Influence of irradiance on water relations and carbon flux during rooting of Shorea leprosula leafy stem cuttings

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Summary Single-node leafy stem cuttings of Shorea leprosula Miq. were subjected to a high, intermediate or low irradiance treatment for 16 weeks in an enclosed mist propagation system. Before rooting, maximum photosynthesis of the cuttings occurred at an irradiance of 400 μmol m⁻² s⁻¹. Although none of the irradiance treatments affected the number of roots produced per cutting, the numbers of cuttings that formed roots were 50 and 30% in the high irradiance (diurnal range of 0--658 μmol m⁻² s⁻¹) and low irradiance (diurnal range of 0--98 μmol m⁻² s⁻¹) treatments, respectively, compared with 62% in the intermediate irradiance treatment (diurnal range of 0--360 μmol m⁻² s⁻¹). Low rooting frequency of cuttings in the high irradiance treatment was associated with water deficits (maximum leaf-to-air vapor pressure deficit (VPD) = 3.6 kPa), whereas cuttings in the low irradiance treatment had a low rooting frequency because they were below the light compensation point most of the time. In the intermediate irradiance treatment, cuttings withstood a daily maximum VPD of 1--2 kPa and recovered overnight from the previous day’s deficit, as indicated by higher relative water content (RWC) and stomatal conductance (gₛ) in the morning than in the previous afternoon and evening. Higher RWC and gₛ of cuttings in all treatments on Days 14 and 21 compared with Day 8 probably indicated recovery from water deficit following severance and insertion of the cuttings in rooting medium. There were negative relationships between stem volume of cuttings and both number of cuttings that rooted and number of roots per cutting.

Keywords: leaf-to-air vapor pressure deficit, photosynthesis, relative water content, stomatal conductance, vegetative propagation.

Introduction

Shorea leprosula Miq. (Dipterocarpaceae) is an important timber species in southeastern Asia (Symington 1974). To sustain the supply of harvestable timber, the species has been planted in deforested areas (Azman et al. 1991), but these planting programs have been hindered by poor supplies of viable seeds (Tang 1971, Tamari 1976, Sasaki 1980). To overcome the shortage of planting stock, a method for propagating the species from stem cuttings has been developed; however, rooting of the cuttings is highly variable. Because irradiance has a pronounced effect on rooting in many species, we hypothesized that the variability in rooting of S. leprosula cuttings is associated with variations in irradiance during propagation.

During propagation, the primary effects of irradiance are on assimilate production and water use of the cuttings (Eliasson and Brunes 1980, Klass et al. 1985, Hartmann et al. 1990). High irradiances increase leaf temperature causing an increase in leaf-to-air vapor pressure difference, and thus an increase in transpiration rate (Grange and Loach 1983a, 1983b, Loach 1988a, 1988b). Rapid transpiration is often lethal to cuttings that have not rooted. Moreover, the warming of air is likely to increase the saturation deficit over the leaf, exacerbating the process of desiccation (Evans 1952, Kemp 1952, Hess and Snyder 1955, Loach 1988a, 1988b, Hartmann et al. 1990). To reduce the effect of high irradiance on cuttings, propagators are shaded (Loach 1977, Loach and Whalley 1978, Loach and Gay 1979); however, there have been few attempts to quantify the effect of irradiance on dipterocarp cuttings during rooting (Moura-Costa and Lundoh 1994, Smits et al. 1994).

To test our hypothesis, we examined the effects of irradiance on leaf-to-air vapor pressure deficit (VPD), relative water content (RWC), stomatal conductance (gₛ) and photosynthetic rate (Pₛ) of S. leprosula stem cuttings during rooting on propagation beds.

Materials and methods

Materials and experimental design

In February 1994, 531 single-node, leafy stem cuttings were taken from seven-month-old stock plants raised in 33% of full sunlight as potted rooted cuttings at the Forest Research Institute of Malaysia. The stock plants comprised 17 clones. The number of cuttings obtained per stock plant varied from 3 to 7 depending on availability of leaves on the node. Cuttings were trimmed to 5 cm in length and 30 cm² in leaf area. The base of each cutting was treated with 20 μg of IBA in ethanol. The alcohol at the cutting base was immediately evaporated in a stream of air from a fan. The initial diameter and node position
of each cutting were recorded, and the volume of each cutting was calculated assuming a cylindrical shape. Prepared cuttings were planted in cleaned river sand in propagators located in a cutting shed.

