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Effects of Dissolved Oxygen Concentrations in Aero-hydroponics on the Formation and Growth of Adventitious Roots

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Abstract. Cuttings of Fictis benjamins L. and Chrvsanthemum x morifolium(Dendranthema grandiflora tzyelev.) were rooted in aero-hydroponics to study the effect of dissolved oxygen concentrations in the range of 8 mg-liter. (ambient saturation) to 0 mg-liter-'. The results of this study indicate that dissolved oxygen is essential to root formation and root growth. Woody (Ficus) and herbaceous (Chrysanthemum) cuttings responded similarly. Lowering the dissolved oxygen concentration increased the time required to form adventitious roots, reduced rooting percentages, reduced numbers of roots formed per cutting, and reduced average root lengths. Comparisons between stirred and unstirred water suggested the development of an area of depleted oxygen concentration (boundary layer) at the stem-water interface on cuttings immersed in unstirred water. Cuttings in water stirred constantly rooted sooner and formed more roots than did those in unstirred water. Maximum rooting occurred in misted (high dissolved oxygen concentrations) sections of cuttings suspended in the aero-hydroponics chambers. Chemical name used: potassium salt of 1H-indole-3-but-vric acid (K-IBA).

Any medium used for rooting must provide mechanical support, water, and oxygen. Whereas a great deal of information is available regarding the importance of water in the rooting process, information on the effects of oxygen is relatively scarce. The actual requirement for 0_2, and its availability in the rooting medium during adventitious root formation have seldom been studied, although the importance of 0_2, in supporting the intensive metabolic processes associated with root formation and subsequent growth is well-recognized.

Zimmerman (14) has shown that cuttings from various plant species required different levels of 0_2, for rooting in water. Willow (Salix pendula) and English ivv (Hedera helix) in tap water required 0_2, concentrations of 1 and 10 mg-liter 0_2, respectively. Zimmerman achieved dissoived 0_2, concentrations greater than ambient saturation levels by bubbling pure 0_2 into water (14). Measurements of 0_2 concentrations were intermittent and, in some instances, after roots emerged, thus affecting dissolved 0_2, concentrations. Tinga (13) injected gas mixtures of N_2 containing 0%, 5%, 10%, and 15% 0_2 into water culture and showed increased rooting of carnation with assumed dissolved 0_2 concentrations (no direct dissolved 0_2 measurements were made). Using Chrysanthemum, he compared rooting in water bubbled with air (21% 0_2) and rooting in water containing no 0_2 (bubbled N_2 in water) and found that rooting occurred only in the presence of 0_2 (13). In none of these studies was the water surrounding the cuttings agitated. If cuttings use dissolved 0_2 from the water during the rooting process as do roots, the lack of agitation might lead to an area of depleted 0_2 concentrations at the stem-water interface.

Regardless of the absence of substantial data on the specific requirements for dissolved 0_2 in the rooting process, it has been shown that periodic aeration with 0_2 of the propagation medium improved survival and rooting of rootstock cuttings (2). Komissarov (4) reported on gravel culture as a successful method of rooting woody cuttings and suggested water culture as a simple and efficient rooting method worthy of practical application. Experiments with water culture, in which the water was changed every 2 days, showed that out of 30 species tested, 20 gave almost equal percentages of rooting in water and in sand. Some species rooted more rapidly in water and developed more vigorous roots than in sand. No reference was made to dissolved oxygen concentrations in the rooting medium.

These findings imply an important role for 0_2 in the formation of adventitious roots and indicate the need for further study of the effects of 0_2 on rooting. The objectives of the present study were three-fold: 1) to determine the effects of dissolved 0_2 concentrations (from air) within the range of its solubility in water on rooting of woody (Ficus) and

herbaceous (Chrysanthemum) cuttings; 2) to assess the importance of a boundary layer that may surround cuttings rooting in water; and 3) to assess the feasibility of water serving as the sole rooting medium.

Materials and Methods

Propagation unit. All rooting experiments were performed using the Ein Gedi System (E.G.S.) mini unit (11) an aero-hydroponics device consisting of an 18-liter reservoir containing 10 liters of recirculating, deionized water (Fig. 1).
Water is drawn from the bottom center of the reservoir via a hollow, rotating impeller and, by centrifugal force, thrown horizontally in to the air space, which creates a mist. The mist descends gently back to the agitated water, facilitating gas exchange. Changing the impeller's rotating speed (maximum of 3000 rpm) changes the flow of water (maximum of 2 liters-min- 1) through the impeller. The continuous flow of water and creation of mist results in water saturated with dissolved 0_2 (according to temperature and barometric pressure) throughout the container.
In the rooting experiments described here, four dissolved-0_2 concentrations were established, each in two propagation units: 0, 2.5, 5.0, and =8.0 mg-liter-1 (actual saturation values were between 7.8 and 8.7, depending on prevailing temperatures).

