# A Comparison of Irrigation System, Basal Temperature and Auxin Concentration on Rooting of Stem Cuttings of *Ilex glabra* L.[1]

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## Abstract

Basal stem temperature and auxin treatment effects on rooting percentage, root number, length of the longest root, and basal stem necrosis of hardwood stem cuttings of *Ilex glabra* L. (inkberry holly) were compared between a recirculating subirrigation propagation system and a conventional intermittent mist propagation system. Recirculating subirrigation maintained basal temperature and medium moisture content better than conventional intermittent mist. While rooting percentages were significantly greater for cuttings rooted in the recirculating subirrigation system, root number, and length of the longest root were higher for cuttings rooted under intermittent mist. A basal temperature of 32C (90F) was supra-optimal, resulting in significantly lower rooting percentages. However, rooting temperature had no effect on root number or basal stem necrosis. Basal stem necrosis of rooted cuttings was greater in the recirculating subirrigation system, and increased with temperature in both irrigation treatments, but appeared to have no significant effect on root number. Increasing auxin concentration increased rooting percentage, root number, and length of the longest root of rooted cuttings, though the response differed with irrigation treatment. Cuttings of *Ilex glabra* L. can be rooted successfully in a recirculating subirrigation propagation system where the rooting process appears to be less sensitive to rooting temperature and auxin concentration than for cuttings rooted under intermittent mist. However, overall root growth was greater on cuttings rooted under intermittent mist, perhaps because of the lower medium moisture content in that system. This suggests that optimal rooting of hardwood cuttings can be achieved without intermittent mist using a recirculating subirrigation system with modifications to the moisture content of the rooting medium.

**Index words:** inkberry holly, auxin, rooting environment, atmospheric environment, intermittent mist, subirrigation.

### Significance to the Nursery Industry

Recirculating subirrigation, a propagation method based on earlier subirrigation systems, circulates heated and aerated water to maintain a constant water table, and provides water to the base of stem cuttings through capillary movement via perlite rooting medium. This propagation system is similar to production and propagation techniques gaining popularity in Europe that eliminate the need for overhead intermittent mist irrigation, which can leach mineral nutrients, increase pathogen incidence and become a management problem due to variability of the microenvironment. In this study, hardwood stem cuttings of *Ilex glabra* L. rooted in a recirculating subirrigation propagation system had higher rooting percentages than those rooted under intermittent mist. The recirculating subirrigation system maintained more consistent control over basal stem temperature and medium moisture content. However, cuttings rooted under intermittent mist had a greater number of roots, longer roots, and less basal stem necrosis. Across irrigation treatments, rooting percentage was highest when cuttings were treated with 16 or 24 mM (3,860 ppm or 5,790 ppm, respectively) KIBA, or rooted at a basal stem temperature of 19C (66F).

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### Introduction

Subirrigation propagation systems have been investigated as alternatives to intermittent mist irrigation (8, 14). Intermittent mist irrigation alleviates water stress before adventitious roots develop by reducing the leaf vapor pressure deficit of stem cuttings. However, depending on frequency and duration, intermittent mist can be excessive or deficient and, even if timed properly, seldom provides uniform application to the entire canopy of the cuttings (19). Intermittent mist irrigation also can leach mineral nutrients from stem cuttings (12), suffer from clogged mist nozzles or other mechanical problems (3), or exacerbate cultural problems such as foliar disease (29). Intermittent mist or overhead irrigation also can contribute to propagule water stress through fluctuations in medium moisture content (16), poor medium drainage (29), or sub-optimal medium temperature (22). Used as a propagation system, recirculating subirrigation builds on approaches to subirrigation techniques introduced in 1946 (26) and more advanced systems, developed in Australia, that use temperature-controlled water reservoirs and allow for incorporation of rooting promoters such as auxin and boron (2).

The recirculating subirrigation system used in the present investigation maintained a constant water table in the propagation tray with water circulated from a heated water reservoir. Subirrigation helps maintain a higher and more constant medium moisture content as compared to the same medium under intermittent mist (25). Water potential of the stem cutting is directly proportional to the moisture content of the rooting medium and has been identified as a primary factor in propagule water stress (13). Loach (20) concluded that basal heating of rooting media under intermittent mist irrigation influences only the minimum basal stem temperature, while average values of medium temperature are determined primarily by water content, irradiance, and air temperature. In the absence of intermittent mist, recirculating subirrigation is influenced less by the surrounding environment and maintains a more constant medium moisture content. Therefore, recirculating subirrigation is an appropriate propagation system in which to evaluate the effects and interaction of basal stem temperature and auxin treatment on adventitious root initiation and development.