Cuttings were subjected to one of three diurnal irradiance regimes adjusted by means of black plastic netting: (1) high irradiance (without net; 0–658 µmol m$^{-2}$ s$^{-1}$); (2) intermediate irradiance (one layer of netting; 0–360 µmol m$^{-2}$ s$^{-1}$); and (3) low irradiance (three layers of netting; 0–98 µmol m$^{-2}$ s$^{-1}$). Cuttings in the high irradiance treatment were not in natural sunlight because the roof of the cutting shed was shaded with a translucent plastic sheet. The mean red:far red ratio in the shaded propagators was 1.1 (SKR 110 660/730, Skye Instruments Ltd., Llandrindod Wells, U.K.), which is close to the value of 1.2 of full sunlight. The cuttings were randomly allocated to nine blocks and there were three blocks per irradiance treatment (117 cuttings per treatment). Each block comprised a closed polyethylene propagator (1 m length × 1 m width × 0.8 m height) with a misting unit in the center that was set to mist for 1 min every hour for 24 h per day. Each time the propagators were opened, the cuttings were sprayed with a hand sprayer to minimize sudden changes in humidity.

**Microclimate in the propagator**

Air and leaf temperatures were measured with thermocouples (Type K chromel-alumel, T.C., Ltd., Uxbridge, U.K.); relative humidity was measured with commercial humidity sensors (MP 100 Rotronic probes, Campbell Scientific Ltd., Loughborough, U.K.) and irradiance was measured with quantum sensors (Skye Instruments Ltd.). The sensors were placed in the center of one randomly chosen block of cuttings in each irradiance treatment. A polyvinyl chloride (PVC) tunnel was made to protect the humidity sensors from direct contact with water. To measure leaf temperature, thermocouples were supported on an aluminum label inserted in the rooting medium so that the sensors touched the underside of the leaf. Signals from the sensors were recorded by a data logger (21X micrologger, Campbell Scientific Ltd.). The data logger was programmed to scan each sensor every 60 s, and to calculate and store mean readings every 5 min. Data were collected from Day 1 to Day 25 of the experiment.

**Relative water content (RWC)**

Leaf RWC was determined as described by Beadle et al. (1987) at 0900, 1300 and 1700 h on Days 1, 8, 14 and 21 on four leaf discs per cutting from six cuttings per treatment. Leaf discs (18 mm) were taken from cuttings with a cork borer, and the fresh (FW), turgid (TW) and dry (DW) weights measured. The turgid weight was determined after floating the discs in distilled water in covered vials for 24 h. Discs were blotted with paper towel before weighing. Dry weight was obtained after drying the discs at 80 °C for 48 h. Leaf RWC was calculated as:

$$ RWC = \frac{FW - DW}{TW - DW} \times 100. $$

**Photosynthetic rate ($P_n$) and stomatal conductance ($g_s$)**

The $P_n$ and $g_s$ of cuttings were measured with an infrared gas analyzer (LCA-3, Analytical Development Co., Hoddesdon, U.K.). Each measurement of gas exchange was recorded when the CO$_2$ differential readings were stable for 30 to 40 s. At each measurement, one leaf was measured per treatment per block. Before each measurement, the leaf surface was blotted with paper towel to remove free water on leaf surfaces. During each measurement, additional artificial light supplied by an incandescent tungsten lamp was used to increase the photosynthetically active radiation (PAR) to ≥ 500 µmol m$^{-2}$ s$^{-1}$. Diurnal changes in $P_n$ were measured for 10 consecutive days after the cuttings were planted in rooting medium. In another set of experiments, $P_n$ and $g_s$ were measured at 0900, 1300 and 1700 h on Days 8, 14 and 21 on four randomly chosen cuttings per treatment per block. Dark respiration ($R_d$) was measured at night and before dawn on the same cuttings.

