduce the volume of air-filled pores. Our measurements revealed that the sites where oaks were declining had very low oxygen diffusion rates in the upper 30 centimeters of the soil profile. On the other hand, areas where trees were vigorous had relatively high ODR values in the upper profile. Tree vigor seemed independent of ODR values in deeper regions of the soil profile.

Research Implications

Abundant shallow roots are considered critical to the health of oak trees. Indeed, in the several landscaped areas we studied where trees were growing vigorously, we found an extensive network of fine roots in the upper 30 centimeters of the soil profile, with more sparse root development at deeper depths. This contrasted sharply with trees undergoing decline. At those sites, root growth in all parts of the profile, particularly the upper zone, was relatively sparse. We found that poor root development seemed to be most closely associated with a very low oxygen diffusion rate in this important part of the soil profile.

We found that ODR gave a better indication of oxygen availability in soil than measurements of gaseous

oxygen. This was because we obtained very low ODR readings around declining trees, even though gas samples collected from small chambers buried in the soil indicated no serious deficiency (concentrations of 14-18 percent). Soil cores collected from those sites revealed very low root densities, indicating that lack of oxygen was indeed limiting to root growth. While the concentration of oxygen in the soil atmosphere appeared satisfactory, the volume of air and the ability of its oxygen component to diffuse through soil was reduced due to the compaction and persistently moist condition of the soil. Thus, we felt that the instrument for measuring soil ODR was very effective in identifying potentially stressful zones within the soil profile.

Turf culture can severely compromise the extensive network of shallow roots surrounding established oaks. The soil beneath turfed areas may be continuously moist and occasionally subjected to heavy foot and vehicle traffic. This combination can lead to significant surface compaction and reduced ODR. Our experiments showed that oxygen levels which are stressful enough to decrease root growth also can increase root susceptibility to pathogen attack, perhaps by decreasing the ability of roots to form effective barriers to pathogen colonization.

Soil aeration management, therefore, is particularly important when landscaping around established trees. A number of methods are employed to improve air entry into compacted soils, or soils overlaid with diffusion barriers such as pavement. While augering, air injection or other practices may open channels that facilitate air entry into the profile, they cannot fully relieve the adverse effects of soil compaction and high moisture content. We experimented with a device which is designed to fracture compacted zones by injecting large volumes of compressed air into soil. While it clearly cracked and lifted parts of the soil, we could detect no significant change in soil ODR. Also, any potential benefits of such treatments can be brief if irrigation and compaction resume after treatment. The best management practice is to prevent initial compaction of soil, and to limit moisture content.

Karel Jacobs is a former graduate student, James MacDonald is Professor, and Terrence Berger is Research Assistant, Department of Plant Pathology, University of California, Davis; Larry Costello is Farm Adviser, University of California Cooperative Extension, San Mateo County; Alison Berry is Associate Professor, Department of Environmental Horticulture, University of California, Davis.