

ROOT AERATION FUNCTION OF BALDCYPRESS KNEES (*TAXODIUM DISTICHUM*)

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Premise of research. The ability of cypress knees to allow easy diffusion of O₂ from the atmosphere to the attached submerged root is supported by a number of past ecomorphological observations yet not by any experimental evidence, despite several past in situ studies that examined this phenomenon.

Methodology. O₂ concentration of air extracted from submerged roots was compared when attached knees were above water and when they were submerged.

Pivotal results. When roots were submerged, the root internal air O₂ concentration was much higher when the knee was above water than when both the knee and root were submerged. This result unambiguously supports past assumptions that cypress knees do indeed function as pneumatophores in supplying submerged roots with oxygen.

Conclusions. Using a simple but unique experimental approach, the results of this study show that cypress knees function as pneumatophores.

Keywords: aeration, anaerobic soil, baldcypress, flooding, gas diffusion, pneumatophores, respiration, roots, swamps, *Taxodium distichum*.

Introduction

In swamps and lake edges in southeastern North America, the gymnosperm baldcypress (*Taxodium distichum*) often produces curious and large (occasionally well over several meters tall; Shaler 1890; Betts 1945; Dennis 1988; Kernell and Levy 1990) woody projections from its attached roots, referred to as cypress knees (Shaler 1887; Wells 1932; Kramer et al. 1952; Brown 1984; Ewel and Odum 1984; Dennis 1988). These structures are often produced in abundance (hundreds to thousands) around the trunk of the tree. The possible function(s) of cypress knees has elicited a number of speculative scenarios in the past century. Unquestionably, the leading hypothetical function has long been that of a root pneumatophore (Dickeson and Brown 1848; Shaler 1887; Wilson 1889; Wells 1932; Dennis 1988), as found in many mangrove species growing along pantropical coastlines (Scholander et al. 1955; Curran 1985; Kitaya et al. 2002). Pneumatophores provide an easy route for O₂ diffusion down to the attached root (and CO₂ in the reverse direction) that is growing submerged underwater in anaerobic conditions.

The circumstantial evidence for a root oxygenation function of cypress knees is quite convincing. For example, knees form in abundance only on trees whose roots are sporadically flooded (Kramer et al. 1952; Hook and Scholtens 1978; Kernell and

Levy 1990); they are less abundant or absent on trees growing in dry soils (Shaler 1887; Brown and Montz 1986; but see Brown 1984; Brown and Montz 1986). Curiously, trees growing where their roots are constantly flooded also lack many knees (Kurz and Demaree 1934; Kernell and Levy 1990; Yamamoto 1992). The latter intriguing observation has not been experimentally investigated. The wood of cypress knees is very light and porous (Dickeson and Brown 1848; Mattoon 1915; Kramer et al. 1952; Hook and Scholtens 1978), reminiscent of aerenchyma tissue, which would provide little resistance to gas diffusion. Furthermore, the apical tips of most knees lack thick bark (Shaler 1887; Brown 1984), which should also reduce diffusional resistance of gases into and out of the knee. Finally, the height of cypress knees usually exceeds the maximal depth of water during periods of flooding (Shaler 1887; Mattoon 1915; Kurz and Demaree 1934; Kramer et al. 1952; Kernell and Levy 1990; but see Brown 1984; Brown and Montz 1986).

Despite the abundance of observational evidence that cypress knees might act as root pneumatophores, only two studies have experimentally examined this hypothetical role, and both studies found no evidence that these structures facilitate the diffusion of oxygen from the air to the submerged attached roots (Kramer et al. 1952; Brown et al. 1984). Kramer et al. (1952) compared respiratory O₂ uptake from knees normally attached to roots with knees that had been severed from their roots in situ in a North Carolina swamp. They found that attached knees did not absorb greater amounts of O₂, as expected if they were pneumatophores. Furthermore, Brown et al. (1984) reported that rates of respiratory CO₂ release of

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knees were no greater than and often equal to those of the woody trunk of the cypress tree. In contrast, Cowles (1975) reported the opposite; respiration rates of knees were much higher than those of the woody tree stem (trunk). Furthermore, Cowles found that knee CO_2 release rates were considerably lower when trees had a full canopy of leaves relative to rates when all leaves had been abscised, implying that root respiratory gases diffused via both the knees and the leaves (where it is likely that such CO_2 was refixed by photosynthesis) when both diffusion pathways were available.

Partly as a result of the latter, several hypotheses unrelated to a pneumatophore function have been forwarded in the past several decades in an attempt to ascribe other potential adaptive benefits of knees to cypress trees that clearly invest a considerable amount of resources in such structures. These include enhanced mechanical stability of these trees in unstable hydric soils (Lamborn 1890; Brown and Montz 1986), access to nutrients in dead cypress stumps (Kummer et al. 1991), litter capture that might eventually increase nutrient availability to the roots (Lamborn 1890; Brown 1984; Kummer et al. 1991), and carbohydrate storage (Brown 1984; Brown and Montz 1986).