**Assessment of cuttings**

Forty-five cuttings per treatment were randomly harvested at 0900 h on Day 1 and leaf and stem dry weights of each cutting were determined after drying at 40 °C for 48 h.

Assessments of rooted, dead and unrooted cuttings as well as number of roots per cutting were made weekly until Week 16. A cutting was considered rooted when it produced a root of 1 cm or more in length, and a cutting was considered dead when the whole stem turned brown. The mean accumulated number of roots per rooted cutting was calculated by dividing the total number of roots produced by the total number of rooted cuttings.

**Statistical analyses**

Analysis of variance was used to test for significant differences in mean accumulated number of roots per rooted cutting at Week 16. Analysis of deviance for stepwise regression was used to determine which variables were significantly associated with accumulated rooting at Week 16. In the regression analysis, the association between each variable and rooting was indicated by regression coefficients and this association is presented graphically. Points on the graph were obtained by grouping observed rooting data with cutting volume (at intervals of 0.25 cm$^3$); the line was constructed by connecting rooting values of individual cuttings computed from the multiple regression model. Because other factors or variables were also fitted, the line drawn through the data points is not smooth.

For the diurnal $P_n$ data, a nonrectangular hyperbola was fitted using the model described by Jarvis et al. (1985), with measured values of PAR, $P_n$ and $g_s$ as the input variables. The parameter optimization utility of the Genstat 5 software package (Payne et al. 1987) was used to estimate parameter values of (i) dark respiration ($R_d$); (ii) mesophyll conductance ($g_m$); (iii) the initial slope of the $P_n$ versus PAR curve ($\alpha$, apparent quantum efficiency); and (iv) the convexity coefficient ($\theta$), which defines the degree of curvature between the initial slope and the asymptotic value of $P_n$. With the parameter estimates, $P_n$ versus PAR curves were plotted using values for the mean $g_s$ measured on leaves of cuttings for each irradiance treatment. Maximum $P_n$ ($P_{n,max}$) was calculated as:

$$ P_n = (C_l - \Gamma)g_m. $$
where $\Gamma$ is the CO$_2$ compensation concentration and was assumed to be 50 µmol mol$^{-1}$ (Dick et al. 1991) and $C_i$ (internal CO$_2$ concentration) was the mean value obtained for PAR higher than 400, 300 and 300 µmol m$^{-2}$ s$^{-1}$ for high, intermediate and low irradiance treatments, respectively. These PAR values were chosen because there was no significant difference in $C_i$ with increasing PAR values.

Treatment differences between the $P_n$ versus PAR curves were determined by a combined curve analysis of variance (Ross 1981), which tests the reduction in residual variance obtained by fitting a set of individual curves that are then compared with the residual variance from the common curve determined from combining the $P_n$ data obtained at all PAR values.

Results were considered to be significant at $P \leq 0.05$.

**Results**

Environmental conditions in the propagators averaged over a 25-day period are given in Table 1. The PAR values in the low, intermediate and high irradiance treatments were 20, 83 and 152 µmol m$^{-2}$ s$^{-1}$, respectively. Mean maximum VPD was highest in the high irradiance treatment and lowest in the low irradiance treatment. The daily maximum VPD in each treatment is shown in Figure 1.

At the start of the experiment, the mean diameters of the cuttings were 0.34, 0.37 and 0.34 cm, and the mean volumes of the cuttings were 0.50, 0.57 and 0.49 cm$^3$ for cuttings in the high, intermediate and low irradiance treatments, respectively. The corresponding values for mean leaf weight were 0.21, 0.20 and 0.21 g and for mean stem weight 0.16, 0.18 and 0.18 g.

The irradiance treatments had a significant effect on the number of cuttings that rooted. Fewer cuttings rooted in the low irradiance treatment than in the other treatments (Figure 2a). Rooting frequency was negatively correlated with initial cutting volume (Table 2 and Figure 2b).