Interactions between basal stem temperature and auxin have been reported to require a lower auxin concentration to yield 100% rooting as temperature increased (2). However, increasing rooting temperature alone does not appear to replace the need for auxin to achieve adequate rooting (27). It has also been shown that the incidence of stem tissue damage (basal stem necrosis) from high concentrations of exogenous auxin may be reduced by increasing basal stem temperature (7), although higher rooting temperatures are also associated with weaker or more branched roots (4) and increased callus formation (24). Dykeman (6), studying the rooting of leafy stem cuttings *Chrysanthemum* spp. (unspecified), concluded that there were separate temperature optima for adventitious root initial formation and root elongation, being a minimum of 5C (9F) higher for root initial formation.

The goals of treating stem cuttings with an optimum auxin concentration and rooting the cuttings at the most favorable basal stem temperature are higher rooting percentage, faster root emergence, and greater root growth resulting in a greater number of high quality cuttings. The objective of this study was to compare the effect of basal temperature and auxin concentration, and their interaction, on the rooting of stem cuttings of *Ilex glabra* L. (inkberry holly) in a recirculating subirrigation system versus an intermittent mist irrigation system.

## Materials and Methods

Seven-hundred-twenty 10 cm (4 in) long hardwood stem cuttings of *Ilex glabra* L. were collected on February 21, 2001, from a clonal field stock block at the Rhode Island Agricultural Experiment Station East Farm, Kingston, RI, (lat. 41° 29'N, long. 71° 31'W). As the cuttings were collected they were misted and placed in polyethylene (PE) bags. The bags were stored at 4C (40F) for 34 hr before the cuttings were inserted into the rooting medium on February 23, 2001. The experiment was conducted in a double layer PE-covered greenhouse at the Rhode Island Agricultural Experiment Station East Farm. Both the intermittent mist benches and the structure insulating the recirculating subirrigation system (25) were walled with a 1 m (39 in) tall single layer of 4 mil clear PE to reduce air movement. Three replications of 10 leafy stem cuttings each, chosen at random, were basal dipped for ≈ 3 seconds in water (0 ppm), or 8 mM (1,930 ppm), 16 mM (3,860 ppm), or 24 mM (5,790 ppm) of the potassium salt of indole-3-butyric acid (KIBA) prepared with de-ionized water. Replications were placed randomly under intermittent mist or in recirculating subirrigation propagation trays filled with de-gassed perlite (Whittemore Co., Inc., Lawrence, MA) (25) that had basal temperature set points of 19C (66F), 26C (78F) or 32C (90F). The experimental design was a 2 × 3 × 4 completely randomized factorial arrangement of treatments (2 irrigation systems × 3 basal temperatures × 4 KIBA treatments).

All cuttings were prepared with a fresh basal stem cut and leaves were removed from the basal 4.5 cm (1.8 in) of each cutting, leaving ≈ 5 leaves per cutting. A heavy basal wound, 2 cm (0.8 in) in length, was made to one side of the stem base, exposing the cambium. The base of each cutting was dipped in water or an auxin treatment to a depth of ≈ 2.5 cm (1 in) for 3 sec. Cuttings in both irrigation treatments were inserted to ≈ 4 cm (1.6 in) depth and watered by hand 4 hr after being inserted into the rooting medium. For the first 7 days of the experiment cuttings in the recirculating subirrigation system were misted by hand at ≈ 3:00 p.m. eastern standard time (EST) using a 1.9 liter/min (0.5 gal/min) Super Fine Fogg-It nozzle (Griffin Greenhouse and Nursery Supply, Tewksbury, MA). Three zones of intermittent mist irrigation were regulated by a mist controller (Phytotronics 1626D, Phytotronics, Inc., Earth City, MO) set to apply mist 6 sec every 6 min from 7:00 a.m. to 4:30 p.m. EST. Intermittent mist was applied at 35 psi to deliver 45.4 liters/hr (12 gal/hr) through Blue Vibro-Mist nozzles (Netafim Irrigation Inc., Altamonte Springs, FL) spaced 1.2 m (4 ft) on center and suspended 0.9 m (3 ft) above the canopy (25). Both intermittent mist irrigation and recirculating subirrigation treatments were watered by hand on February 27, 2001, and cuttings under intermittent mist were irrigated every 3 days. Cuttings in the subirrigation system were hand watered only as needed to maintain a full reservoir (≈ 90 liters; 23.8 gal).