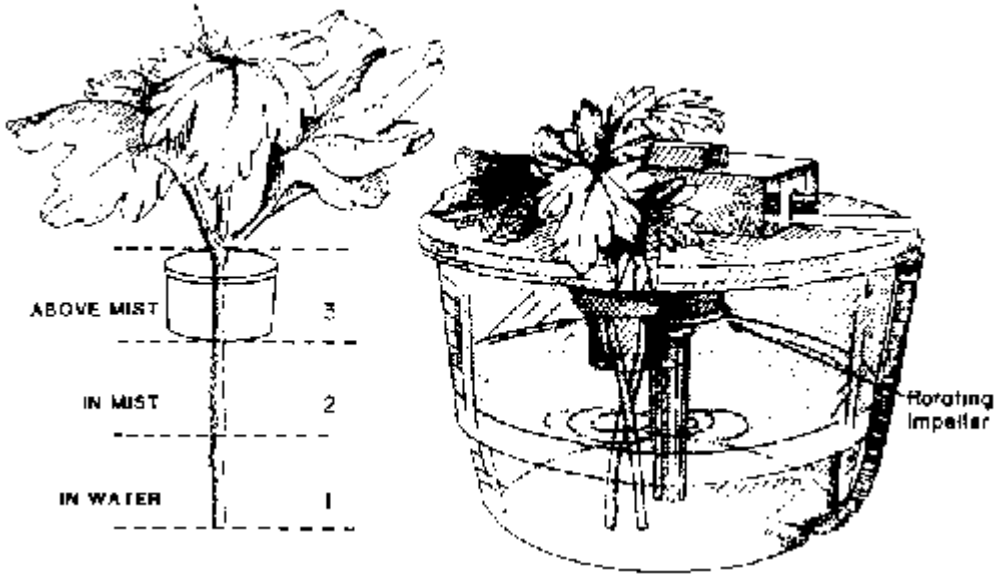

fig. 1. The Ein Gedi System propagation unit and the three designated segments of a cutting in relation to water and mist.

The subsaturation concentrations were established by a barostatically controlled flow of N_2 gas bubbled into the containers. In two additional units the impeller motor was turned off, thus creating no mist and leaving the water inside unstirred to determine the effects of stirring on root initiation (0_2 concentrations ranged between 8.0 and 7.5 mg-liter-1). Dissolved 0_2 concentrations were measured continuously with an 8500 Dissolved Oxygen monitor (Nester Instruments), the probe of which consumes no 0_2, during 0_2, measurements and is unaffected by water turbulents.
The cuttings were placed in the units so as to create three distinct segments of each stem. The lower 5 cm of the stem (segment 1) was immersed in water. The center 5 cm (segment 2) was exposed to mist, and the upper 5 cm (segment 3) was above the mist (Fig. 1). The remainder of the cutting (leaves and terminal apex) was outside the propagation unit.
Plant material. Hardwood cuttings (25 cm long) of weeping fig were collected in Sept. 1986 from greenhouse-crown (Oki Nurserv, Sacramento, Calif.) stock plants. All but the upper three to four leaves were removed from the cuttings. The bottom 2 cm of the cuttings was dipped in 5000 mg-liter K-IBA for 15 sec and immediately placed in the aero-hydroponic propagation units in groups of 10. There were three groups of 10

cuttings in each of the two propagation units at each dissolved oxygen concentration (60 cuttings per treatment). The experiment was located in a greenhouse (20° to 25°C and 60% to 100% RH). The water temperature of the aero-hydroponics units was 24° +/- 1° during the experiment. The experiment with weeping fig was unrepeated.
Herbaceous cuttings of 'Bright Golden Anne', 'Intrepid White', and 'Intrepid Gold' chrysanthemum were collected from plants (obtained from Yoder Bros. Nursery, Salinas, Calif.) grown in the greenhouse under long days. Cuttings (25 cm long) with three to four leaves were dipped (bottom 2 cm submerged) for 5 sec in 3000 mg-liter-1 K-IBA and then placed in groups of 10 in each of the 10 propagation units (20 cuttings per treatment). The rooting experiment with the chrysanthemum cultivars was repeated six times, three times with 'Bright Golden Anne', twice with 'Intrepid White', and once with 'Intrepid Gold'. The propagation units were placed in a growth chamber at a controlled temperature of 24°C, 60% to 70% RH, and 14- hr photoperiod with photosynthetic photon flux (PPF) of 400 µmol.s-¹.-m-² from metal halide lamps.

