

Rooting Responses of Three Oak Species to Low Oxygen Stress¹

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Abstract: Rooting characteristics were compared in blue (*Q. douglasii*), valley (*Q. lobata*), and cork oak (*Q. suber*) seedlings under hypoxic (low oxygen) conditions. A 50 percent reduction in root growth occurred in all species at an oxygen level of 4 percent, or an oxygen diffusion rate of $0.3 \mu\text{g cm}^{-2}\text{min}^{-1}$. Blue oak formed few lateral roots regardless of oxygen level, but valley and cork oak root production decreased under hypoxic conditions. Four percent soil oxygen might be viewed as a minimum requirement for sustaining root growth of oaks in the field, and differences in root branching morphology may be correlated with tolerance of root hypoxia.

Blue oak (*Quercus douglasii* Hook. and Arn.), valley oak (*Q. lobata* Née), and cork oak (*Q. suber* L.) vary in tolerance to flooding, with blue oak considered the least tolerant and valley oak the most tolerant (Harris and others 1980, Whitlow and Harris 1979). Cork oak is considered moderately tolerant to flooding in its native, Mediterranean habitat (Cooke and others 1961). Blue and valley oaks are indigenous to California and are deciduous species in the subgenus *Leucobalanus* ("white oaks"). Blue oak occurs at elevations between 300 and 1,250 m, primarily on xeric slopes of the Sierra Nevada-Cascade foothills and coastal mountain ranges. Valley oak occurs at elevations between sea level and 1,200 m and is especially prevalent in deep, alluvial soils (Griffin and Critchfield 1976, Miller and Lamb 1985). Cork oak is an evergreen species in the subgenus *Erythrobalanus* ("black oaks"). It grows on coastal hills and mountains in the Mediterranean region, akin to the distribution of California live oak (*Q. agrifolia* Née) in California. It is also found in interior regions of Spain and Portugal at elevations from 500 to 1,300 m (Velaz de Medrano and Ugarté 1922). Cork oak was introduced into California in the mid-1800's for cork production and ornamental purposes (Metcalf 1947), and the species has become a common landscape tree there.

Oaks are exposed increasingly to soil compaction, back-filling, turf irrigation, and other stresses associated with urban development in California. These practices can reduce soil aeration and oxygen diffusion to roots (MacDonald 1993). Low soil oxygen, or hypoxia, inhibits root growth and diminishes tree vigor (Kozlowski 1985). Moreover, hypoxia stress may predispose a plant to disease and insect pests, particularly root rots (Heritage and Duniway 1985, Miller and Burke 1977). *Phytophthora* root rot of cork oak and coast live oak occurs in mature trees grown under conditions of low soil aeration (Mircetich and others 1977). Jacobs and others (these proceedings) showed that oxygen levels below 3-4 percent, or an oxygen diffusion rate (ODR) of $0.3 \mu\text{g cm}^{-2}\text{min}^{-1}$, significantly increased the incidence of *Phytophthora cinnamomi* root disease in cork oak seedlings. Costello and others (1991) and MacDonald (1993) note that even lower oxygen diffusion rates occur in irrigated turf sites associated with declining coast live and cork oaks.

The objective of this study was to compare root growth and morphology of blue, valley, and cork oak seedlings subjected to different oxygen concentrations. The information gained might offer insight regarding the variation observed in flooding tolerance among the species and aid in managing oaks in urban landscapes.