Leaf shedding and mortality of cuttings were significantly higher in the low irradiance treatment than in the other treatments (Figure 2c). Irradiance effects on Shorea cuttings.
ments (Figures 3a and 3c). Regression analysis indicated that mortality and cutting volume were positively associated (Figure 3b). The number of roots per rooted cutting was not significantly affected by the irradiance treatments (Figure 4a); however, it was significantly and negatively affected by initial cutting volume (Figure 4b).

The RWC of the cuttings decreased with increasing irradiance (Figure 5a). Across all treatments, RWC varied during the day with a high of 86% at 0900 h, falling to 83% at 1300 h and recovering slightly at 1700 h. On Day 8 after insertion of cuttings in rooting medium, RWC was 78% compared with values of 87 and 86% on Days 14 and 21, respectively. Stomatal conductance, $g_s$, followed the same diurnal pattern as RWC. It was highest at 0900 h with a value of 302 mmol m$^{-2}$ s$^{-1}$ followed by 115 and 123 mmol m$^{-2}$ s$^{-1}$ at 1300 and 1700 h, respectively.

Photosynthetic rate of cuttings was significantly lower in the low irradiance treatment than in the other irradiance treatments (Figure 5b) and the carbon balance was negative in cuttings in the low irradiance treatment. Combined curve analysis of variance of curves fitted using the theoretical model of Jarvis et al. (1985) indicated that there were significant treatment

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**Figure 2.** (a) Effect of irradiance on rooting rate of stem cuttings ($n = 60$ per treatment). (b) Relationship between rooting and cutting volume of stem cuttings. Circles represent groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model.

**Figure 3.** (a) Effect of irradiance on percentage of dead stem cuttings ($n = 60$ per treatment). (b) Relationship between dead cuttings and cutting volume. Circles represent groups of observed data and line was drawn by connecting predicted values computed from the multiple regression model. (c) Effect of irradiance on the rate of leaf shedding of stem cuttings ($n = 60$ per treatment).

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**Table 2.** Analysis of deviance for a stepwise regression to determine the effects of irradiance treatments and morphological characteristics on rooting ability of stem cuttings at Week 16 ($n = 60$ per treatment; $^*$ = significant at $P \leq 0.01$; and ns = not significant at $P \leq 0.05$).

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Deviance</th>
<th>Mean deviance</th>
<th>Deviance ratio</th>
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</thead>
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<tr>
<td>Blocks</td>
<td>2</td>
<td>3.26</td>
<td>1.63</td>
<td>1.27 ns</td>
</tr>
<tr>
<td>Irradiances</td>
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<td>12.86</td>
<td>6.43</td>
<td>5.02*</td>
</tr>
<tr>
<td>Cutting volume</td>
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<td>10.60</td>
<td>10.60</td>
<td>8.28*</td>
</tr>
<tr>
<td>Residual</td>
<td>174</td>
<td>222.25</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>248.97</td>
<td></td>
<td></td>
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</tbody>
</table>

1 Regression coefficient.
differences in the overall predicted $P_n$ curves (Table 3). The PAR required for maximum photosynthesis was around 400 $\mu$mol m$^{-2}$ s$^{-1}$, and higher PAR did not result in further increases in $P_n$ of cuttings (Figures 6a–c). Estimated values of $g_m$, $\alpha$, $P_{n,\text{max}}$ and $R_d$ of cuttings in each irradiance treatment are given in Table 4. Dark respiration rate was significantly higher in cuttings in the high irradiance treatment than in the other treatments.

