METABOLIC ACTIVITY OF FLOAT-TOBACCO TRANSPLANT ROOTS[1]

L. V. Caruso, R. C. Pearce, F. F. Fannin and L. P. Bush[2]

The nutrient solution in the tobacco-transplant float system is not usually circulated or aerated, and may thus become stagnant and deficient in oxygen. In this system, plants produce media, water and spiral roots. The metabolic activity of these root types during seedling development was investigated in a standard float system and under a low oxygen (near-anaerobic) regime. Oxygen consumption by tobacco seedling root tips was measured using a microsensor. The effects of near-anaerobic conditions on seedling development in a float system reduced dry matter accumulation of both media and water roots. Media roots and spiral roots had a similar metabolic activity rate, and a much greater rate of oxygen uptake than did water roots. The near-anaerobic condition in the float system was less deleterious to media roots than water roots.

ADDITIONAL KEY WORDS: anaerobic environment, oxygen uptake, root protein analysis

INTRODUCTION

Most tobacco, *Nicotiana tabacum*, transplants are produced in greenhouses using a float transplant-production system. Three distinct kinds of roots are produced by tobacco seedlings in this system. "Media roots" refer to the roots that grow in the soilless medium within a float tray cell. They have an appearance similar to roots produced by field-bed grown transplants. "Water roots" are the roots that grow out of the soilless medium, through the bottom of the tray and into the water solution. They differ in appearance from media roots; they tend to be fragile and are often torn from the plant as it is removed from the tray during transplanting (5). "Spiral" or "aerial roots", do not penetrate the surface of the growing medium, and are a common problem observed in direct-seeded float systems. The cause of spiral roots is not completely understood; however, it may to be related to inadequate medium aeration (too little air or too much water), which creates a stress environment for the young plant root. Seedlings with spiral roots often survive, but they usually lag behind other plants in development resulting in few usable transplants.

Research has established a link between media compaction and the subsequent increase in water-holding capacity with the occurrence of spiral roots (7,21). Media compaction destroys macroporosity and air is displaced as the resulting small pores are filled with water once trays are floated in the water tank. Over compaction of media creates a stressful environment for seedling root growth and development, thereby producing a condition that may lead to spiral root formation. Roots of common everlasting (*Gnaphalium polycephalum)*, when grown in poorly drained soils (anaerobiosis), often exhibit negative geotropism, whereas roots in well-drained soils exhibit positive geotropism (11).

Tobacco is particularly susceptible to injury caused by anaerobic conditions (19). Harris and van Bavel (12) investigated the growth of tobacco root systems and concluded that excessive CO_2 in the root zone was the major contributing factor to plant injury. Aerobic respiration in plant roots is necessary for growth, mineral uptake, and indirectly, may affect water uptake (18). Previous studies have demonstrated that low-oxygen partial pressures retard root growth by inhibiting respiration, reducing root branch formation, and even initiating root death (15,17,24). As a consequence, the effect of limiting oxygen supply to plants is a reduction in leaf area and chlorophyll content of the leaf, which subsequently reduces plant growth (3,25). Excess water in the root environment of plants can be injurious because it blocks the transfer of O_2 between the soil and the atmosphere (9). Since air exchange into and out of the stagnant water surface of the tobacco float system occurs very slowly, water roots generally develop in an oxygen deprived (anaerobic) environment, which affects their appearence and function. Tobacco seedlings growing in small cells have been confined in such a way that media roots also may be under restricted oxygen supply because of the slow transfer of dissolved O_2 into the water-filled pore space of the soilless medium.

Many investigators have put considerable efforts into establishing critical oxygen concentrations below which respiration is restricted. Amoore (2) observed a hyperbolic relationship between respiration rate of pea root tips and oxygen concentration, thus concluding that respiration of root tips was limited by slow diffusion of oxygen through the tissue at an oxygen concentration of less than 20%. The composition of the gas mixture in contact with the roots of tobacco plants seriously affects physiological functions, particularly when CO_2 exceeded O_2 in the mixture (13). Harris and van Bavel also investigated the nutrient uptake of tobacco plants and concluded that an O_2 partial pressure lower than 10% reduced nutrient solution uptake (14). Anaerobiosis decreased water uptake by tobacco roots and affected many plant physiological functions (27). These studies clearly show that if the concentration of oxygen in the surrounding atmosphere or soil solution of the root falls below a critical threshold, the O_2 consumption rate in respiration will decline.