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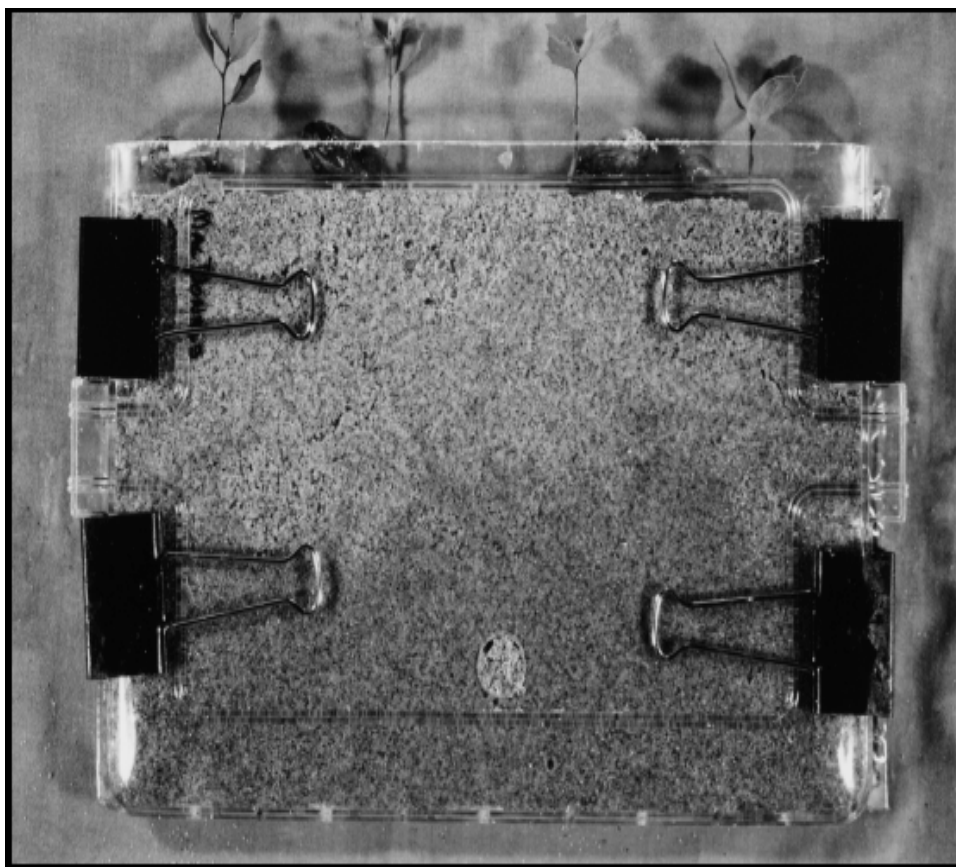
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Materials and Methods

Acorns were collected from several trees of each species during October and November of 1987 through 1990. Acorns were soaked overnight in water, air-dried, and stored in plastic bags in lots of 50, at 4-6 °C. Seeds were removed from cold storage as needed and germinated in vermiculite-filled flats in a greenhouse at 20-25 °C.

Mini-rhizotrons were made from 24- by 32- by 2-cm plastic crisper lids with a removable plexiglass plate clamped to one side (*fig. 1*). Holes were drilled in the

Figure 1—Mini-rhizotron showing polarographic oxygen sensor port in base (arrow). Roots are facing away from camera.



bottom of the lids for drainage, and at the base of the mini-rhizotrons for measuring oxygen (described below). A graded, coarse-textured sand (#0/30) (RMC Lonestar, Pleasanton, Calif.) was selected as the soil medium in order to encourage rapid drainage and oxygen diffusion and have similar moisture conditions in each mini-rhizotron. The water- holding capacity of the sand was determined by constructing a moisture release curve, and, the sand was autoclaved for 1 hour and thoroughly wetted with distilled water before use.

Five to seven germinated acorns were transferred to each mini-rhizotron when radicles were approximately 5 cm long (e.g., 2-6 weeks old). The shoots had typically emerged but were still in the cotyledonary stage of growth, although this varied somewhat with species. Seedlings were placed on the open surface of the sand-filled mini-rhizotron, and the plastic plate was carefully clamped over the exposed roots, leaving the shoots exposed. Mini-rhizotrons were placed in a 25 °C growth chamber with a 12-hour daylength for one week before treatment and were kept at a 45° angle to encourage root growth along the plastic plate. The mini-rhizotrons were watered daily. On the day the oxygen

treatments were imposed, root growth that occurred along the removable plastic plate was traced onto an acetate sheet. Mini-rhizotrons were drained to container capacity (comparable to field capacity) and placed at a 45° angle into airtight treatment chambers kept in a 25 °C controlled environment room. The incubation period lasted for 5 days.

