

The Effect of Low Oxygen Stress on *Phytophthora cinnamomi* Infection and Disease of Cork Oak Roots¹

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Abstract: The incidence and severity of *Phytophthora cinnamomi* Rands root disease was quantified in cork oak (*Quercus suber* L.) roots subjected to low oxygen (hypoxia) stress. Seedling root tips were inoculated with mycelial plugs of the fungus and incubated in ≤ 1 , 3-4, or 21 percent oxygen for 5 days. Ninety-four percent of roots became necrotic in the ≤ 1 percent oxygen treatment, compared to 60 and 46 percent in the 3-4 and 21 percent oxygen treatments, respectively. Root colonization and necrosis did not differ significantly between treatments. Fifty percent of inoculated roots remained asymptomatic in the atmospheric oxygen treatment, compared to 6 percent of roots in the near anoxic treatment. The asymptomatic roots were characterized by continued tip extension growth and lateral root formation above the inoculation point. Hypoxia increased the likelihood that roots became diseased, but not the severity of symptoms.

Root and crown rots caused by *Phytophthora* spp. are especially problematic in wet soils in which oxygen availability to roots may be restricted. Low oxygen (hypoxia) may directly injure roots by impairing root function (Drew and Lynch 1980, Levitt 1980), and may enhance disease by predisposing plants to infection by fungal pathogens (Shoenoweiss 1986, Stolzy and Fluhler 1984). A number of plant species including avocado (*Persea americana* Mill.), cherry (*Prunus serotina* Ehrh.), citrus (*Citrus sinensis* L.), eucalyptus (*Eucalyptus* sp.), rhododendron (*Rhododendron* sp.), and safflower (*Carthamus tinctorius* L.) exhibit increased susceptibility to *Phytophthora* sp. when their roots suffer from hypoxia directly, or indirectly as a result of flooding (Blaker and MacDonald 1981; Davison and Tay 1987; Heritage and Duniway 1985; Stolzy and others 1965, 1967; Wilcox and Mircetich 1985).

Several oak species, including cork oak (*Q. suber* L.), are susceptible to *Phytophthora* root and crown rot in California. Although more than one *Phytophthora* species is involved, *P. cinnamomi* Rands is the principal causal agent on cork oak (Mircetich and others 1977). The disease occurs where trees are grown in irrigated turf grass and compacted soils, and the symptoms include a blackened, sometimes "bleeding" inner periderm and cambium of the trunk, root necrosis, foliar chlorosis, and drying.

Costello and others (1991) reported that urban soils in which cork and coast live oak (*Q. agrifolia* Née) were declining had oxygen diffusion rates (ODR) below $0.3 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ in the upper soil profile. An ODR below 0.2 corresponds to very low soil oxygen concentrations in clayey soils, i.e. 0-2 percent (Letey and Stolzy 1967, Valoras and others 1964). Root growth in cork oak seedlings is reduced significantly at an oxygen level of 4 percent or less (Jacobs and others, these proceedings). In that study, 3-4 percent oxygen corresponded to an ODR of 0.3.

The purpose of this study was to determine whether hypoxia increases the susceptibility of cork oak roots to *P. cinnamomi* root rot.

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Materials and Methods

Experimental Procedures

We collected acorns for 3 years from several cork oaks on the campus of the University of California at Davis in October and November of 1987-1990. Seeds were soaked overnight, air-dried, and stored at 4-6 °C for at least 3 months. To initiate germination, batches of seeds were removed from cold storage and placed in trays containing vermiculite in the greenhouse. Acorns were dipped 4-5 minutes in a dilute solution of the broad-spectrum fungicide Imazalil (Jannsen Inc., Belgium) before germination, to suppress the growth of saprophytic fungi that might have contaminated seed coats.

A strain of *P. cinnamomi* that had been recovered from a diseased cork oak on the campus of the University of California at Davis was used as the source of inoculum for the study. We re-isolated the strain from diseased seedlings after each trial to sustain its virulence throughout the study. Stock cultures of the fungus were maintained on corn meal agar (Difco, Detroit, Mich.) at 6 °C, and in sterile water containing autoclaved hemp seeds at 25 °C.

Germinated acorns were transferred to mini-rhizotrons when radicles were approximately 50 mm long. The mini-rhizotrons were constructed from 24- by 32- by 2- cm plastic lids and had a removable surface to permit root viewing and inoculation. The mini-rhizotrons were kept at a 45° angle to encourage root growth along the removable surface. Before treatment, we placed the mini-rhizotrons containing seedlings in a growth chamber at 25 °C for 1 week to allow the seedlings to acclimate. At the end of 1 week the mini-rhizotrons were opened, and we selected unbranched, 1- to 2-mm diameter roots for inoculation. Inoculation was done by inserting each root tip into a 10 mm × 5 mm diameter glass tube containing an agar plug of *P. cinnamomi*. Tips of control roots were inserted into water agar plugs. We then placed the mini-rhizotrons into airtight chambers that were vented continuously with a gas mixture of known oxygen concentration between 0 and 21 percent, as described in Jacobs and others (these proceedings).