Roots grown under anaerobic conditions contain much more space between tissues than normal roots. In normal roots, an uninterrupted epidermis surrounds the compact cortex and the stele (conducting tissue) occupies 25% of the

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[2] Graduate Student, Associate Extension Professor, Research Analyst, and Professor; Agronomy Dept., University of Kentucky, Lexington, Kentucky 40546-0091.
Corresponding author email: lpbush@uky.edu.

root diameter. Gas-filled intercellular space may comprise nearly 90% of the axis volume of anaerobic grown roots (10). Hypothetically, O_2 should flow through the less resistant pathways (gas-filled spaces) without penetrating significantly into the cells. Plants that grow naturally in flooded soils have a well-developed and extensive internal gas space system called aerenchyma, allowing diffusion of O_2 to the roots (16). According to Willey (27), tobacco roots are sensitive to aeration, and lack the central air cavities in the stem that could transport gas between aerial plant parts and the roots.

Most important to efficient field establishment of tobacco transplants is an understanding of how water roots and media roots function at transplanting. The objective of this study was to measure the metabolic activity of different roots found on float tobacco plants by measuring oxygen consumption of these roots.

MATERIALS AND METHODS

Plant culture

Tobacco plants were grown in the greenhouse of the College of Agriculture, University of Kentucky, Lexington, KY. Pelletized burley tobacco seeds, cv. NC 129, were sown in trays and floated on tanks of nutrient solution. The growth medium was a commercial (Southern States brand), non-fertilized, peat-based medium that contained vermiculite and perlite. Polystyrene trays (67.5 x 34.5 x 7.5 cm), each containing 200 cells, were used. Tray cells were open-ended, with a volume of 27 cm^3 in an inverted pyramid shape. Trays were manually filled with premoistened media and then pelletized seeds were direct-seeded with a vacuum seeder into a dibble (round shape with 12.5 mm depth). Care was taken during seeding to ensure that one seed was placed in each cell and that the seed was at the bottom of the dibble. Temperature at the tray level was maintained at 20 to 24°C. The water tank was fertilized with 100 mg L^{-1} nitrogen from 20-10-20 water soluble fertilizer.

To obtain a reduced O_2 concentration in the tobacco transplant root system environment, the nutrient solution nearly 90% of the axis volume of anaerobic grown roots was purged continuously with nitrogen gas. Flexible plastic tubing (3.175 mm id) was placed in the water in a serpentine design and fixed at the bottom of the float tank. Perforations in the plastic tubing were made using 20.5 gauge hypodermic needles equidistant from each other. The flow rate of the nitrogen was adjusted to maintain dissolved oxygen levels in the float water at about 50% of the control tanks.

Oxygen partial pressure in the water was measured in samples taken periodically from different locations in the float bed. Water samples were transferred to a glass vial, sealed with a plastic cap and taken to the laboratory for analysis. The pH of the float tank nutrient solution was monitored with pH indicator paper. The nitrogen treatment was imposed for five weeks from seeding until transplant time. Plants were randomly selected from each treated tank for further laboratory analysis and measurements.

Oxygen Partial Pressure Measurements

Oxygen partial pressure (pO_2) in water samples and oxygen consumption by the tobacco seedling roots were measured using a Diamond General Microsensor II using a Clark style oxygen electrode microsensor (Cat. # 731, 46 mm length). Since oxygen diffusion rates vary with temperature, microsensor calibration was carried out at the same temperature (29°C) as that of the experimental material. Two-point calibration was adequate for the measurements made. The points were a 21% pO_2 (ambient) obtained with a solution of 0.9% sodium chloride, and 0% (pO_2) obtained with a 2% solution of sodium sulfite.