Varying mixtures of nitrogen and compressed air were used to generate oxygen concentrations between 0 and 21 percent, and gas mixtures entered the chambers at a flow rate of 16 l/hr. One-milliliter gas samples were collected from three points in the apparatus to monitor oxygen levels (*fig. 2*), and samples were

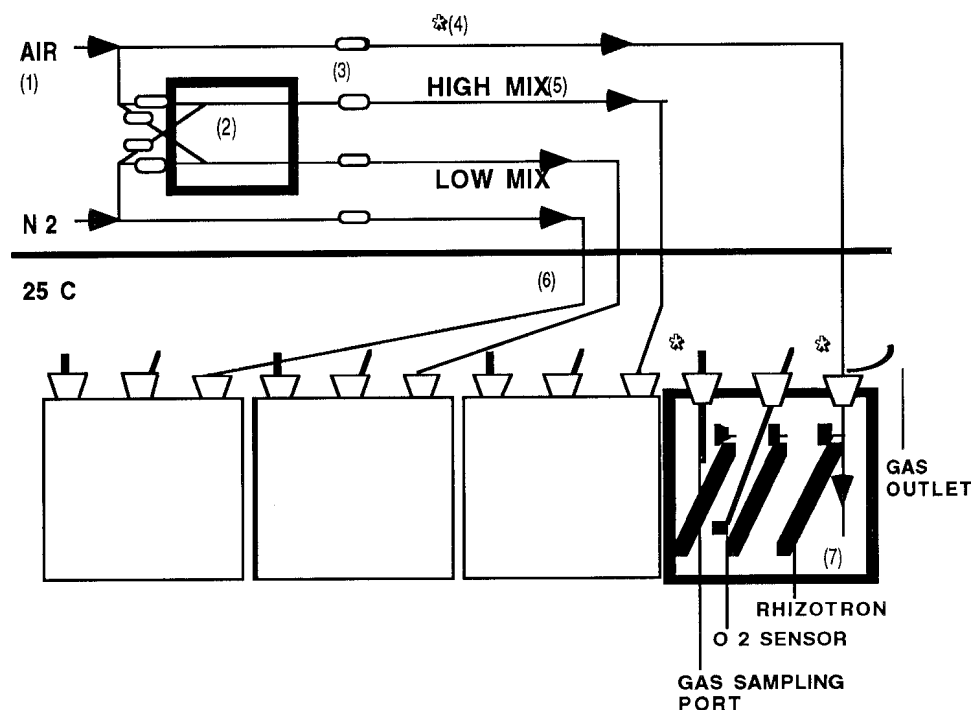


Figure 2—Experimental apparatus used to impose oxygen treatments. (1) Gas inlet lines supplying compressed air and pure (99.99 pct) nitrogen. (2) Gas mixing board. (3) Capillary tubes used to regulate flow rate of gases. (4) Stars indicate points where gas samples were taken to monitor oxygen levels. (5) Up to four oxygen treatments were run simultaneously: a high oxygen mix, low oxygen mix, 0 percent oxygen (= nitrogen), and 21 percent oxygen (= control). (6) Each gas line led to an airtight incubation chamber inside a controlled environment room. (7) Side view of three mini-rhizotrons placed at a 45° angle in a treatment chamber. A polarographic oxygen (O_2) sensor was attached to one mini-rhizotron in each treatment chamber. Seedlings were incubated for 5 days.

measured electrochemically using an oxygen analyzer (Saltveit and Strike 1989). Polarographic oxygen sensors (Jensen Instruments, Tacoma, Wash.) were used to monitor oxygen concentrations inside one mini-rhizotron in each treatment chamber. The sensors were inserted into holes drilled into the base of the mini-rhizotrons. Oxygen diffusion rate (ODR) (see Birkle and others 1964) was monitored inside the mini-rhizotrons for the 21 percent, 3-4 percent, and ≤ 1 percent oxygen treatments using platinum microelectrodes (Jensen Instruments, Tacoma, Wash.). We did not monitor ODR in later trials because oxygen concentration correlated directly with ODR measurements, and, in our system, the polarographic sensors varied less and were easier to use than the microelectrodes.

Three to four treatment chambers were run simultaneously, and a minimum of 15 seedlings of each species were treated for each oxygen level. More treatments were included at low, rather than high, oxygen concentrations because root growth was found to be unaffected until oxygen levels fell below 6 percent.