Discussion and conclusions

The low irradiance treatment significantly reduced the number of $S$. leprosula stem cuttings that rooted (0–98 $\mu$mol m$^{-2}$ s$^{-1}$; about 5% full sunlight). Poor rooting at low irradiances has been observed previously. For example, Eliasson and Brunes (1980) reported that cuttings of $Populus$ tremula L. $\times$ tremuloides Michx. and $Salix$ caprea L. $\times$ viminalis L. did not root at irradiances of less than 2 W m$^{-2}$ (about 0.3% full sunlight). Similarly, only 9% rooting was observed in $Prosopis$ alba Griseb. cuttings grown at irradiances of less than 150 $\mu$mol m$^{-2}$ s$^{-1}$ (about 8% full sunlight) (Klass et al. 1985). Both Eliasson and Brunes (1980) and Klass et al. (1985) concluded that current photosynthate was essential for rooting, although no measurements of photosynthesis were made in either study. In our study, poor rooting in the low irradiance treatment was
Table 4. Estimated parameter values from the theoretical model of Jarvis et al. (1985) and maximum photosynthetic rate ($P_{n,max}$). Measurements of photosynthetic rate ($P_n$) were made for 10 days after cuttings were planted in rooting beds in a high, intermediate or low irradiance treatment propagator. During measurements, the cuttings were artificially illuminated by a tungsten lamp to increase the maximum irradiance in each treatment to $\geq 500 \mu\text{mol m}^{-2}\text{s}^{-1}$ ($n = 228$ and 48 for $P_n$ and $R_d$ measurements, respectively; $\alpha = $ initial slope of $P_n$/PAR curve (apparent quantum efficiency); $g_m =$ mesophyll conductance; $R_d =$ dark respiration; and $\theta =$ convexity coefficient).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\alpha$ ($\text{mol CO}_2,\text{photon}^{-1}$)</th>
<th>$g_m$ ($\text{mol CO}_2,\text{m}^2\text{s}^{-1}$)</th>
<th>$R_d$ ($\mu\text{mol CO}_2,\text{m}^2\text{s}^{-1}$)</th>
<th>$\theta$</th>
<th>$P_{n,max}$ ($\mu\text{mol CO}_2,\text{m}^2\text{s}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High irradiance</td>
<td>0.06</td>
<td>0.0092</td>
<td>0.85</td>
<td>0.00</td>
<td>2.54</td>
</tr>
<tr>
<td>Intermediate irradiance</td>
<td>0.03</td>
<td>0.0083</td>
<td>0.77</td>
<td>0.94</td>
<td>2.29</td>
</tr>
<tr>
<td>Low irradiance</td>
<td>0.04</td>
<td>0.0083</td>
<td>0.71</td>
<td>0.13</td>
<td>2.43</td>
</tr>
</tbody>
</table>

Figure 6. Curves of $P_n$ versus PAR for stem cuttings before rooting at (a) high irradiance, (b) intermediate irradiance, and (c) low irradiance ($n = 228$). Measurements were made for a period of 10 days; maximum irradiance for each treatment was achieved with a tungsten lamp.

Associated with low $P_n$, Because the rooting of cuttings of several tree species is influenced by the carbon balance of the cuttings at the time they are severed and inserted in rooting medium, it has generally been concluded that photosynthesis must precede the onset of rooting (Davis 1988, Leakey and Storeton-West 1992, Newton et al. 1992, Hoad and Leakey 1993, Mesen 1993). Furthermore, it has been postulated that low $P_n$ indirectly reduces rooting by slowing basipetal transport of auxin and other rooting cofactors that may originate from leaves and buds (cf. Davis 1988).

In certain species, e.g., *Hibiscus rosa sinensis* L., leafy cuttings root equally well in darkness or moderate irradiance (van Overbeek et al. 1946), indicating that current photosynthetic is not a prerequisite for rooting of cuttings of all species. However, *Hibiscus* cuttings root within one week, which may explain the independence of rooting on current photosynthate. By contrast, in species characterized by a longer rooting period, even cuttings with substantial carbohydrate reserves, such as *Picea abies* L. (Karst.), may require additional photosynthate to compensate for the depletion of carbohydrate reserves during the rooting process (Strömquist and Eliasson 1979). We conclude that *S. leprosula* stem cuttings require current photosynthate because the rooting period is 6 to 8 weeks in this species. Furthermore, mortality of cuttings was high (63%) in the low irradiance treatment and was associated with a high percentage of leaf shedding (60%).

