Decline in stomatal response to leaf water deficit in *Corylus maxima* cuttings

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Summary Many woody species can be propagated from leafy cuttings. However, following rooting, cuttings of *Corylus maxima* Mill. cv. Purpurea do not always survive the transition from a highly supportive rooting environment (e.g., fog) to a more natural environment where evaporative demand is higher. We found that it is not the supply of water to leaves, but stomatal dysfunction that leads to severe water deficits in the rooted cuttings. Two hours after well-rooted cuttings were transferred from the rooting environment, we were able to relate visible signs of leaf water deficit to high stomatal conductance (gₛ) and low relative water content (R). Small expanding leaves (L3) had unusually high gₛ and lower R than fully expanded leaves (L1). Although high cuticular conductances (gᵦ) were occasionally observed in L3, SEM confirmed that increased total leaf conductance (g) was mainly a result of abnormally wide stomatal opening. We measured changes in the ability of stomata to control water loss during rooting by determining stomatal responsiveness to leaf water deficit in detached L1 and L3 harvested from cuttings during the first 75 days after severance from stock plants. Reduced stomatal responsiveness was observed within 7 days of severance, prior to adventitious root formation, and was more pronounced in L3 than in L1. A period of acclimatization after rooting (no leaf wetting, but a vapor pressure deficit of 0.20 kPa) reduced gₛ by 50% in L3 but not in L1, and partially restored stomatal responsiveness in L1 but not in L3. After rooting, the original leaves on the cutting retained substantial capacity for photosynthesis (e.g., in L1, 8 µmol m⁻² s⁻¹ at a photosynthetic photon flux density of 400 µmol m⁻² s⁻¹). The implications of the results for post-rooting acclimatization procedures are discussed.

Keywords: acclimatization, conductance, cuticle, fog, relative water content, transpiration, weaning.

Introduction Ornamental trees and shrubs are often vegetatively propagated from leafy cuttings. During rooting of cuttings, transpiration must be minimized to prevent desiccation in the absence of a root system. A combination of low irradiance, elevated humidity, and leaf wetting are commonly used to reduce vapor pressure deficit (Dₛ), and hence transpirational water loss from leaves of cuttings. Mist nozzles create fine water droplets that gently wet the leaves, whereas fog-producing nozzles produce even finer droplets (< 50 µm) that can raise humidity close to saturation, further reducing Dₛ as well as keeping leaves wet. For some difficult-to-root cuttings such conditions are necessary to prevent desiccation and significantly improve rooting success rates (Harrison-Murray and Thompson 1988). Avoidance of water deficit stress also ensures that carbohydrate supply is maintained to the cutting through carbon fixation. In several species, it has been shown that leaves of cuttings are photosynthetically active before and after adventitious root formation (Smalley et al. 1991, Svenson et al. 1995).

Commercial plant propagators attempt to reduce losses of cuttings during transfer from the rooting environment by using a transition environment with intermediate evaporative demand. During this transition, relatively poorly rooted cuttings often die; however, even well-rooted cuttings of *Corylus maxima* Mill. cv. Purpurea frequently exhibit severe water deficit stress and up to 50% of cuttings often die. Because *C. maxima* cuttings represent an extreme example of the practical problem of acclimatization, this species was selected as the experimental model to study the physiology underlying the development of severe water deficits in transplanted rooted cuttings.

In other species, it has been proposed that an inadequate supply of water from the newly formed adventitious root system can lead to water deficits (Sasse and Sands 1996, Fila et al. 1998). However, this does not appear to be the case in *C. maxima*, because hydraulic conductance of the adventitious root system is similar to that of *Weigela florida* Bunge. cuttings, which rarely show signs of water stress despite bearing a similar leaf area (Ford and Harrison-Murray 1997). Therefore, the primary problem may be poor control of water loss. Studies of stomatal conductance (gₛ) during the rooting of cuttings have been reported (e.g., Gay and Loach 1977, Smalley et al. 1991, Newton and Jones 1993) but none have followed changes in stomatal response to water deficit throughout the rooting and acclimatization phases.