At the end of 5 days, mini-rhizotrons were removed from the treatment chambers, and root growth was evaluated. Root growth was measured on a per-seedling basis as the ratio of root length that occurred during incubation to total root length. The ratio was used to help account for initial variation in root length between seedlings. New root growth was traced over the pre-incubation tracings, and initial and total root lengths were input into a computer using a digitizing

tablet (model MM1202, Summagraphics Corp., Fairfield, Conn.) and MacMeasure software (Research Services, National Institute of Mental Health, Bethesda, Md).

Root tracings were also used to measure daily taproot growth for 10 seedlings with aerated shoots per species in a 25 °C growth chamber with 12-hour daylength. Measurements were made until taproots reached the base of the mini-rhizotrons (approximately 1 week).

The number of lateral roots that formed along a single taproot during the incubation period was determined for six seedlings per species at each of five oxygen levels: 0-2, 2-5, 5-7, 7-10, and 21 percent. Only seedlings that developed single taproots were measured, and we grouped similar oxygen concentrations together in order to have sufficient seedling numbers for the analysis.

The experiment was set up as a split-plot design with oxygen concentration as the main-plot, replicated over time, and species as the sub-plot. Differences in root length were compared utilizing regression analysis and curve fitting. To compare lateral branching, the oxygen treatment was considered a fixed effect, and the analysis of variance procedure with Tukey-Kramer and Duncan’s means separation tests were used (SAS Institute 1991).

Results

Apparatus

The moisture release curve indicated that the soil medium was very well drained with a moisture content ranging from 17 percent at the top of the mini-rhizotron (25 mbar tension) to 23 percent at the bottom of the mini-rhizotron (0 mbar tension). It was important to have the same soil moisture content in all mini-rhizotrons because of the potential impact of moisture on root branching, and oxygen diffusion, discussed later.

Polarographic oxygen sensors indicated that the oxygen concentration inside mini-rhizotrons equilibrated with the surrounding atmosphere within 5 hours. The relationship between oxygen concentration, measured by the sensors, and oxygen diffusion rate, measured with platinum microelectrodes, was determined for three treatment levels:

<i>Oxygen concentration</i>	<i>Oxygen diffusion rate</i>
<i>(pct)</i>	<i>(µg cm⁻²min⁻¹)</i>
21	0.7-0.8
3-4	0.3
≤1	0.1

Growth Response with Aerated Shoots

The average taproot extension rate under atmospheric conditions did not differ significantly (*P* = 0.05) between species:

<i>Species</i>	<i>Root growth (mm/day)</i>
Blue oak	17
Valley oak	14
Cork oak	13

The number of first order lateral roots that formed along the apical 15 cm of tap root differed significantly (*P* = 0.05) between species:

<i>Species</i>	<i>Lateral roots cm⁻¹ taproot</i>
Blue oak	0.16
Valley oak	0.91
Cork oak	0.63

Growth Response to Reduced Oxygen

Root growth began decreasing at oxygen levels below 6 percent in blue, cork, and valley oak seedlings, and the response was explained best by a saturation growth curve (fig. 3). Valley oak root growth decreased more gradually than cork and blue oak roots as oxygen levels fell, and this is reflected in a higher coefficient of correlation ($R^2 = 0.87$) for the valley oak curve fit. Cork oak root growth dropped off precipitously at about 4 percent oxygen, and considerable variation in growth rate occurred in both cork and blue oak at the lower oxygen levels.

The oxygen concentration that brought about half-maximum root growth, i.e., a 50 percent reduction in the root growth that occurred at 21 percent oxygen, was approximately 4 percent for all species (fig. 3).