Seedlings were exposed to the oxygen treatments for 5 days after which time we opened the mini-rhizotrons and evaluated roots. Approximately 80 roots were inoculated for each of three oxygen treatments: ≤1, 3-4, and 21 percent oxygen. The oxygen treatments were repeated 14-18 times, and for statistical analyses each repetition was considered a replicate composed of several observations (inoculated roots). We assessed the variation between replications by determining soil moisture contents via water release curves, and continuously monitored soil oxygen levels inside the mini-rhizotrons.

Disease Assessment

Disease incidence was calculated as the percent of inoculated roots that exhibited necrosis originating from the point of inoculation, and from which the pathogen was successfully recovered onto the semi-selective medium PARP (Kannwisher and Mitchell 1978). Disease severity was assessed by measuring the length of root necrosis originating from the point of inoculation and the length of root tissue from which *P. cinnamomi* was re-isolated. We obtained the latter measurement by plating consecutive, 10-mm segments of each root onto PARP medium (fig. 1).

If inoculated roots continued growing through the inoculum plug but did not become necrotic, we confirmed the viability of the fungus by plating the inoculum plug onto PARP medium. If the inoculum was viable, roots were considered to have "escaped" disease. Inoculum viability was verified in asymptomatic roots from the 21 and ≤1 percent oxygen treatments only.



Figure 1—Evaluation of root colonization by *P. cinnamomi*. One-cm segments of an inoculated root were plated sequentially onto the semi-selective medium, PARP, beginning with the root tip (arrowhead). Photo shows a root colonized 60 mm.

Data Analysis

Disease incidence and severity were evaluated by analysis of variance and Tukey-Kramer and Duncan tests for means separation. Oxygen level was considered a fixed effect, and infection frequency, length of colonization, and necrosis were analyzed as continuous variables. We also summarized the disease incidence data as frequency counts and analyzed them using chi-square tests (SAS Institute 1991).

Results

Ninety-four percent of inoculated roots that were incubated at ≤ 1 percent oxygen became diseased, as evidenced by necrosis originating from the point of inoculation and successful pathogen recovery from root tissue. By contrast, only 60 and 46 percent of roots incubated at 3-4 percent and 21 percent oxygen, respectively, became diseased (fig. 2). A significantly higher ($P = 0.05$) mean frequency of disease occurred in the ≤ 1 percent oxygen treatment compared to the 3-4 and 21 percent treatments. The chi-square tests also indicated that disease frequency varied significantly ($P = 0.01$) between oxygen treatments.

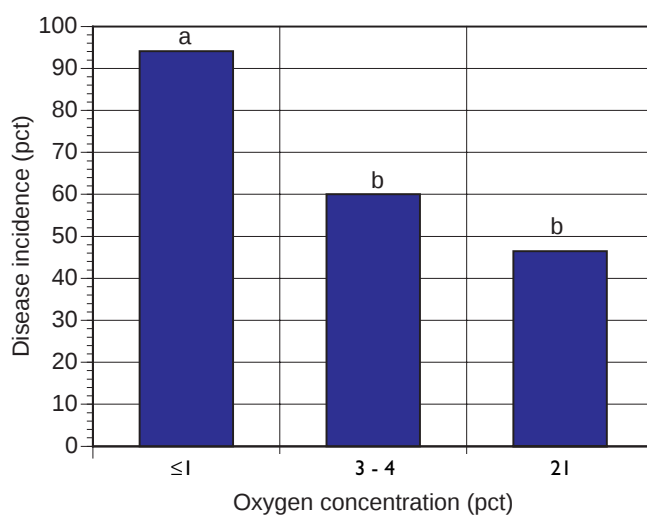
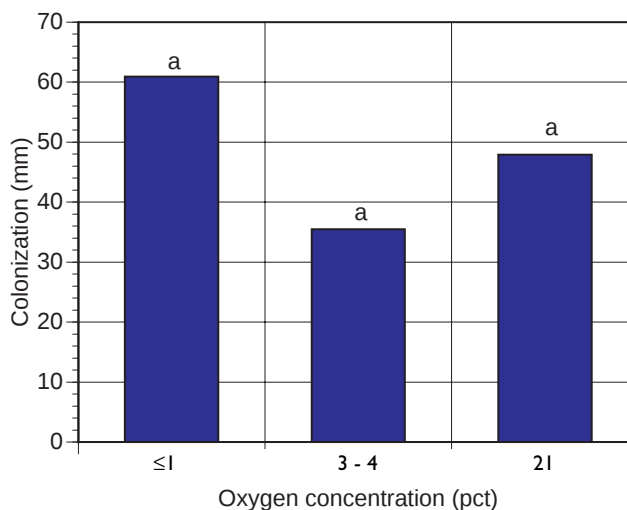


Figure 2—Disease incidence measured as the percent of inoculated roots that became diseased during a 5-day incubation at different oxygen concentrations. Lowercase letters above bars indicate significant differences between treatments at the $P = 0.01$ level. Approximately 80 inoculated roots (230 total) were used for each oxygen concentration.