Given the still-puzzling nature of the possible adaptive nature of these fascinating root structures coupled with the relative dearth of experimental studies on their possible function, the objective of this study was to revisit, using a heretofore-untried simple yet powerful experimental approach, the possible pneumatophore-like function of cypress knees.

Material and Methods

Plants and Collection Site

Knees with their attached roots were collected from cypress trees (*Taxodium distichum* [L.] Richard; Cupressaceae) growing in an intermittently flooded creekbed at the Little River National Wildlife Refuge (lat. 33°57'44"N, long. 94°37'36"W) on the outskirts of Broken Bow, Oklahoma. Typical environmental conditions at the site include 33°C maximum monthly high temperature, -2°C minimum monthly low temperature, and 11-cm maximum monthly precipitation. Knees were collected from April to December 2012.

On each of 6 collection dates, 2 cypress knees were collected, each within 3 m of the trunk of each of 2 trees that were at least 5 m apart (oxygen concentrations in air extracted from the roots were similar on all collection dates except for knees collected in November; O_2 concentrations in the air of these knees were, for inexplicable reasons, substantially higher than the others). Thus, the sample size of the study was 12 knees, 1 from each of 12 different trees, although definitive tests (digging and following roots or genetic analyses) were not performed to ensure the latter. Knees were removed by cutting (with a shovel and large pruning shears) the attached main root (typically 20–30 cm below the soil surface) approximately 30–40 cm on either side of the knee, as well as all other smaller roots. The knee and attached main root were then placed in a plastic bag in which several paper or cloth towels and liters of water were added, after which they were transported by automobile (approximately 7–8 h) to the University of Kansas, whereupon they remained wet in a greenhouse 1.5 d until use in the laboratory.

Experimental Approach

Once in the laboratory, 2 mL of air was extracted from the root using a syringe for O_2 analysis (see below). Then the root was placed into a glass aquarium (39-L volume) with the longitudinal root along the bottom and the knee projecting vertically. Deionized degassed water was then added to the aquarium, submerging the entire root (including its cut ends), but only to the bottom of the knee. The water was degassed in a desiccator (lacking desiccant) under constant vacuum while vigorously stirring for 1 h. After 30 min of root submergence, another 2-mL air sample was extracted from the root for O_2 analysis. Then additional deionized degassed water was added to the aquarium to entirely submerge both the root and the knee. Following 30 min of submergence, another root air sample was removed from the root for O_2 analysis. At this point, water was removed from the aquarium such that the root remained submerged while the entire knee was exposed to the air. The aquarium opening was partially covered to minimize evaporation yet allow easy gas diffusion. After 24 h, a final air sample was removed from the root for O_2 analysis. Each root/knee structure was still alive at this time, as all exhibited the appearance of new roots.

All root air samples were extracted with a syringe (27-gauge [0.4-mm-diameter] needle; 3.2 cm long) while the root remained underwater. Although piercing the woody root with the syringe needle was difficult, a ready supply of fresh needles guaranteed eventual success. The syringe needle was inserted approximately 1–2 cm into the center of the 3–4-cm-diameter root and approximately 5 cm from the base of the connected knee. Once the root was pierced, extraction of the air samples was not particularly difficult, so it is highly unlikely that enough suction was generated in the root to pull atmospheric gas into and through the exposed knee. Furthermore, when the knee was submerged, air (without water) was still easily extracted from the root. The needle remained in the same extraction hole of the root throughout the experiment.

Measurement of O_2 Concentration

Root air samples (2 mL) were injected into 4 mL of deionized water in a temperature-controlled (25°C) glass vessel with a YSI (Yellow Springs Instruments, Yellow Springs, OH) model 5331 O_2 probe inserted into the constantly stirred water. The stirred water was not exposed to room air by sealing with an O-ring around the electrode and a rubber bung at the injection port. The increase in O_2 concentration due to the injected air was measured by a YSI model 5300 biological O_2 monitor and recorded by computer, and once a stable reading was obtained after the injection, the total O_2 content of the injected air was calculated by integration of the curve generated by the injection. Following each injection of root air, the O_2 concentration of the vessel water was reduced by bubbling N_2 gas into the water until a lower O_2 concentration was obtained. The latter was necessary to avoid oxygen saturation of the vessel water.

Statistical Analysis

Because the data were not normally distributed (Sokal and Rohlf 1981), all mean root O_2 concentrations were compared

using the nonparametric Kruskal-Wallis one-way ANOVA on ranks. Individual pairs of means were then compared with the Tukey pairwise multiple comparison test. Data were analyzed using the SigmaPlot 12.5 (Systat, San Jose, CA) statistical software package.