Plants propagated *in vitro* typically exhibit a reduced ability...
to control water loss from their leaves, which is associated with increased stomatal (Ziv et al. 1987, Santamaria et al. 1993, Zacchini and Morini 1998), and possibly cuticular (Sutter 1988), conductance. Stomata on leaves of in-vitro-derived plantlets often fail to close in response to leaf water deficit and other stimuli that normally induce stomatal closure, such as darkness, abscisic acid and calcium ions (Ziv et al. 1987, Santamaria et al. 1993). The reduced stomatal function of in-vitro-derived plantlets has been attributed to the very low $D_a$ in the culture vessels that renders stomatal closure almost redundant. This idea is supported by studies in which evaporative demand of the in vitro environment has been increased. For example, reduction of relative humidity (RH) in the culture vessels improved control of water loss from leaves of plum plantlets (Sciutti and Morini 1995).

It is possible that a similar loss of normal stomatal function occurs in cuttings of species such as *C. maxima* as a result of prolonged exposure to the low $D_a$ necessary for adventitious root formation. The present study was therefore undertaken to test this hypothesis and to determine the rate of development and the reversibility of such stomatal dysfunction. Measurements of $g_s$ and relative water content ($R$) of well-rooted cuttings that were exposed to moderate $D_a$ until leaves started to wilt, indicated disruption of the normal stomatal response to leaf water deficit. To study the development of stomatal dysfunction over time, the response of stomata to water deficit was measured in detached leaves, harvested from cuttings during the first 75 days after severance from stock plants. To determine whether stomatal dysfunction can be reversed by acclimatization, stomatal responsiveness was measured 57 days after rooted cuttings were moved from fog to an acclimatization chamber ($D_a$ of 0.20 kPa) in which evaporative demand was not sufficient to cause damage. Changes in photosynthetic rates were also measured.

**Materials and methods**

**Plant culture**

Well-established hedges of *C. maxima* were pruned in early spring 1997. Cuttings were collected between April and June, in the early morning, and prepared in a cool, humid environment. Cuttings were trimmed to 3–4 unfolded leaves and dipped in an aqueous suspension of 2 g l$^{-1}$ benomyl to inhibit fungal attack. The basal 1 cm of stem was then dipped for 5 s in a solution of the root-promoting auxin indolylbutyric acid (IBA; 1.25 g l$^{-1}$ in 50% aqueous acetone v/v) and allowed to dry. The cuttings were planted in 9-cm pots containing rooting medium (50:50 v/v peat: fine bark) with 1 g l$^{-1}$ of fertilizer (16:10:10 N,P,K), and placed in a controlled propagation environment (CPE).

**Propagation environment**

The CPE consisted of a chamber 3.4 (L) × 2.0 (W) × 2.2 m (H), made from transparent polyethylene and illuminated by four high-pressure sodium lamps (400 W SON-T Plus, Philips Electronics N.V., The Netherlands), suspended above the chamber. Photosynthetic photon flux density ($Q$) at cutting height was 121 ± 10 µmol m$^{-2}$ s$^{-1}$ over a 12-h photoperiod (0900–2100 h). The CPE was located in a temperature-controlled room set at 20 ± 2 °C. Fog from a pneumatic fogging nozzle (Type 052 Sonicore, Jeff Donovan Ultronics, Otley, U.K.), providing a water deposition rate of 150 × 30 µm h$^{-1}$ during the light period (see Ford and Harrison-Murray 1997), kept the air in the chamber saturated.

**Environment for measuring responsiveness of stomata**

A growth cabinet (600G3/TL, Fisons Scientific Apparatus, Loughborough, U.K.), which was maintained at 23 ± 1 °C and provided a $Q$ at plant height of 120 µmol m$^{-2}$ s$^{-1}$, was modified to provide humidity control by means of an ultrasonic fog system (Vindon Scientific Ltd, Oldham, U.K.).