Disease severity (i.e., the length of root colonized by *P. cinnamomi*) ranged from 30 to 60 mm and did not differ significantly ($P = 0.05$) between oxygen treatments (fig. 3). Root necrosis ranged from 15 to 45 mm from the point of inoculation. Colonization exceeded visible root necrosis by about 15 mm in all oxygen treatments, based upon recovery of the fungus from root sections.

Figure 3—Average length of root from which *P. cinnamomi* was re-isolated. Lowercase letters above bars indicate no significant differences ($P = 0.01$) between oxygen treatments.



We refer to necrotic roots as “diseased” rather than “infected” because some asymptomatic roots were superficially colonized by the fungus (Jacobs 1991). These asymptomatic or “escape” roots differed from diseased roots in other ways: the root tip continued growing through the inoculum plug, and lateral roots were produced above the inoculation point in escape roots (fig. 4). Forty-six percent of inoculated roots from the 21 percent oxygen treatment escaped disease compared to only 2 percent of roots from the ≤1 percent oxygen treatment. In 4 percent of inoculated but asymptomatic roots, *P. cinnamomi* was not recovered from the inoculum plug; consequently, the roots were not considered to be escapes.

Figure 4—Morphology of inoculated seedlings that became diseased (right) and remained asymptomatic (left) during incubation at 21 percent oxygen. Note root tip growth through inoculum plug, and development of lateral roots above the inoculation point on left seedling. Seedling on right shows typical disease response in which root tip ceased growing and no laterals were formed.



Discussion

Disease incidence (percent of inoculations that resulted in symptomatic roots) was greater in the hypoxic treatments than the atmospheric oxygen treatment, but a significant increase in disease occurred only in the ≤ 1 percent oxygen treatment. Total root growth of cork oak seedlings began declining at 6 percent oxygen, and a 50 percent reduction in root length occurred when oxygen levels fell below 3-4 percent (Jacobs and others, these proceedings). Thus, roots became more susceptible to *P. cinnamomi* only at oxygen levels below that which retards root growth.

The cause(s) of increased susceptibility of plants to *Phytophthora* root rot in hypoxic conditions is not well understood. Inhibited root growth and regeneration and the resulting lack of compensatory roots are thought to be primary reasons for increased susceptibility (Duniway 1983). Our results further indicate that in addition to poor root regeneration, hypoxia renders already inoculated roots more susceptible to disease than if the roots were in well-aerated conditions.

Disease severity (i.e., root colonization by *P. cinnamomi*) did not differ between treatments despite the increase in disease incidence in the hypoxic treatments. Also, when *P. cinnamomi* was grown in pure culture, colony diameters were equal after 5 days in ≤ 1 , 3-4 or 21 percent oxygen (Jacobs, unpublished⁶). Several *Phytophthora* spp. grow well at low oxygen tensions (Mitchell and Zentmyer 1971). The fact that growth of *P. cinnamomi* was apparently unaffected by hypoxia suggests that host factors were responsible for the greater susceptibility of hypoxic roots to disease. Low oxygen impairs root function, including the formation of physical barriers to pathogen ingress, i.e., suberin and lignin (Drew and Lynch 1980). Levels of barrier compounds (e.g. polymerized phenolics) differed between cork oak roots incubated at near-anoxic oxygen and atmospheric oxygen (Jacobs 1991).

It may be possible to limit *Phytophthora* root rot of cork oak in the field by maintaining soil conditions that support adequate root growth. Under our study conditions, soil oxygen levels above 3-4 percent permitted adequate root growth. However, oxygen diffusion in the mini-rhizotrons was not impeded because of the uniform texture and good drainage of the soil medium. Under field conditions, aerated shoots may help to oxygenate the rhizosphere and allow plants to tolerate root hypoxia longer (Crawford 1982), so lower oxygen levels may suffice. Alternatively, if soils are poorly drained, compacted, or otherwise present impediments to oxygen diffusion, 3-4 percent oxygen may not be adequate to support root growth and prevent predisposition to *P. cinnamomi*. MacDonald and others (1993) note that in soils where oxygen diffusion is limited, the ODR is a better indicator of soil aeration than oxygen concentration. An ODR of 0.3 and higher corresponded to 3-4 percent oxygen in the mini-rhizotrons, and this could be considered the threshold diffusion rate needed to minimize root rot.

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⁶Unpublished data, K.A. Jacobs.

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