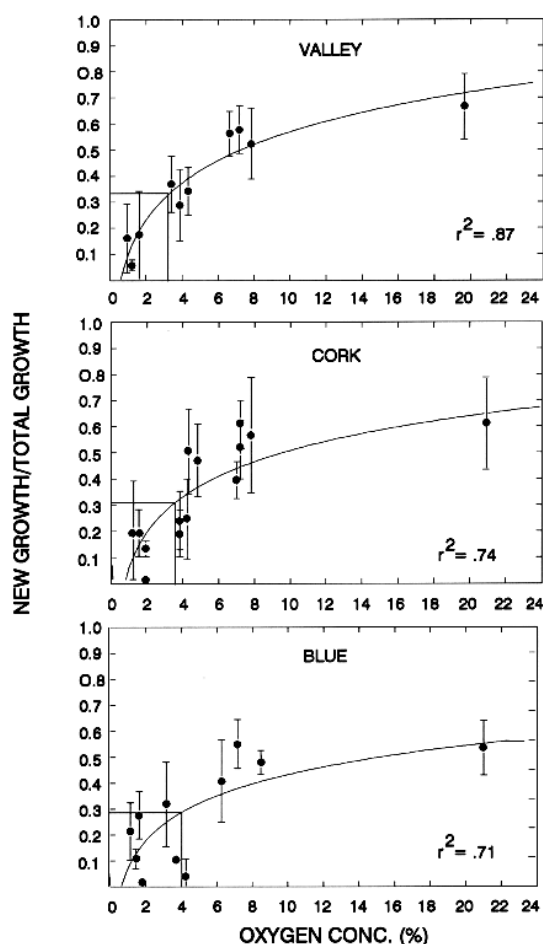
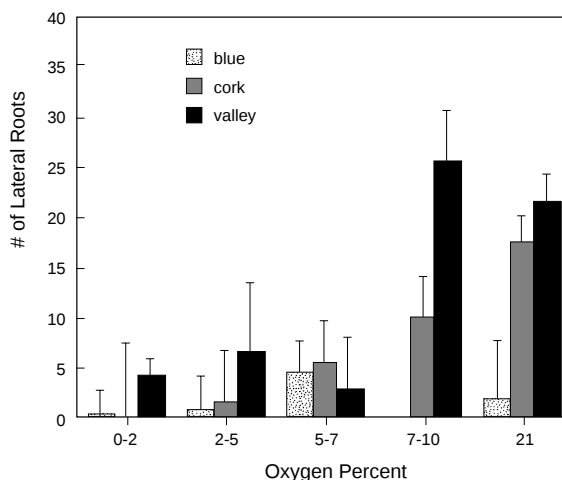


Figure 3—Root growth of valley, cork, and blue oak (new growth/total growth) affected by oxygen concentrations ranging from 0 to 21 percent. Correlation coefficients indicate the closeness of fit of the data to a saturation curve. Each point represents an average of at least 15 seedlings. Half-maximum root growth from that occurring at 21 percent oxygen is indicated by a line drawn from curve to x and y axes.

The number of lateral roots produced on a single tap root decreased significantly ($P = 0.05$) in cork and valley oak as oxygen concentrations fell (fig. 4). In contrast, the number of lateral roots produced by blue oak seedlings did not vary with oxygen concentration and was always less than cork and valley oak.

Figure 4—Total number of lateral roots produced along the apical 15 cm of tap root at different oxygen concentrations. A total of 30 seedlings per species were evaluated (6 seedlings/oxygen level/species), and only seedlings producing a single tap root were measured.



Discussion

Root growth of blue, valley, and cork oak seedlings responded similarly to decreasing oxygen in that a 50 percent reduction in growth occurred at 4 percent oxygen. We are not aware of other studies that identify an oxygen concentration corresponding to half-maximum root growth; however, root growth is generally thought to decrease by one third at approximately 6 percent oxygen (Greenwood 1968) and sometimes ceases at or below 5 percent oxygen (Stolzy 1974). Extrapolation from the root growth curves in our study (*fig. 3*) indicates that a 30 percent reduction in oak root growth did occur around 6 percent oxygen, but this varied somewhat among species. Four percent oxygen, and the corresponding ODR of $0.3 \mu\text{g cm}^{-2}\text{min}^{-1}$, was a definitive “threshold” below which root growth of the three species was significantly inhibited.

The oxygen concentration of wet, clayey soils has been measured at 2 percent (Letey and Stolzy 1967), and compacted soils underlying turf may have ODR values as low as $0.2 \mu\text{g cm}^{-2}\text{min}^{-1}$ (Costello and others 1991, MacDonald 1993). Our results suggest that oak root growth would be severely restricted under these conditions. Therefore it may be useful to monitor soil aeration in problematic sites (e.g., urban and developed landscapes, over-grazed lands) and if the oxygen concentration is below 4 percent, or the ODR is below $0.3 \mu\text{g cm}^{-2}\text{min}^{-1}$, improvements to soil drainage and porosity could be attempted.