Propagation trays in the recirculating subirrigation system were modified from 26.4 liters (7 gal), 54 × 39 × 14 cm (21.3 × 15.4 × 5.5 in) Rubbermaid® storage boxes (Rubbermaid, Inc., Wooster, OH) (Fig. 1). A water table height of ≈ 8 cm (3.1 in) was maintained by positioning a drainage pipe consisting of a 44.5 cm (17.5 in) length of perforated polyvinyl chloride pipe covered with a 1 mm (0.04 in) mesh screen that returned the solution to the reservoir and allowed the solution to be aerated as it fell from the propagation tray to the reservoir (Fig. 1). A 402 Powerhead™ centrifugal pump (Rolf C. Hagen Corp., Mansfield, MA) recirculated heated water at 3 liters/min (0.8 gal/min) from 125-liter (33 gal) Sterilite™ tote water reservoirs (Sterilite Corp., Townsend, MA). All nine recirculating reservoirs were insulated with styrofoam packing pellets. Plastic Jumbo Trays™ (TFI Plastic Manufacturing Ltd., Westford, MA), 52 × 26 × 6 cm (20.5 × 10.2 × 2.4 in) were used as propagation trays under intermittent mist because of their similar available rooting area to the trays used in the recirculating subirrigation system.

In the recirculating subirrigation system, a basal stem temperature of 19C (66F) was maintained using one 100 W Rena Cal™ aquarium heater (Aquarium Pharmaceuticals, Inc.,

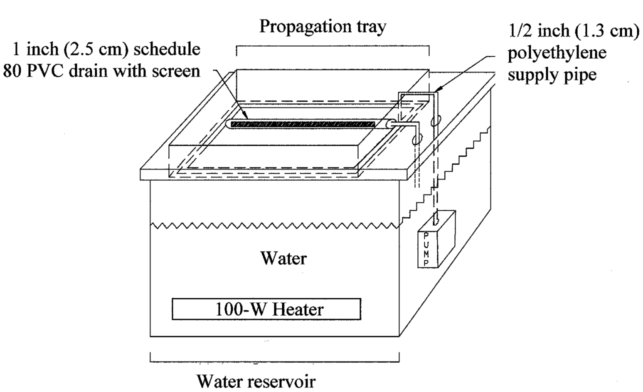


**Fig. 1. Three dimensional schematic of a recirculating subirrigation propagation system.**

Table 1. Significance of three treatments (irrigation system, basal temperature, and auxin) on percentage rooting, root number, length of longest root, and basal necrosis of stem cuttings of *Ilex glabra* L. propagated in a PE covered greenhouse February 23 to March 27, 2001.

| | Root percentage | | | Root number | | | Length of longest root | | Basal stem necrosis | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | df | F value | Sig. | df | F value | Sig. | F value | Sig. | F value | Sig. |
| Irrigation (I) | 1 | 4.7 | 0.036 | 1 | 12.4 | 0.001 | 97.6 | 0.001 | 25.3 | 0.001 |
| Basal temperature (T) | 2 | 33.5 | 0.001 | 2 | 1.0 | NS[z] | 2.2 | NS | 7.9 | 0.001 |
| KIBA concn. (A) | 3 | 16.0 | 0.001 | 3 | 11.1 | 0.001 | 7.0 | 0.001 | 2.7 | 0.044 |
| I × T | 2 | 3.0 | NS | 2 | 3.2 | 0.042 | 1.5 | NS | 6.9 | 0.001 |
| I × A | 3 | 1.2 | NS | 3 | 3.2 | 0.023 | 2.9 | 0.036 | 1.6 | NS |
| T × A | 6 | 1.0 | NS | 6 | 1.6 | NS | 1.6 | NS | 1.4 | NS |
| I × T × A | 3 | 0.4 | NS | 3 | 2.3 | NS | 2.5 | NS | 0.6 | NS |
| Error | 48 | | | 307 | | | | | | |

[z]NS Nonsignificant at $P \geq 0.05$.

Chalfont, PA). The higher temperatures of 26C (79F) and 32C (90F) were attained by using two 100-watt aquarium heaters. A second pump circulated the water within the reservoirs to ensure uniformity. A BioTherm® Microclimate heating system (BioTherm Hydronic, Inc, Petaluma, CA) was used under misted trays. MicroClimate Tube® was spaced 5 cm (2 in) on center to reach temperatures set at 19C (66F) and 26C (79F) or spaced 2.5 cm (1 in) on center to achieve a basal stem temperature of 32C (90F).