Results and Discussion

Root initiation of weeping fig occurred on the 13th day in air-saturated water (8 mg-liter-¹ 0_2). Roots appeared on the 15th day in 5.0 mg-liter-1 of 0_2 and on the 18th day in 2.5 mg-fiter-¹, a delay of 2 and 5 days after the cuttings in 8 mg-liter-¹ 0_2 rooted. Roots initiated from the base of the cuttings.
On day 21, there was a relationship between 0_2 concentration and rooting expressed in percent rooting, number of roots, and length of the longest root (Table 1). Between days 21 and 38, rooting in 5.0 and 2.5 mg-liter-¹ 0_2 improved considerably compared with the cuttings rooting in 8 mg-liter-¹ 0_2. All cuttings were in deionized water for the entire period. No changes occurred in pH or EC of the deionized water. The cuttings in 0 Mg-liter-¹ 0_2 remained essentially unchanged throughout the 38-day period. No callus or root development was observed.

Table 1. Effect of the dissolved 0_2, concentration on rooting of Ficus benjamina cuttings at 15, 21, and 38 days. Means +/- SD of two replications, 20 to 30 cuttings each.

Oxygen concn mg.liter-¹)	Rooting (%)	No.roots/rooted cutting	Avg. length of longest root (cm)
DAY 15			
0 2.5 5.0 8.0	0 0 0 85.0		
DAY 21			
0 2.5 5.0 8.0	0 6.7 33.3 96.7	--- 1.5 +/- 0.7 2.5 +/- 1.8 4.8 +/- 3.3	--- 055 +/- 0.25 1.88 +/- 1.46 5.06 +/- 3.25
DAY 38			
0 2.5 5.0 8.0	0 83.3 88.0 100.0	--- 7.6 +/- 2.3 12.3 +/- 3.2 8.0 +/- 2.7	--- 7.6 +/- 2.6 19.4 +/- 3.9 23.4 +/- 3.9

Exposing cuttings in one of the two 0 mg-liter-¹ dissolved 0_2 propagation units to 8 mg-liter-¹ 0_2 at day 21 by eliminating N_2 gas flow for the following 17 days resulted in root formation (percent rooting = 32.0, number of roots/cutting = 4.5, and average length of the longest root = 0.82 cm). This response indicates that the potential for rooting in weeping fig can be restored after long periods of complete 0_2 absence from the rooting medium, as long as cuttings remain intact. Chrysanthemum cuttings responded similar to weeping fig cuttings to dissolved 0_2 concentrations; number of roots (Figs. 2 and 3) and

total root length (Fig. 3) increased as dissolved 0_2 concentrations increased. The three chrysanthemum cultivars differed little in their response to concentrations of dissolved O_2. Chrysanthemum cuttings rooted along the entire stem, although there were some differences in the rooting response among the three segments. There was no rooting in any of the segments in the complete absence of 0_2; however, roots formed in the misted, center section of the stem and a few formed above the mist line in the 2.5 mg-liter-' 0_2 treatment (Fig. 3).

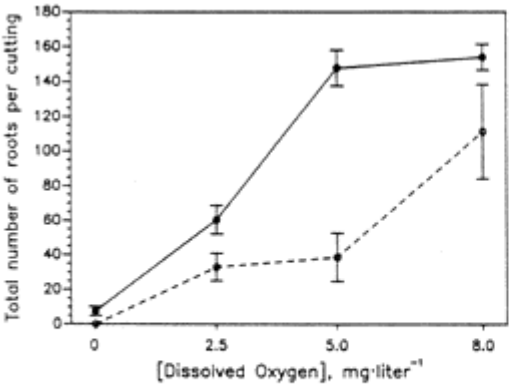

Fig. 2. Effect of dissolved O_2 concentration on the formation of roots in cuttings of two chrysanthemum cultivars. (●——●) 'Bright Golden Ann' (after 10 days). (○- - - -○) 'Intrepid White' (after 14 days). Values are means of 10 cuttings ± SD.

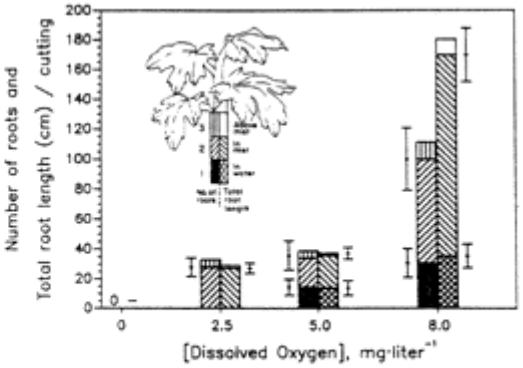

Fig. 3. Effect of dissolved O_2 concentration on number of roots and total root length of stem segments of 'Bright Golden Anne' chrysanthemum cuttings. Values are means of 10 to 20 cuttings ± SD.