**Experiment 1: stomatal response in intact rooted cuttings**

Ten well-rooted, 75-day-old cuttings were moved from the CPE to the growth cabinet ($D_a$ of 1.55 kPa). The first roots normally emerge after 20–30 days and the additional period in the CPE ensured that almost 100% of cuttings had an extensive root system. Leaves were dried gently with paper tissue and pots were sealed in polyethylene bags to prevent evaporation from the medium. Cuttings were weighed at 30-min intervals. After 120 min, when the rate of weight loss had stabilized, $g_s$ was measured on all leaves. Leaves were then excised and weighed ($W_f$) for determination of $R$. Leaves were rehydrated for 12 h under hessian shading in the CPE, to obtain the fully turgid weight ($W_i$), before drying in an oven for 3 days at 80 °C to determine the dry weight ($W_d$). Leaf $R$ was calculated as:

$$R = (W_i - W_f) / (W_i - W_d) \times 100.$$  

(1)

**Experiment 2: changes in stomatal behavior during rooting**

The response of stomata to leaf water deficit was determined on six occasions over the first 75 days after cuttings had been severed from stock plants. The response was quantified by monitoring the decline in transpiration rate of detached leaves as their water deficit increased (i.e., constructing a transpiration decline curve, Slavik 1974). With this method, the range of water content that could be imposed did not change as cuttings rooted. By adjusting $D_a$ it was possible to control the rate at which leaf water deficit increased.

The stem of whole shoots was cut under water (deionized and degassed) just above any adventitious roots (25–30 mm from the base) to ensure that leaves remained well hydrated at this stage. Before leaves were detached, the entire shoot was placed in the growth cabinet for 3 h to equilibrate, by which time rates of water loss (determined gravimetrically) had stabilized.

Two leaves were then excised: the oldest fully expanded leaf (designated L1), and the youngest leaf with an unfolded lamina (designated L3, typically the second above L1) had mean projected areas of 98 ± 3.6 and 27 ± 1.0 cm$^2$, respectively. The leaves were weighed at 5-min intervals until they had lost ≥ 30% of their initial, fresh weight ($W_i$). The $W_f$ was
then determined as described above and \( R \) estimated.

Rate of weight loss (i.e., transpiration, \( E \)) of each leaf was plotted against \( R \). The shape of the curve provided a measure of the responsiveness of the stomata to increasing leaf water deficit. To facilitate comparisons, a responsiveness index was derived from each curve, defined as the change in \( E \) between 90 and 70% \( R \) (\( E_{90} \) and \( E_{70} \), respectively):

\[
\text{Responsiveness index} = \frac{(E_{90} - E_{70})}{E_{90} \times 100}.
\]

Both \( E_{90} \) and \( E_{70} \) were estimated from regression equations derived with Genstat 5. Straight lines were fitted unless there was evidence of curvature, in which case an asymmetrical sigmoid curve (Gompertz curve) generally provided a good fit. Where neither of these was appropriate, a third-order polynomial was used.

**Experiment 3: stomatal and photosynthetic responses to acclimatization**

In Experiment 3, 24 well-rooted, 77-day-old cuttings were transferred to an acclimatization chamber. Chamber conditions were set so that there was no leaf wetting and RH was maintained at 91–98% (day/night RH equivalent to a \( D_a \) of 0.20/0.05 kPa) but in other respects the conditions were similar to the propagation environment described above. After 57 days of acclimatization, we tested stomatal responsiveness in detached leaves as described above. Data were subjected to analysis of variance (ANOVA) with Genstat 5.

**Measurements of net photosynthetic rate and leaf conductance**

A steady-state porometer (PMR-1; PP-Systems, Herts, U.K.) was used for all leaf conductance measurements (\( g \)). Corylus maxima is hypostomatous, therefore measurements on the abaxial surface give a measure of stomatal conductance (\( g_s \)). Measurements of \( g_s \) included a component of cuticular conductance (\( g_c \)), estimated by measuring conductance on the adaxial surface. Measurements of \( g_s \) were made at the end of the equilibration phase, just before leaf detachment, and at the end of the transpiration decline curve procedure when water deficit was maximal (hereafter referred to as pre-\( g_s \) and post-\( g_s \), respectively).