A CR21X Micrologger in combination with an AM416 multiplexer (Campbell Scientific, Inc., Logan, UT) recorded output from all sensors at 15 min intervals for the duration of the experiment. Air temperature and relative humidity (RH) were measured with a CS500 temperature and RH probe (Campbell Scientific, Inc.). Thermocouple probes (105T; Campbell Scientific, Inc.) recorded recirculating subirrigation media temperatures and water reservoir temperatures. Media temperatures under intermittent mist were recorded using a HOBO® H8 Industrial 4 channel external logger with TMC-HA series temperature sensors (Onset Computer Corp., Pocasset, MA). The photosynthetic photon flux (PPF) in □mol·$m^{-2}$·$s^{-1}$ photosynthetically active radiation (PAR) and photosynthetic total flux (mmol·$m^2$ PAR) were recorded at canopy level using LI-190SA quantum sensors (LI-COR, Inc., Lincoln, NE). An IRTS-S Infra-red Transducer™ connected to a Fluke® 50D Series II contact thermometer (Apogee Instruments, Inc., Logan, UT) with a 1:1 field of view was used to sample single leaf temperatures at four dates. Sample medium moisture content was measured at three dates with a ThetaProbe™ soil moisture sensor (Dynamax, Inc., Houston, TX).

After 33 days (March 27, 2001), cuttings were harvested and rooting percent of replicated samples, root number, length of the longest root, and basal distance to first root of rooted cuttings was recorded. Rooted cuttings had at least one root ≥ 1 mm in length. Basal distance to the first root of rooted cuttings was used as a measure of basal necrosis. Rooting data was statistically analyzed to evaluate treatment effects and interactions, and Tukey's honest significant difference (HSD) post hoc tests were performed when appropriate, using SPSS™ 11.0 (SPSS Sci., Chicago, IL). A significance level of $P < 0.05$ was used for all analyses. Leaf temperature and visual observations were used to estimate plant stress.

## Results and Discussion

There was a significant main effect of irrigation type, basal temperature, and auxin concentration on all rooting variables with the exception that basal temperature did not significantly affect root number or length of the longest root (Table 1). Irrigation method interacted with basal temperature to affect root number and basal stem necrosis of rooted cuttings. Irrigation method also interacted with auxin concentration to affect root number and length of the longest root of rooted cuttings (Table 1). The significant mean differences reported in Table 2 show a 30 point increase in rooting percentage when cuttings were rooted in the recirculating subirrigation system, but a greater mean root number and length of the longest root occurred on cuttings rooted under intermittent mist irrigation. The extent of basal necrosis was almost three fold greater on cuttings rooted in recirculating subirrigation. When interpreting the treatment main effects it is important to note that non-treated cuttings at a medium temperature set point of 26C (79F) did not root. Cuttings treated with 0 mM (0 ppm) or 8 mM KIBA (1,930 ppm) and rooted at 32C (90F) under intermittent mist also did not root. Therefore, though basal necrosis appears to be reduced under intermittent mist, the data may be deceptive because fewer cuttings actually rooted and those unrooted cuttings were not included in the estimation of basal necrosis. After only 12 days, leaves of stem cuttings at a basal temperature of 32C (90F) under intermittent mist became chlorotic regardless of auxin treatment (Fig. 2).

Rooting percentages were significantly lower when cuttings were rooted at 32C (90F). Rooting percentages were also higher on cuttings treated with 16 mM (3,860 ppm) or 24 mM (5,790 ppm) KIBA (Table 2). Mean root number and length of the longest root of rooted cuttings were greater when cuttings were treated with 16 mM (3,860 ppm) or 24 mM (5,790 ppm) KIBA, and length of the longest root was significantly less on non-treated cuttings (Table 2).

The effect of auxin on root number and length of the longest root was more pronounced on cuttings rooted under intermittent mist irrigation than on those rooted in recirculating subirrigation (Fig. 3). In recirculating subirrigation, a significant difference existed only between the root number of non-treated cuttings and those treated with 24 mM (5,790 ppm) KIBA. Figure 4 shows the significant difference in root number on cuttings propagated under intermittent mist in response to increasing basal temperature ($F = 4$, $P < 0.05$), but not on cuttings propagated in the recirculating subirrigation system. Basal stem necrosis of cuttings rooted in the recirculating subirrigation system ($F = 41$, $P < 0.05$) increased with basal temperature, but not for cuttings rooted under intermittent mist irrigation. In analyzing the simple