There were no roots in the basal segment immersed in water. Roots developed in all three segments in the 5.0 mg-liter-¹ 0^2, treatment; however, the number of roots and the root length was only slightly greater than those in 2.5 mg-liter-¹ 0_2- In 8.0 mg-liter-¹, the number and length of roots increased in each of the three segments, and the majority developed in the center, misted segment. When root initiation occurred, roots always appeared first on the lowest segments, even if more subsequently developed above (data not shown).

One more treatment was tested in two other propagation units in which no mist was present and the water remained unstirred during the entire rooting period. The initial 0_2 concentration was 8.0 mg-liter-¹ (ambient saturation) and had dropped slightly to 7.5 mg-liter-¹ by the end of the experiment 38 days later. Weeping fig roots initiated after 32 days in the unstirred propagation units, compared to 13 days in the stirred units. Only 50% of the weeping fig cuttings rooted in unstirred water; all rooted in the units with stirred water. Rooting of chrysanthemum cuttings was delayed 2 days in the unstirred propagation units (Table 2). Root number and root length in immersed sections of weeping fig and chrysanthemum were greater in stirred water than in unstirred water (Table 2). Section 2 of cuttings in stirred units was misted, whereas those sections in unstirred units were not. More roots formed and the root length increased in misted sections of 'Bright Golden Anne' cuttings (Table 2) compared to non-misted. Further study is needed to understand fully this apparent beneficial effect of misting on the cutting stem.

The results of this study indicate that dissolved 0_2 is essential to root formation. Both woody (weeping fig) and herbaceous (chrysanthemum) cuttings responded favorably, but somewhat differently, to increased 0_2 concentrations in the water. Oxygen affected the timing of rooting, rooting percentage, number of roots, and root length.

It seems apparent, based on the rooting of the immersed segment of cuttings in stirred and unstirred units, that the dissolved 0_2 concentration of greatest physiological interest is at the interface between the cutting's stem surface and the water. Therefore, measurements of air volume in a given rooting substrate or O_2, concentrations in a liquid medium are of minor importance to the rooting process unless the 0_2 measurements are representative of the stem-water interface surrounding the stem. This measurement is best done in water that is agitated vigorously. Failure to do so may explain the poor correlation between adventitious root formation and volumetric air content of rooting

media (1, 5-7, 9, 12). Volumetric air content may make little difference in terms of root formation if the cutting has an aqueous layer surrounding it that inhibits the diffusion of 0_2 from the air to the interior of the cutting. In liquid culture, the interface between the stem and unstirred water may consist of a depletion gradient of dissolved 0_2 concentration, similar to that described and associated with mineral uptake by roots (3, 8). An additional explanation may be that substances exuded from the cutting stem may accumulate around the stem when placed in unstirred water. The interface becomes thinner as the turbulence of the surrounding liquid increases, hence the stirring effect (10). A propagation device in which water is the sole medium might serve efficiently, provided the water is saturated with dissolved 0_2 and agitated sufficiently to minimize or eliminate the boundary layer. Aero-hydroponics has the advantage of initiating roots either in mist or in 0_2-saturated water and subsequent root growth and development in well-aerated nutrient solution.

Table 2. Stirred and unstirred water effect on rooting of *Ficus benjamina* and *Chrysanthemum* ×*morifolium* 'Bright Golden Anne' cuttings. Values are means of 10 to 20 cuttings.

	Ficus benjamina				Bright Golden Anne chrysanthemum			
Stem	No. roots		Longest root (CM)		No. roots		Total root lenth (cm)	
treatment	Stirred	Unstirred[z]	Stirred	Unstirred[z]	Stirred	Unstirred	Stirred	Unstirred
Immersed	8	5.1	23.4	3.1	30.5	21.2	37.8	16.5
Misted	---	---	---	---	69.6	3.0	137.0	0.5
Above mist	---	---	---	---	11.3	---	10.7	---
Mean[y]	8 a	5.1 b	23.4 a	3.1 b	111.4 a	24.2 b	185.5 a	17.0 b
Percent rooting	100	50	100	50	100	100[x]	100	100[x]

[z]Means of rooted cuttings.
[y]Mean separation by Duncan's new multiple range test, p = 5% (*Ficus*) or 1% (chrysanthemum).
[x]A delay of 2 days for root initiation.

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