Photosynthetic rate (\( A \)) was measured with a portable LCA 2 IRGA (ADC BioScientific Ltd., Hoddesdon, U.K.) equipped with a PLC-B leaf cuvette (ADC BioScientific Ltd.) at a range of \( Q \) provided by low voltage halogen lamps at a variable voltage. Second-order polynomials provided the best-fit curves for the light response to photosynthetic rate and were fitted with Genstat 5.

**Low temperature scanning electron microscopy (SEM)**

Samples were frozen-hydrated and viewed with a Hexland CT1000 Cryotrans System attached to a Cambridge Instruments S200 scanning electron microscope (SEM). Leaf segments were attached to the sample holder with Leit-C (Neubauer, Münster, Germany) conductive carbon cement, then frozen by plunging in nitrogen slush at about –210 °C, transferred to the SEM with the vacuum transfer device and viewed uncoated at 10 kV. Contaminating surface ice was sublimated (etched) with a cold stage heater, set at –75 °C. Samples were gold sputter coated in the cryo-preparation chamber for 5 min (1.5 kV and 1.5–2 mA) and returned to the SEM cold stage. Photography was at about –180 °C, using an accelerating voltage of 10 kV.

**Results**

**Experiment 1: stomatal response in intact rooted cuttings**

Previously, we observed that when well-rooted cuttings of *C. maxima* were moved out of the fog environment in which they had rooted, the majority showed visible signs of leaf water deficit such as wilting. Therefore, we measured leaf water status and \( g \) (\( g_s + g_c \)) over a 2-h period following transfer to a controlled environment at a \( D_a \) of 1.55 kPa (Figures 1A and 1B). After 2 h, some leaves were severely curled, whereas others showed no signs of stress (Figure 1A). At this time,

![Figure 1](https://academic.oup.com/treephys/article-abstract/21/8/489/1684809/181684809)

**Figure 1.** Relationships between leaf area, relative water content (R), and visible symptoms of stress (A), leaf area and conductance (B) in 75-day-old well-rooted cuttings of *C. maxima* (Experiment 1). Leaf conductance (\( g \)) was measured on the adaxial surface to provide a measure of cuticular conductance (\( g_c \)), and the abaxial surface for stomatal (\( g_s \) + cuticular conductance. Cuttings were transferred from fog and placed in a growth cabinet for 130 min at a \( D_a \) of 1.55 kPa before measurement. Data are for individual leaves from 10 cuttings.
whole-cutting $E$ was almost constant ($26.6 \pm 1.71$ mg m$^{-2}$ s$^{-1}$). Visible symptoms of water deficit were generally more frequent and severe in the younger, smaller leaves than in the older, larger leaves. Although this observation was confirmed by the $R$ data (Figure 1A), it is evident from the values for individual leaves that the degree of leaf curl was not a reliable indicator of the magnitude of leaf water deficit.

There was a strong negative correlation between $R$ and $g_s$ ($P < 0.001$; Figure 1), suggesting that stomata were failing to close sufficiently to limit leaf water deficit, particularly in the younger leaves. Measurements of the upper, adaxial surface indicated that $g_s$ averaged $17 \pm 4.1$ mmol m$^{-2}$ s$^{-1}$, which was higher than expected based on other studies (e.g., 1–5 mmol m$^{-2}$ s$^{-1}$; Kerstiens 1996). We note that the high mean $g_s$ was partly a result of occasionally high values of $g_s$ in some smaller leaves, which was most likely attributable to localized damage to the leaf surface (e.g., cracks or fungal colonies).

Root dry weight, which has been shown to relate to root system hydraulic conductance (Ford and Harrison-Murray 1997), was variable (range 0.100–0.810 mg, data not shown) compared with the leaf area it was supplying. There was no correlation between $R$ and root dry weight ($P = 0.89$), indicating that variation in water supply to the base of the stem was not a significant source of variation in water deficit.

**Experiment 2: changes in stomatal behavior during rooting**

To follow the development of stomatal dysfunction, stomatal response to leaf water deficit was measured in detached leaves on six occasions between 0 and 75 days after the cuttings had been severed from the stock plants. The response was measured as the decline in $E$ of detached leaves as $R$ decreased (i.e., the transpiration decline curve).