Root respiration was measured as relative oxygen uptake on a root tip basis. Tobacco seedlings were taken to the laboratory and the soilless medium was gently washed through a set of sieves. After being cleaned, the root samples were separated from the medium and determined as media roots and water roots. The apical 0.5 cm of the root system was removed with a stainless steel razor blade. Prior to oxygen consumption analysis, root tips were collected and placed into 2.0 ml screw top plastic vials with septa

Figure 1. **Time-course of the relative oxygen consumption by float tobacco roots. Each sample contains 10 roots (0.5 cm each). Root tips = apical portion of the root.**

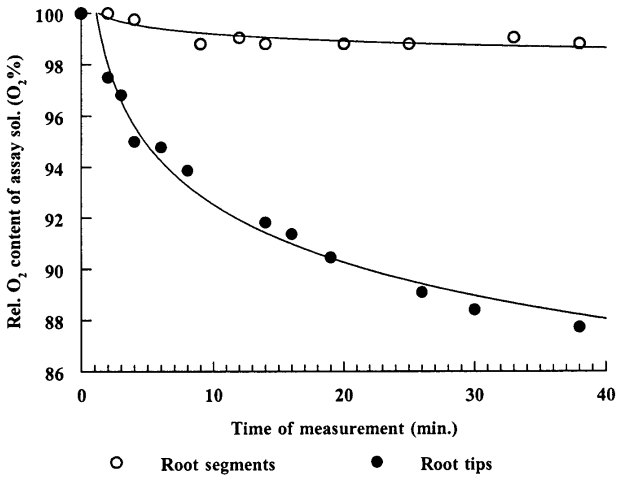
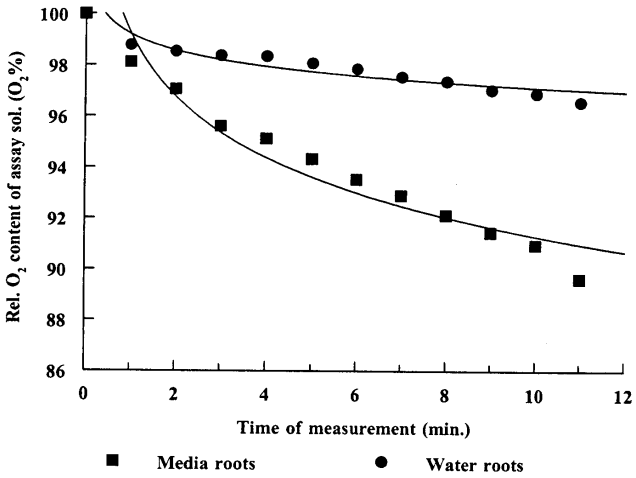


Figure 2. **Time-course of the relative oxygen consumption by float tobacco roots at transplanting time. Data points are mean values of three measurements (replications); each sample contained 10 root tips (0.5 cm each).**



inserted into the caps. The vial was filled with oxygenated water, equilibrated to air at standard atmospheric pressure, and the samples kept at 29°C in a water bath. Each sample consisted of 10 root tips.

Oxygen consumption measurements were obtained by inserting the microelectrode into a vial while stirring. Oxygen uptake was calculated from the slope of oxygen depletion over time. A constant slope was typically obtained within 2 to 3 min of sealing the glass container. After O_2 measurements were completed, all root tip samples were frozen for later protein analysis. Dry weight of duplicate samples was also measured by drying in a forced-air oven at 65°C. All data were subjected to analysis of variance to determine differences between treatment means at $\alpha = 0.05$.

Root Protein Analysis

Soluble proteins of root tip samples were determined by a micro assay following the Bradford method (4). Samples were thawed at room temperature a few hours prior to analysis. Each root sample of 10 root tips was suspended in 1 ml of 0.1 M NaOH and heated for 30 min in a boiling water bath. Extractions were centrifuged at 1500 g for 5 min, the supernatant was saved, and the pellet was reextracted. Three extractions were necessary to obtain at least 90% of the total soluble protein in the roots. One ml of the combined supernatant was transferred to another tube and neutralized with 19 µl of 6 N HCl. One ml of Coomassie Brilliant Blue Dye Reagent (Bio-Rad protein assay dye reagent concentrate diluted with 4 volumes of distilled H_20) was added and immediately mixed vigorously. The standard curve for protein determination was prepared using bovine serum albumin.

RESULTS AND DISCUSSION

Oxygen uptake by roots in standard float system

Two weeks after seeding, 15% pO_2 was detected in the float water. Short-term measurement of oxygen consumption by media roots of tobacco transplants showed that the apical portion of the root consumed oxygen at a much higher rate than portions of the root at a greater distance from the root tip (Figure 1). According to Wilkins (26), the gradient of oxygen consumption (respiration) by roots decreases with distance from the root cap. This observation is logical, because more rapid metabolism occurs near the root tip where meristematic activity is localized.