The irradiance treatments resulted in differences in the estimated parameters obtained from the $P_n$ model. The $g_m$ value was slightly higher in the high irradiance treatment than in the other treatments. In general, the estimated $g_m$ values for *S. leprosula* (mean $g_m = 0.008 \mu\text{mol CO}_2\,\text{m}^{-2}\text{s}^{-1}$) were comparable to those for other climax species; e.g., *Blighia sapida* K. König and *Strombosia pustulata* Oliv. (Riddoch et al. 1991). The value of $\alpha$ in the high irradiance treatment was slightly greater than in the other treatments, suggesting that the quantum efficiency of *S. leprosula* is sensitive to differences in irradiance. These results are in contrast with those obtained by Riddoch et al. (1991), Ramos and Grace (1990), and Kweesiga et al. (1986) who found that $\alpha$ is insensitive to environmental conditions. The rate of dark respiration was lower at low irradiance than at high irradiance. Similarly, Bazzaz and Picket (1980) observed that respiration rates of shade-acclimatized leaves of climax species were lower than those of unshaded leaves of pioneer species.

Lower rooting at high irradiance than at intermediate irradiance could be the result of water deficits in cuttings in the higher irradiance treatment. Also, at high irradiance, carbohydrates may be used for maintenance respiration rather than for root formation (Hartmann et al. 1990). Mesen (1993) showed that rooting of *Cordia alliodora* (Ruiz & Pav.) Oken. stem...
cuttings was significantly reduced at high irradiances (0–1460 μmol m⁻² s⁻¹) compared with low irradiances (0–339 μmol m⁻² s⁻¹), and concluded that the reduction in rooting was associated with water deficits in the cuttings and with damage to the photosynthetic apparatus as indicated by a decline in leaf chlorophyll fluorescence.

Rooting of *S. leprosula* stem cuttings was negatively associated with the initial volume and diameter of the cuttings. The larger cuttings may have undergone secondary growth and greater lignification than the smaller cuttings, and lignin is known to create a physical barrier to root initiation (Hartmann et al. 1990). Lignified cuttings often root poorly and die when their carbohydrate reserves are depleted (Hartmann et al. 1990). An alternative explanation for the negative relationship between rooting and the initial volume of cuttings is that rooting may not depend on the carbohydrate reserves of the cutting. It is also possible that the carbohydrate reserve was not converted to sugar for root initiation.

At night (1900–0700 h), VPD in all irradiance treatments was low; however, periods of temporary water deficit occurred during the day, especially in the intermediate and high irradiance treatment propagators. These temporary water deficits did not affect rooting of *S. leprosula* stem cuttings in the intermediate irradiance treatment and even in the high irradiance treatment, 50% of the cuttings rooted despite a daytime maximum VPD of 3.6 kPa. Overnight recovery from these water deficits was reflected in a high RWC and g, the following morning. Increased RWC on Days 14 and 21 compared with Day 8 probably reflects recovery of the cuttings from a water deficit experienced at the time of severance and insertion into the rooting medium (cf. Mesen 1993, Newton and Jones 1993).

We observed large reductions in rooting and high mortality of *S. leprosula* stem cuttings grown in low irradiance and conclude that a reduction in photosynthesis may be the cause. Because photosynthetic activity of stem cuttings of *S. leprosula* saturated at a PAR of about 400 μmol m⁻² s⁻¹ (about 20% of full sunlight), we conclude that an irradiance of 0–360 μmol m⁻² s⁻¹ (about 0–18% full sunlight; propagator with one layer of netting) is most suitable for the propagation of cuttings of this species because it kept VPD low while supporting photosynthesis at about 80% of its maximum rate. Similar irradiance regimes are optimal for the rooting of other dipterocarp cuttings. For example, more than 60% rooting of *S. acuminata* Dyer and *S. parvifolia* Dyer cuttings was obtained at an average irradiance of 27 W m⁻² (about 3% of full sunlight, Noraini and Ling 1993); and 73 to 88% of *Dryobalanops lanceolata* Burck stem cuttings rooted in 290 μmol m⁻² s⁻¹ (about 15% full sunlight, Moura-Costa and Lundoh 1994).

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References