Results and Discussion

Oxygen concentrations of the air extracted from the cypress tree roots to which knees were attached were very low (fig. 1) compared with atmospheric O_2 concentrations as well as with values obtained in a similar manner for mangrove roots by Scholander et al. (1955). A likely reason for such low concentrations is the result of the analysis technique used in this study, in which the O_2 concentration of the root air sample was measured with an aqueous O_2 electrode once the air was dissolved in water. In contrast, Scholander et al. (1955) measured the O_2 concentration of root air samples directly as a gas concentration. It is possible that the oxygen in the samples analyzed in water was not fully solubilized and as a result was not detectable. It is also possible that gas resistances in the wood of the two different trees differed dramatically, although no such comparative information is available. Nevertheless, whenever the cypress knee was above water, the O_2 concentration in the attached knee underwater was much higher relative to the knee O_2 concentration when the knee was submerged underwater. This provides unequivocally strong evidence that cypress knees do indeed allow the diffusion of oxygen to the submerged attached root. These results run counter to the results of two previous investigations of the potential pneumatophore-like function of cypress knees. Different experimental approaches were employed in all three studies. The approach used here was simple and similar to that previously used to certify the O_2 -

diffusional capacity of mangrove pneumatophores (Scholander et al. 1955). Kramer et al. (1952) measured O_2 uptake by attached and detached knees in situ in a North Carolina swamp. They found low rates of O_2 uptake by the knees but, more importantly, no additional O_2 uptake by attached knees relative to detached knees. The results of their approach of measuring the uptake of O_2 by intact and severed knees in situ are difficult to interpret because the measurements were likely prone to complications from respiratory O_2 uptake by the (numerous) organisms living on the surface of the knee. Also, measurement of CO_2 release by the knees in situ by Brown et al. (1984) was also prone to the same problem of interference from respiratory CO_2 release by these same living tissues and organisms. Furthermore, Brown et al. (1984) found that intact woody knees released the same amount of or even less CO_2 than the woody trunk of the same tree, although Cowles (1975) found the opposite. Although it may be argued that the ex situ approach used here might have yielded erroneous findings as a result of the excision and removal of the knee and root from their environment, such an argument is difficult to justify given the simplicity of the experimental approach used, its avoidance of contaminating gas exchange by other organisms, and the fact that the root/knees were alive during the entire experiment (see above). It is possible that root gases may have diffused from the cut ends of the root into the surrounding water, yet there is little reason to assume that such diffusional loss would explain the results obtained in this study for two reasons: (1) such losses would be very low due to the extremely slow rate of gas diffusion in water and (2) there is little reason to assume that more O_2 might diffuse out of the root when the knee was submerged relative to such diffusion when the knee was above water (which would explain the results obtained here). In fact, if such root-to-water O_2 diffusion occurred, this would only strengthen the results shown above.

Of course, it is possible, if not likely, that cypress knees evolved in response to a number of selective factors. Nonetheless, the results of this study provide strong evidence that one of those factors was aeration of roots growing in constantly or intermittently anaerobic environments. This conclusion is further supported by the ecomorphological observations described above.

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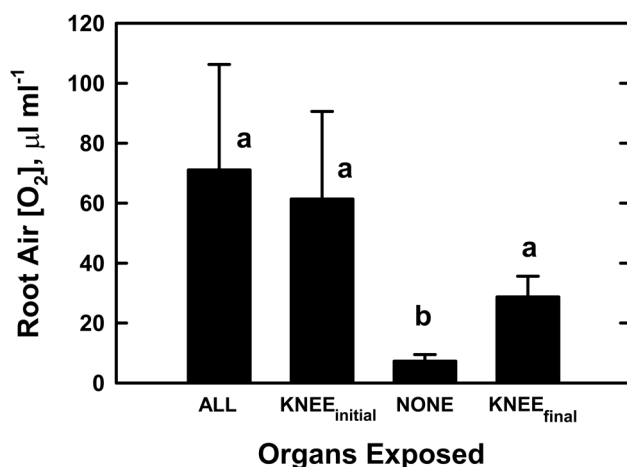


Fig. 1 Mean (extending, capped lines are SE; $N = 12$) oxygen concentration of air ($\mu L O_2/mL$ air) extracted from baldcypress (*Taxodium distichum*) roots with knees attached when both the root and the knee are exposed to air (ALL), when only the knee is exposed (KNEE), and when no organs are exposed to the air (NONE). Measurements for KNEE_{initial} and KNEE_{final} were made at the beginning and end of the experiment, respectively (see "Material and Methods"). Means with shared letters are not significantly different ($P \leq 0.05$).

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