Media roots consumed more oxygen than did water roots (Figure 2). The relative uptake of O_2 by both media and water roots tips, when exposed to a well-oxygenated solution, exhibited a nearly linear depletion of pO_2. These two different kinds of roots grow and develop under distinctly different environmental conditions as previously discussed. Growth of water roots may be restricted under conditions of poor aeration, since the rapidly metabolizing zone of cell division near the root tip is the first to be affected by suboptimum aeration conditions. In the float system, the presence of a stagnated layer of water in permanent contact with the surfaces of water roots may serve to impede oxygen diffusion to root cells. This suggests that a significant reduction in relative oxygen uptake could result from the death of a minor portion (tips) of the root system. The lower oxygen consumption by the water roots may also indicate that roots represent an additional sink for that portion of the tobacco seedlings having normal metabolic activities.

Since spiral and media roots contained approximately the same amounts of protein (data not shown), oxygen consumption was evaluated based on the amount of protein present in these roots. Spiral roots had a slightly higher oxygen uptake than media roots (Figure 3). This observation was unexpected because spiral root tips are injured during spiral formation that occurs approximately 2 weeks after seeding. However, the root tips may have suffered only a superficial damage as the spiral pattern was formed. Injury often decreases overall respiration, but may O_2 consumption may also have been stimulated at the site of damage as part of wounding response (23).

From these results, it may be concluded that tips of

Figure 3. **Time-course of the relative oxygen consumption by float-tobacco spiral roots. Data points are mean values of three measurements (replications); each sample contained 10 root tips (0.5 cm each).**

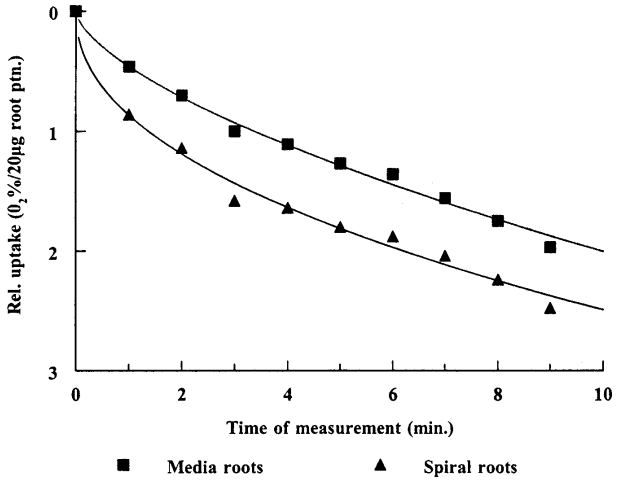
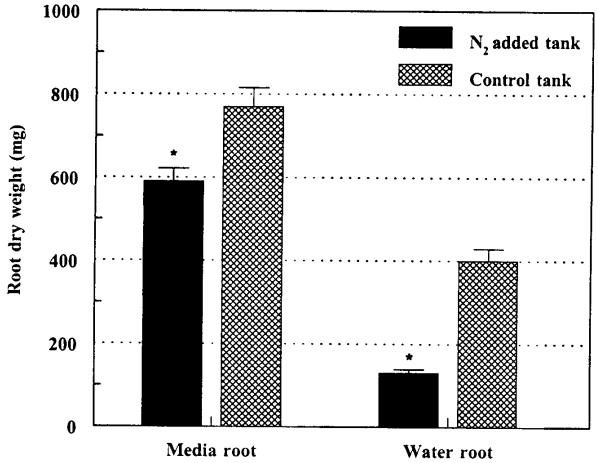


Figure 4. **Dry matter accumulation by tobacco roots in a N_2-treated and control-water float tank. N_2 tank= water float tank treated with nitrogen-gas; Control tank= regular water float tank; error bars represent the standard error of the mean; (*) = significant at P≤ 0.05.**



media roots had significantly greater respiration rate and consequently a higher metabolic activity than tips of water roots. In the tobacco float system, spiral root formation has been associated with excess moisture (water logging), which possibly reduces the amount of O_2 in the soilless media. However, based on the relative O_2 uptake, spiral roots seem more likely to behave as media roots. It would suggest that the cause of this physiological disorder is not exclusively related with deficiency of O_2 in the float system, but a combination of O_2 deficiency and other factors present during the stages of root development.