Table 2. **Main effect means of percentage rooting (n = 3) and root number, length of the longest root length and distance to first root of stem cuttings of *Ilex glabra* L. treated with four auxin concentrations (0, 8, 16, 24 mM KIBA) and subirrigated or misted with a basal stem temperature set point of 19C (66F), 26C (79F), or 32C (90F).**

| Treatment | | Rooting response | | | | |
|---|---|---|---|---|---|---|
| | | Percentage | Number | Length of longest root (mm) | Distance to 1st root (mm) | n[z] |
| Irrigation | Sub | 80.2[y] | 26[y] | 9[y] | 8[y] | 181 |
| | Mist | 50.7 | 44 | 22 | 3 | 147 |
| Basal temperature (C) | 19 | 80.2a[x] | 34[w] | 14[w] | 3[v] | 154 |
| | 26 | 66.2a | 32 | 16 | 8 | 126 |
| | 32 | 24.5b | 38 | 17 | 10 | 48 |
| KIBA concn. (mM) | 0 | 27.1c | 12b | 7c | 5a | 39 |
| | 8 | 50.7bc | 23b | 13b | 5a | 72 |
| | 16 | 77.8a | 36a | 18a | 8a | 113 |
| | 24 | 72.2ab | 47a | 17ab | 5a | 104 |

[z]The number (n) is given for root number, length of the longest root, and distance to first root from stem base because of differences in the number of cuttings that rooted between treatments.

[y]Means were significantly different by analysis of variance ($P \leq 0.05$).

[x]Mean separation with columns for a treatment by Tukey's HSD after analysis of variance ($P \leq 0.05$).

[w]Main effect not significant, $P \geq 0.05$.

[v]No post hoc test preformed because of disordinal nature of significant interaction.

effects of all other interactions no significant mean separations were found using Tukey's HSD.

The recirculating subirrigation system was superior to intermittent mist in edaphic environmental control, maintaining basal stem temperatures with greater accuracy, particularly at higher temperature set points (Table 3). Basal temperatures under intermittent mist may have been influenced more by ambient air temperature, air movement and irradiance, or by fluctuations in bottom heat provided by the Biotherm® system. Medium moisture content showed the same trend, fluctuating more under intermittent mist irrigation and even decreased below 5% when mean basal stem temperatures were ≥ 24C (75F) and not given supplemental irrigation (Table 4). When the perlite medium under inter-

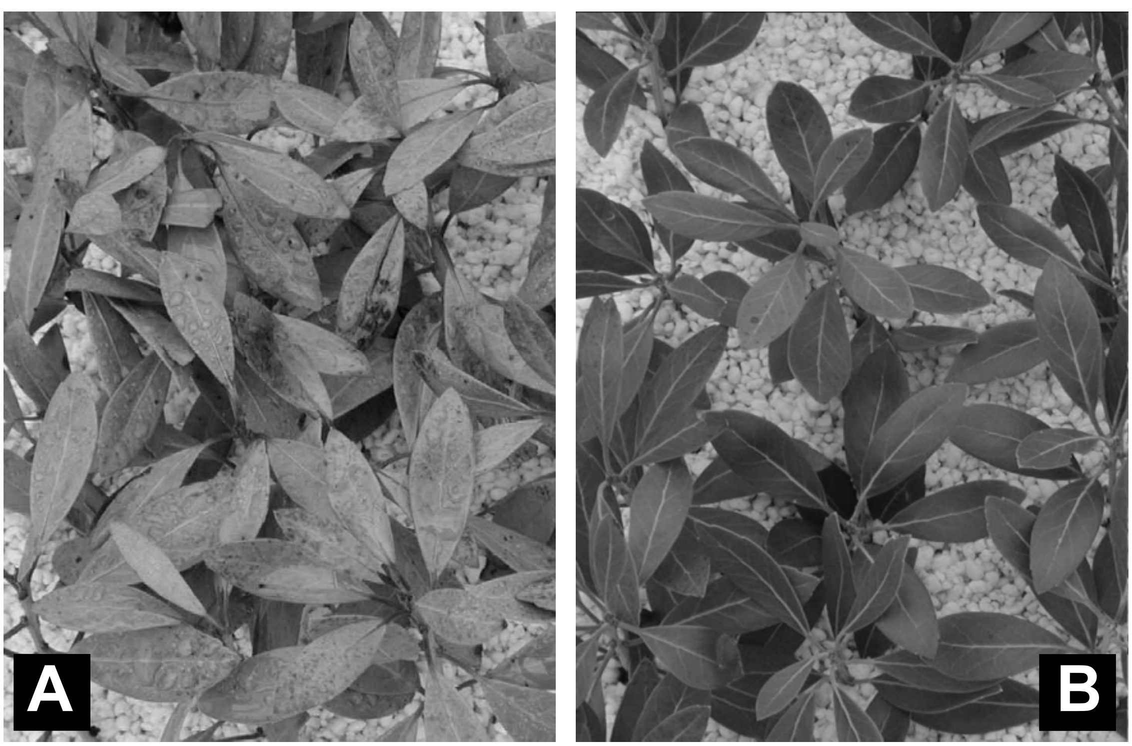


Fig. 2. **Photographic observations of the cutting canopy under mist (A) or in recirculating subirrigation units (B) across auxin (KIBA) concentrations made 12 days after cuttings were inserted into the rooting medium (March 6, 2001) with a basal temperature set point of 32C (90F).**