Figure 2 shows the relationship between $E$ and $R$ for leaves taken at the beginning and end of the experiment and illustrates marked changes in stomatal behavior. On Day 0, $E$ dropped rapidly as $R$ decreased, reaching < 4 mg m$^{-2}$ s$^{-1}$ at $R = 70\%$, whereas on Day 75, $E$ remained > 10 mg m$^{-2}$ s$^{-1}$ at the same $R$. This reduction in responsiveness was more marked in L3 than in L1. There was also an increase in $E$ at high $R$ (i.e., the first measurement following detachment) that was more marked in L3 than in L1.

A responsiveness index was derived from the transpiration decline curves (cf. Figure 2) based on the proportionate decrease in $E$ as $R$ decreased from 90 to 70%. Figure 3 shows that a decrease in responsiveness of L3 was already evident at Day 7, long before the first roots emerged (Days 20–30). Compared with L3, the decrease in responsiveness was less pronounced in L1 and was significant only on Day 75. When $D_a$ of the air was reduced fivefold, to reduce the rate at which the leaf water deficit developed, there was a small but consistent increase in the responsiveness index, except on Day 0 (Figure 3).

In addition to the gravimetric measurement of $E$, $g_s$ was measured before and after the transpiration decline procedure (i.e., at high and low $R$). These measurements showed that the decline in responsiveness in L3 over the first week was accompanied by a fourfold increase in $g_s$ in the intact cutting (pre-$g_s$, Figure 4A). Mean $g_s$ in L3 continued to increase and was $534 \pm 30.5$ mmol m$^{-2}$ s$^{-1}$ by Day 75. In contrast, $g_s$ in L1 showed a

![Figure 2. Relationships between transpiration rate ($E$) and relative water content ($R$) at a $D_a$ of 0.70 kPa on Day 0 (A and B) and Day 75 (C and D) for L1 (A and C) and L3 (B and D) leaves (Experiment 2). Curves were fitted by regression as described in the text. Different symbols are used for each replicate leaf.](https://academic.oup.com/treephys/article-abstract/21/8/489/1684809)

![Figure 3. Changes in responsiveness index of L1 and L3 between Days 0 and 75 after cuttings had been severed from the stock plants (Experiment 2). The responsiveness index was calculated from the proportionate decrease in transpiration associated with a reduction in leaf $R$ from 90 to 70%. Each value is the mean of three measurements ± SEM derived from transpiration decline curves for individual leaves at a $D_a$ of 0.70 or 0.14 kPa.](https://academic.oup.com/treephys/article-abstract/21/8/489/1684809)
smaller initial increase than in L3 ($P < 0.01$), remained essentially constant and was only $328 \pm 28.2 \text{ mmol m}^{-2} \text{s}^{-1}$ by Day 75. On Day 0, $g_s$ in L3 was only half that in L1 ($P < 0.001$; Figure 4A), consistent with measurements made on plants in the field.

Stomatal conductance at the end of the transpiration decline curve procedure (post-$g_s$, Figure 4B) provided an additional measure of the ability of stomata to control water loss. Both L1 and L3 showed a gradual increase in post-$g_s$ during rooting, the increases being significantly greater in L3 than in L1.

Porometer measurements cannot unambiguously distinguish high $g_s$ from high $g_c$, but scanning electron micrographs (SEM) showed that the unusually high $g$ of the abaxial surface of L3 was associated with stomata approaching their maximum aperture (Figure 5B). Apertures were in the region of 6.5 µm, compared with 0.5–1.5 µm in L3 in the field (Figure 5A).

**Experiment 3: stomatal and photosynthetic response to acclimatization**

Acclimatization increased the responsiveness index of L1 from 26 to 42% ($P < 0.05$), but had no significant effect on responsiveness of L3 (Figure 6). Furthermore, even after acclimatization, the responsiveness of L1 remained only about half that of freshly harvested cuttings in Experiment 2 (i.e., 70–90%, Figure 3). Acclimatization reduced pre-$g_s$, in L3 by more than half ($P < 0.05$), but had no effect on pre-$g_s$ in L1 (Figure 7), indicating that acclimatization reduced water loss of both types of leaf, but in different ways. In L3, acclimatization reduced $g_s$ before detachment, whereas in L1 it enhanced the response to leaf water deficit that developed after detachment.