Root function in induced anaerobic conditions

Twenty four hours after beginning the addition of nitrogen gas to the float water, treated water had a reduction in O_2 concentration of approximately 60% (Table 1). Levels in the treated float water were kept lower than 10% pO_2 until the end of the experiment. The pO_2 in the control float water did not change significantly over the experimental time period. After four weeks, the average was 17% pO_2. Values of pH changed according to measurements in both treated and untreated float systems. Even with a possible increase of CO_2 evolution due to root respiration and nutrient depletion in the water solution, pH values were around 6.5 to 6.0 for both treated and control float tanks. Optimum pH for root respiration during early root elongation is 6.5, while in late root growth, the optimum pH is 7.5 (6). Thus, pH values in the float trays were near optimum for root respiration.

During the first 4 weeks, neither the N_2-treated nor control tobacco seedlings produced water roots. However, 4 weeks after seeding, the number of water roots produced by the control seedlings increased rapidly; N_2-treated seedlings had few water roots. Lowering the oxygen content in the float water clearly affected root development of tobacco transplants. At the end of 6 weeks, the seedlings grown in the nitrogen treated float tanks had significantly less root dry weight accumulation (730 mg vs. 1160 mg $plant^{-1}$, respectively) (Figure 4) than control plants. Most of this difference was due to differences in the water roots. The greater dry weight of the control roots may also be the result of the greater supply of oxygen available to their apical meristems.

The near-anaerobic conditions in the N_2-treated float tanks reduced the shoot growth compared with control plants (visual observation). Tobacco plants had a much smaller shoot with reduced leaf sizes. Nagao (20) observed that translocation of ^{14}C in roots of tobacco seedlings was greater in an environment containing a high O_2 concentration. Leaf weight of tobacco plants was positively correlated with root activity (respiration) during the stage of maximum growth. In addition, Nagao (19) reported that leaf weight was correlated with root weight during maximum growth and flowering. Smit et al. (25) also concluded that root hypoxia reduced leaf growth. Undoubtedly, the growth of tobacco seedlings can be negatively influenced by near-aerobic conditions in the float system.

Oxygen consumption, based on the total amount of soluble protein (Figure 5) in the media and water root tips, further illustrates differences between the N_2-treated and control tobacco transplants (Figure 6). Roots from the N_2 treatment had less soluble protein compared to the control roots. Short-term oxygen consumption in a well-oxygenated solution was appreciably lower for water roots from the N_2-treated tanks compared to water roots from a conventional float tank. Clearly the water roots were damaged by the low pO_2 in the float tank and even after being exposed to higher oxygen concentration in the assay vial did not increase their short-term metabolic activity. Media roots from both N_2-treated and control float tanks consumed more oxygen than their respective water roots. However, a compensation factor was observed after 4 min in the assay vial. This suggests that media roots, once exposed to a well-aerated solution, are able to provide oxygen to all meristematic cells concentrated in the apical portion of the root. Media roots may have been less affected, mainly because these roots have access to a significant percentage of pore spaces having direct access to atmospheric O_2 in the substrate.

Water roots have a low respiratory capacity in a conventional float system. In addition, evaluation of roots in a float system treated with N_2-gas suggests that oxygen consumption of water roots declined at a much higher rate than in the control. This general decline in oxygen uptake indicates that part of the water root system was injured by the N_2 treatment. Williamson and Splinter (28) examined the gaseous composition of the tobacco root environment during root development and growth and concluded that water absorption is affected by changes in cell and cell membrane characteristics that result from oxygen deficiency. Further, our observations of possible root injuries made when plants were removed from the float tanks revealed that approximately 2 to 3 mm of most root tips were slightly brownish. This damage could have been a significant factor in the reduced respiration rates measured

Figure 5. **Total soluble proteins extracted from tobacco root tips grown in a N_2-treated and control water float tanks. N_2 tank= water float tank treated with nitrogen-gas; Control tank= regular water float tank; error bars represent the standard error of the test; (*) = significant at P≤ 0.005.**