Fig. 3. **Mean root number (± standard error) (A) and length of the longest root of rooted stem cuttings (B) (n = 9 to 57) of *Ilex glabra* L. treated with 0 (water), 8, 16 or 24 mM KIBA concentrations and then propagated for 33 days under intermittent mist irrigation or in a recirculating subirrigation system.**

mittent mist was hand watered every 3 days it maintained a medium moisture content of ≈ 23%. Medium moisture content decreased as rooting temperature increased in both irrigation treatments, but never fell below 36% in the recirculating subirrigation system, even at a mean basal temperature of 33C (91F), over 33 days (Table 4).

Fig. 4. **Mean (± standard error) root number (A) and distance to first root (± standard error) (B) of rooted stem cuttings (n = 22 to 78) of *Ilex glabra* L. propagated for 33 days under intermittent mist irrigation or in a recirculating subirrigation system with basal stem temperatures set at 19C (66F), 26C (79F), or 32C (90F).**

High leaf temperature may be an indicator of propagule stress (31). Under intermittent mist irrigation, leaf temperatures averaged 19C (66F) across treatments, significantly lower than the mean leaf temperature of 24C (76F) for cuttings in the recirculating subirrigation system (F = 216, $P < 0.05$, n = 27) when determined on 3 days with equivalent irradiance, February 27, March 16, and March 20. On an overcast day (March 6, average *PPF* = 105 □mol·m$^{-2}$·s$^{-1}$ PAR)

Table 3. **Mean (± standard deviation), minimum, and maximum values of environmental parameters and degassed perlite medium temperatures, misted or subirrigated, recorded between February 23 to March 27, 2001.**

| Environmental paramenter | Day[z] Mean | Day[z] Min. – Max. | Night Mean | Night Min. – Max. |
|---|---|---|---|---|
| Air temperature (C) | 18.5 (2.2)[y] | 14.7 – 26.7 | 16.8 (0.9) | 15.3 – 19.8 |
| Rel. humidity (%) | 58.3 (18.0) | 17.5 – 88.7 | 59.8 (9.6) | 37.0 – 83.7 |
| Surface temp.(C)[x] | 16.5 (3.5) | 9.7 – 30.1 | 13.7 (1.2) | 10.6 – 17.6 |
| *PAR* (□mol·m$^{-2}$·s$^{-1}$) | 219 (164) | 0 868 | — | — |
| *PAR* max[w] (□mol·m$^{-2}$·s$^{-1}$) | 710 (78) | | | |
| **Basal temp. set point (C)** | **Subirrigation Mean** | **Subirrigation Min. – Max.** | **Mist irrigation Mean** | **Mist irrigation Min. – Max.** |
| 19 | 23.7 (1.1) | 20.3 – 26.6 | 22.0 (1.8) | 11.8 – 25.2 |
| 26 | 28.3 (0.5) | 25.7 – 31.1 | 24.0 (1.2) | 13.3 – 28.3 |
| 32 | 33.4 (0.6) | 30.9 – 35.2 | 29.4 (2.4) | 18.3 – 37.0 |

[z]Daytime temperatures were recorded from 7:30 a.m.–4:30 p.m. EST.

[y]Standard deviation is in parentheses, following the mean of parameter measurements every 15 min. for 33 days.

[x]AM416 multiplexer black surface at canopy height oriented near recirculating subirrigation units.

[w]PAR total flux over 33 days = 131 mol/m$^2$.