Oxygen diffusion rate was directly related to soil oxygen concentration in our study because we used a coarse, well-drained sand that was uniformly porous. However, in many urban and field situations the soil is wet and compacted, and consequently, oxygen diffusion to the roots may be impeded. The result is that bulk soil oxygen concentration will probably not reflect accurately the oxygen available for root uptake, as would oxygen diffusion rate (MacDonald 1993). This is because in wet soils, oxygen diffusion is as little as 1/10,000 of that which occurs in dry soils (Letey and Stolzy 1967), and the so-called “water jacket” effect necessitates that oxygen concentrations be higher than that needed for root growth and respiration (Armstrong and Gaynard 1976, Crawford 1982, Letey and Stolzy 1967). Similarly, in compacted soils underlying turf the lack of pore space can diminish ODR despite there being a high bulk oxygen concentration (MacDonald 1993). A potential drawback to ODR is that in dry soils the lack of a continuous water film disrupts electrical conductivity between the electrodes, and erroneous and variable measurement of ODR may

result (Birkle and others 1964, Jacobs unpublished⁷). Therefore, ODR should be used to monitor soil aeration in wet and compacted soils, and the measurement of oxygen concentration with polarographic sensors should be reserved for dry, porous soils.

Interspecific differences were found in rooting morphology that might influence tolerance to hypoxia (and flooding) in blue, valley, and cork oak. Despite similar taproot growth rates at atmospheric oxygen concentrations, valley oak formed the most lateral roots, and blue oak formed the least. Our findings agree with reported accounts of root branching in blue and valley oak (Matsuda and McBride 1986). There is little information on the rooting morphology of cork oak, but the root system of coast live oak, a similar xerophytic, evergreen oak, is highly-branched and shallow (Cooper 1926).

In blue oak, the tendency to form a root system with few laterals might confer a degree of sensitivity to hypoxia because of the minimal root surface area available for oxygen uptake. The flooding sensitivity of some tree species, including *Eucalyptus* spp. and *Picea* spp., has been related to a sparsely branched root system (Coutts and Phillipson 1979, Kozłowski 1985). The converse would be true of valley oak, and to a lesser extent cork oak, because of their greater tendency to form lateral roots. A highly-branched root system is associated with plants adapted to flooded conditions (see Whitlow and Harris 1979) and is thought to be an adaptation of valley oak to wet soils and riparian habitats in California (Wolfe 1969).

Although valley oak is considered the most flood tolerant of the species studied, there is evidence suggesting that it is not tolerant of root hypoxia. Cooper (1926) describes mature valley oaks as having a dual root system with both deep tap and primary roots, and shallow, highly-branched lateral roots. He notes that the species is rarely found in nature in topographical positions where soil aeration is limiting. Callaway (1990) found that lateral rooting in blue and valley oak seedlings was highly dependent on soil moisture, and that blue oak adapted to changing moisture conditions better than valley oak. Because of the plasticity of its root system, blue oak might tolerate flooding and hypoxia better than valley oak.

Several of the plant responses believed to confer tolerance to flooding and hypoxia, including lateral and adventitious root proliferation, aerenchyma, and hypertrophied lenticel formation, depend upon ethylene translocation from anoxic roots to aerial shoots (Bradford and Yang 1981, Kawase 1981). These processes were inhibited in the low-oxygen treatments in our study because shoots were enclosed. However, in related experiments, blue, valley, and cork oak seedlings had their root systems flooded continuously for 5 days and all formed hypertrophied lenticels and aerenchyma (Jacobs 1991). The magnitude of the responses were similar among species, suggesting that all three have a similar capacity to respond to flooding as long as shoots remain aerated. Comparing seedling root systems in soils that are hypoxic, but not flooded, e.g. compacted sites, would help to assess the impact of aerated shoots on hypoxia tolerance, and better define how root morphology in blue, valley, and cork oak relates to hypoxia tolerance.

Acknowledgments

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⁷Unpublished data on file at The Morton Arboretum, Route 53, Lisle, IL 60532.

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