Photosynthetic rates of L1 and L3 were measured before and 17 days after acclimatization (Figure 8). At the $Q$ of the rooting environment, $A$ was $3.2 \pm 0.21 \text{ mmol m}^{-2} \text{s}^{-1}$. Photosynthetic rates were significantly higher ($P < 0.001$) in L1 than in L3, irrespective of acclimatization (Figure 8). Following acclimatization, both leaf types had significantly higher ($P < 0.001$) $A$ than leaves taken directly from rooted cuttings growing in the fog rooting environment.

**Discussion**

*Corylus maxima* leaves frequently show signs of severe water deficit when rooted cuttings are removed from the wet and humid rooting environment required to achieve high rooting success. We tested the hypothesis that such water stress results from stomatal dysfunction, preventing sufficient stomatal closure to keep transpiration within the capacity of the new root system to supply water. The positive correlation between water deficit and $g_s$ with $g_c > 500 \text{ mmol m}^{-2} \text{s}^{-1}$ in leaves that were visibly wilting, provided strong support for stomatal dysfunction. We also explored the time course of development of stomatal dysfunction and its reversibility as a first step toward understanding the physiology of the dysfunction.

Because of the many reports of increased $g_s$ in plantlets cultured *in vitro* (e.g., Wardle et al. 1979, Sutter 1988), we examined the possibility that high $g_s$ could contribute to impaired control of water loss in *C. maxima* cuttings. Some increase in $g_c$ of the adaxial surface was detected but $g_{\text{adaxial}}$ remained an order of magnitude lower than $g_{\text{abaxial}}$. Although it is theoretically possible for $g_c$ to increase more on the adaxial surface than on the adaxial surface, the SEMs showed a high density of extremely wide-open stomata (Figure 5), indicating that the increase in $g_c$ was unlikely to have contributed substantially to $g_{\text{abaxial}}$.

The stomatal pores in L3, but not in L1, became almost circular, a feature associated with stomatal dysfunction *in vitro* (Ziv et al. 1987, Zacchini et al. 1997), perhaps because development of these stomata was completed in the unnaturally wet and humid rooting environment. This finding is consistent with the results for *Prunus cerasicera L.*, showing a gradual decline in stomatal function during leaf development *in vitro* (Zacchini and Morini 1998). Furthermore, it would explain why stomatal behavior changed much more rapidly in L3 than in L1 where stomata had developed *in vivo*. The mechanism for such stomatal dysfunction remains unclear, but may be as-

![Figure 4](https://academic.oup.com/treephys/article-abstract/21/8/489/1684809/1684809)
sociated with reduced cellulose deposits during guard cell development (Marin et al. 1988), possibly leading to an alteration in cell wall mechanics and a reduced ability of stomata to close (Ziv et al. 1987).

A shoot excised as a cutting is isolated from its normal supply of root-derived hormones such as abscisic acid (ABA) and cytokinins, as well as its normal source of water and nutrients. Any of these perturbations, alone or in combination with the unnatural rooting environment, could be involved with disruption of normal stomatal function. For example, lack of root-derived ABA might contribute to the rapid increase in $g_s$ in L3 (Figure 5). However, a rapid decrease in $g_s$ in cuttings is normally associated with a leaf water deficit (Gay and Loach 1977, Svenson et al. 1995). Under the conditions of the highly controlled environment used to root cuttings in our experiments, _C. maxima_ leaves did not suffer from substantial leaf water deficits. Before root formation, midday water potentials of L1 and L3 were about $-0.6 \pm 0.03$ MPa, compared with $-1.5 \pm 0.02$ MPa in plants growing in the field (data not shown). Maintenance of such high water potentials appears to be necessary to maximize rooting percentage (R.S. Harrison-Murray, unpublished data), but may have contributed to the observed increase in $g_s$ and the loss of stomatal responsiveness.