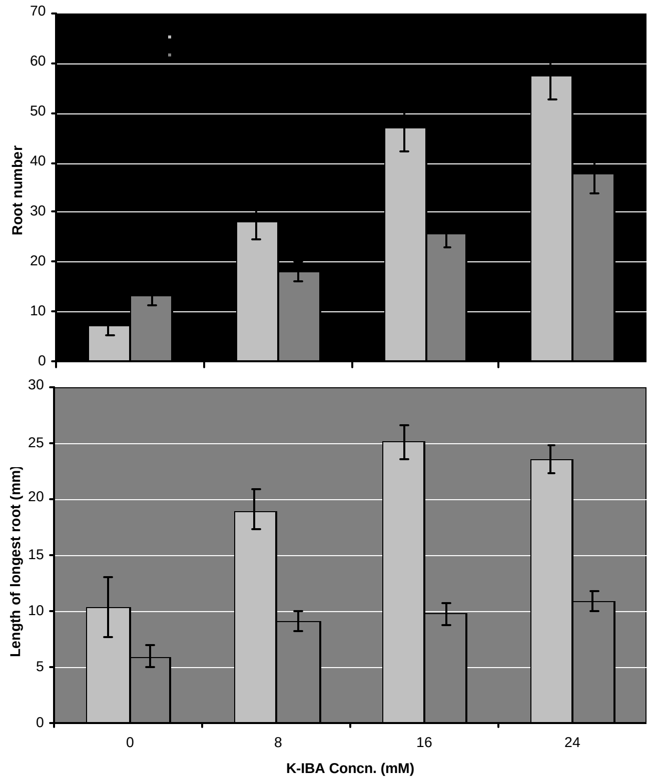


**Fig. 3.** **Mean root number (± standard error) (A) and length of the longest root of rooted stem cuttings (B) (n = 9 to 57) of *Ilex glabra* L. treated with 0 (water), 8, 16 or 24 mM KIBA concentrations and then propagated for 33 days under intermittent mist irrigation or in a recirculating subirrigation system.**

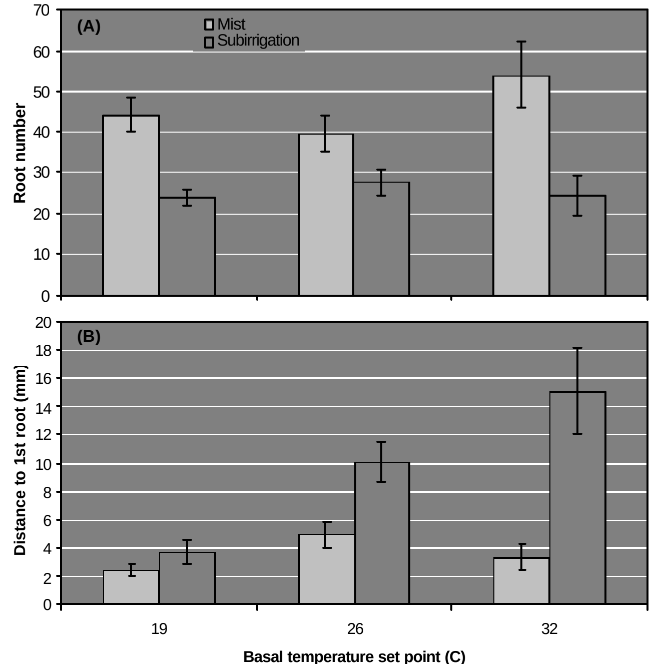


**Fig. 4.** **Mean (± standard error) root number (A) and distance to first root (± standard error) (B) of rooted stem cuttings (n = 22 to 78) of *Ilex glabra* L. propagated for 33 days under intermittent mist irrigation or in a recirculating subirrigation system with basal stem temperatures set at 19C (66F), 26C (79F), or 32C (90F).**

**Table 4. Mean leaf temperature (± standard deviation, n = 3) of *Ilex glabra* L. and soil moisture content (± standard deviation, n = 3) of degassed perlite for both recirculating subirrigation and intermittent mist irrigation set at three basal temperature set points [19C (66F), 26C (79F), 32C (90F)]. Sample data recorded between February 27 and March 20, 2001.**

| | | Medium moisture content ($m^3*m^{-3}$) | | | Leaf temperature (C) | | | |
|---|---|---|---|---|---|---|---|---|
| **Irrigation** | **Temp. (C)** | **Feb. 27** | **Mar. 6** | **Mar. 20** | **Feb. 27** | **Mar. 6[z]** | **Mar. 16** | **Mar. 20** |
| Mist | 19 | 17.1 (2.8) | 25.6 (5.0) | 22.5 (4.0) | 17.1 (–0.6) | 17.3 (0.1) | 21.1 (1.8) | 17.7 (0.7) |
| | 26 | 0.8 (1.4) | 25.2 (6.6) | 23.8 (2.5) | 16.7 (1.0) | 17.6 (0.1) | 20.6 (1.4) | 17.4 (0.6) |
| | 32 | 4.8 (3.4) | 24.7 (0.3) | 16.2 (1.9) | 19.5 (1.6) | 17.7 (0.8) | 21.8 (1.4) | 17.4 (1.1) |
| Subirrigation | 19 | 41.3 (2.9) | 40.0 (5.2) | 41.0 (8.2) | 21.9 (1.7) | 16.9 (0.2) | 27.3 (1.2) | 21.8 (0.2) |
| | 26 | 41.0 (2.8) | 39.2 (3.4) | 41.4 (2.4) | 22.7 (0.9) | 17.3 (0.5) | 27.2 (2.9) | 21.8 (0.5) |
| | 32 | 39.8 (1.7) | 37.4 (2.7) | 36.1 (2.7) | 24.2 (0.1) | 18.4 (1.1) | 28.0 (2.2) | 22.6 (0.7) |