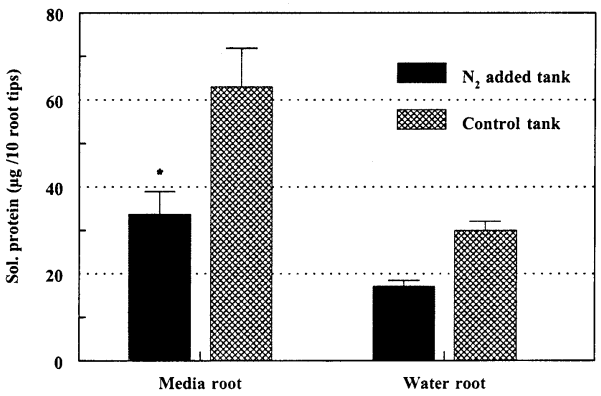


Table 1. **Oxygen levels in float tanks with and without nitrogen gas application.**

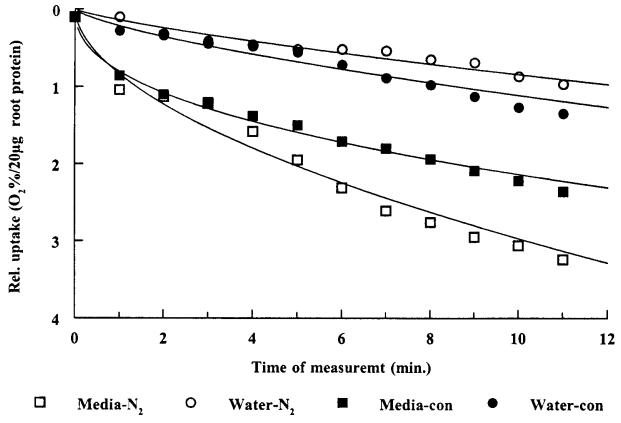
	Partial Pressure of Oxygen (%)				
Treatments	Day 1[a]	Day 4	Day 7	Day14	Day30
Nitrogen tank	7.7	6.3	7.3	8.7	8.8
Control tank	19.7	15.0	11.5	15.8	17.0

[a] Values represent means of five samples on each day.

by the water roots compared with media roots in both treated and control water tanks (Figure 6). Berry (1944), cited by Amoore (1), demonstrated that there are no intercellular air spaces in the meristematic region of root tips. Therefore respiration must be naturally limited by the slow diffusion of oxygen through the liquid phase. Ohmura and Howell (22) reported a repressive effect of water on the respiration of different plant tissues. Their evidence suggested that inhibition was due to the presence of water in the normally air-filled intercellular spaces of the tissues, thereby dramatically slowing the entrance of oxygen to the cells. In addition to this physical aspect, when the cells of water roots start to become anaerobic, their glycolysis and fermentation pathways are activated. The fermentation process is not totally injurious because the end products (mostly ethanol) diffuse across the plasma membrane to the surrounding medium. However, the net yield of ATP in fermentation is only 2 mol per mol of hexose compared with 36 mol per mol in fully aerobic respiration. This process quickly reduces the energy status of cells to a very low level (8). Since growth of roots and uptake of water and nutrients are dependent upon energy obtained by respiration, it may be assumed that reduction in respiration activity is the first step in the growth limiting effects of insufficient aeration.

In summary, the combined results of this study provided important information on the growth and relative activity of both media and water roots of float tobacco transplants. Based on this data, one may conclude that water roots had significantly less respiration and metabolic activity than media roots. The lowered pO_2 condition normally created in the float system, mostly due to the presence of a large body of stagnate water, had little effect on the growth of media roots, which yielded a compensatory response when placed in a well-oxygenated system for assay. Therefore, growth and development of media roots should be stimulated to the greatest extent possible in tobacco transplants produced in float system. Water roots and spiral roots did not seem to contribute significantly to transplant development.

Figure 6. **Time-course of the relative oxygen consumption by tobacco root tips grown in a N_2-treated and control water float tank. Media-N_2 = media roots grown in water float tank treated with nitrogen-gas; Water- N_2 = water roots grown in water float tank treated with nitrogen-gas; Water-C = water roots grown in a control water float tank; Media-C = media roots grown in a control float tank; Data points are mean values of three measurements (replications).**



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