Various forms of acclimatization (e.g., reduced $D_a$) have been shown to reduce conductance and improve survival rates of in-vitro-grown plants (Díaz-Pérez et al. 1995, Sciutti and Morini 1995, Fila et al. 1998). We found that a long period
without leaf wetting, at a $D_a$ of 0.20 kPa, resulted in only small changes in stomatal behavior. In L3, initial $g_s$ was halved in response to acclimatization but there was no restoration of responsiveness. In L1, acclimatization caused a small but significant restoration of responsiveness, but no decrease in initial $g_s$. These results suggest that the main benefit of acclimatization for $C.\text{maxima}$ cuttings is an increase in water supply from the roots as a result of continued growth of the adventitious root system. If this suggestion is correct, then the benefit of acclimatization will accrue quite slowly and at a rate determined by $A$.

Photosynthetic light response curves showed that both L1 and L3 could maintain a substantial $A$ at 94 days after severance, that L1 was significantly more efficient than L3, and that assimilation increased up to a $Q$ of $\geq 400 \mu\text{mol m}^{-2}\text{s}^{-1}$. This indicates that the supply of assimilates for root growth would tend to be limited by the maximum irradiance that was compatible with avoidance of severe dessication. This implies that the most effective acclimatization environment for $C.\text{maxima}$ should be one in which $D_a$ is kept close to zero while increasing irradiance.

Whether an individual cutting survives the transition from the rooting environment depends on the complex interplay of many variables that influence its water balance. The rate of water loss from the plant as a whole depends on total leaf area, mean leaf conductance, and leaf to air vapor pressure difference ($D_{l-a}$), which itself depends on $D_a$ and irradiance. When stomatal function is impaired, as has been shown in $C.\text{maxima}$ cuttings, avoidance of damaging water deficits depends on the hydraulic conductance of the root system (and the associated root–soil interface), which determines the maximum rate at which water can be supplied to the leaves. Under these circumstances, boundary layer conductance ($g_b$) may take the place of $g_s$ as the major constraint on water loss. Therefore, minimizing air movement, and consequently $g_b$, in the acclimatization environment should reduce the risk of dessication despite increases in $D_{l-a}$. Thin polyethylene sheeting, laid directly on cuttings is widely used and should achieve low $g_b$ as well as high humidity. However, given the great variability of natural irradiance, such systems provide poor control of $D_{l-a}$ and therefore rather variable results. For this reason, artificial lighting, such as used in the CPE, provides opportunities for more reliable acclimatization of rooted cuttings.

In conclusion, control of water loss in cuttings of $C.\text{maxima}$ rooted in fog is severely disrupted by changes in stomatal function. Stomata open unusually wide and fail to respond sufficiently to leaf water deficit to protect against permanent wilting when evaporative demand is high. Because stomatal dysfunction was not reversed by exposure to an acclimatization environment and was more severe in leaves that completed their development during rooting than in leaves that were fully

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**Figure 6.** Responsiveness index of L1 and L3 following 57 days of acclimatization of the cuttings at $D_a$ of 0.20 kPa compared with non-acclimatized cuttings (Experiment 3). Error bar represents the least significant difference (LSD) at $P = 0.05$. (Probabilities of $F$ values from ANOVA: leaf type = 0.029; acclimatization = 0.056; leaf × acclimatization = 0.047).

**Figure 7.** Stomatal conductance before leaf detachment (pre-$g_s$) and at the end of the transpiration decline curve procedure (post-$g_s$) in L1 and L3 from non-acclimatized (Non-acc) and acclimatized (Acc) cuttings (Experiment 3). Values are means of six leaves and error bars represent the LSD at $P = 0.05$ for comparisons within pre-$g_s$ or post-$g_s$ measurements.

**Figure 8.** Photosynthetic light response curves for L1 and L3 on well-rooted cuttings growing in fog (---, Non-acc), and after 17 days in an acclimatization environment (----, Acc) that provided a $D_a$ of 0.20 kPa (Experiment 3).
formed before rooting began, we believe that the dysfunction is associated with permanent structural changes rather than to temporary disturbance of ABA supply or other chemical factors. Studies are in progress to test this hypothesis and explore the relevance of these findings in the context of other species and other environments.

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