[z]March 6, 2001 was an overcast day (mean *PPF* = 109 ± 97 □$mol \cdot m^{-2} \cdot s^{-1}$, minimum = 3 □$mol \cdot m^{-2} \cdot s^{-1}$, maximum = 352 □$mol \cdot m^{-2} \cdot s^{-1}$ PAR).



the mean temperature of leaves in the recirculating subirrigation system and under intermittent mist irrigation were equivalent (Table 4). Therefore, it could be concluded that recirculating subirrigation supplied sufficient water for transpirational cooling under low irradiance because of reduced vapor pressure deficit.

Under intermittent mist irrigation, the perlite medium at a basal temperature set point of 32C (90F) reached 37C (99F) and dried to < 5%, resulting in leaf chlorosis, necrosis, dessication, and finally death of all the cuttings. Under these conditions the rooting medium was unable to sustain adequate moisture content. Perlite conducts more heat than other rooting media (9), and it has been suggested that daily overhead watering under intermittent mist irrigation could be inadequate (23). In retrospect, the 3 day watering regime might not have been adequate to maintain an optimum moisture content of the media under intermittent mist at the highest root zone temperatures (16.2 $m^3/m^{-3}$ on March 20). Media components such as sand or gravel, with high air-filled porosity and low water holding capacity, have been used successfully in subirrigation propagation even though they contribute to higher leaf temperatures and vapor pressure deficits, both of which are indicative of propagule stress (1). Perlite, like these inert propagation media, allows for greater stem cutting water uptake (13), higher rooting percentages (8), and increased length of the longest root (23) because of freely available water supplied by the constant water table in subirrigation systems. Water in peat- or vermiculite-based media mixtures is not as freely available (13).

The high rooting percentage and better appearance of *Ilex glabra* L. cuttings in the recirculating subirrigation system suggests that the stem cuttings were better able to maintain cell turgor and competence to initiate and develop adventitious roots. This runs contrary to studies that suggest that water uptake from the base of stem cuttings is impeded by tyloses blocking vessel elements (28). Wounding has also been shown to contribute to increased water uptake through the cutting stem base, but only within the first days after cuttings were inserted into the rooting medium (13). Unlike intermittent mist irrigation, but similar to systems reported by Loach and Whalley (21), recirculating subirrigation can allow for overnight recovery of turgor in water stressed propagules. However, higher mean leaf temperatures in the recirculating subirrigation could indicate that stem cuttings were under greater water stress during daytime hours, possibly slowing root development (as shown by lower root numbers and lengths).

Stem necrosis on stem cuttings of *Ilex glabra* L. was greater in the recirculating subirrigation system, possibly resulting from a combination of supra-optimal medium moisture content (28) and high basal stem temperature (30). It is unlikely that stem necrosis resulted from anaerobic conditions, because the subirrigated cuttings would have exhibited signs of water stress due to an inability to absorb water (11). Though no interaction was found, increased basal necrosis in recirculating subirrigation could have been a product of increased auxin uptake and toxicity from the combination of wounding (15), high temperatures (18), high medium moisture content, and increased transpiration (17) in the absence of mist. Gislerød (10) reported that root number and root length of stem cuttings of *Euphorbia pulcherrima* Willd. ex Klotzch increased with higher basal stem temperature and lower medium moisture content, similar to the response observed in this study.

The increase in root number of rooted cuttings with increasing auxin concentration may reflect more stimulation of root primordial sites. Studies have shown that the optimal auxin concentrations and basal temperatures for rooting differ among taxa (5, 6, 7). Owen (25) suggested, contrary to others, that a single basal stem temperature might suffice for both root initial formation and root growth and development as opposed to needing separate temperature optima for adventitious root initation and root growth and development. Recirculating subirrigation propagation, with modifications to the edaphic environment, represents a viable alternative method of propagation for many taxa. Recirculating subirrigation offers better control of root-zone temperature, and the possibility of manipulating the subirrigation solution through the addition of auxin or mineral nutrients.

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