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Survival and growth of drought hardened *Eucalyptus pilularis* Sm. seedlings and vegetative cuttings

D. S. Thomas

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Abstract Forestry requires low mortality of transplanted seedlings. Mortality shortly after planting is often associated with inadequate hydration of transplants. Seedlings can be hardened to the drought conditions they may experience after transplanting by exposing them to controlled drought conditions in the nursery. *Eucalyptus pilularis* Sm. seedlings were drought hardened by providing nil (severe treatment) or half (mild treatment) the daily irrigation routinely received (control treatment) for up to two non-consecutive days per week during the last 4 weeks of growth in the nursery. Drought hardening reduced stem diameter, seedling leaf area, leaf area per root biomass and seedling quality measured by the Dickson quality index, but increased root:shoot ratio. Hardened seedlings had lower stomatal conductance and leaf water potential on the days they received less irrigation that the control treatment. Hardened seedlings had greater stomatal conductance and were less water stressed than seedlings experiencing drought for the first time indicating hardened seedlings had adjusted physiologically to drought. Survival after transplanting in the controlled drought environment in a glasshouse was enhanced by the hardening treatments. Non hardened seedlings that had had their upper leaves manually removed immediately prior to transplanting to reduce leaf area (top-clipped) had similar survival to hardened seedlings. Stomatal conductance and leaf water potential after transplanting were higher in hardened and top-clipped seedlings than unhardened control seedlings or vegetative cuttings. Survival in the field trial was over 95% for all treatments, possibly as rain fell within 4 days of planting and follow-up rain occurred in the subsequent weeks. Neither the hardened or top-clipped seedlings planted in the field trial had reduced growth, increased propensity to form double leaders or worse stem form than control seedlings when measured at age 3 years.

Keywords Eucalyptus · Nursery · Survival · Hardening · Growth

D. S. Thomas (✉)
Forests NSW, Land Management and Forestry Services, P.O. Box J19, Coffs Harbour Jetty, NSW 2450, Australia
e-mail: danet@sf.nsw.gov.au

Introduction

Plantation resources are becoming increasingly important to world timber supplies. One of the major costs of plantation forestry are costs incurred in plantation establishment. These costs consist of procuring and planting seedlings, but if seedling mortality is high then additional costs of replanting will be incurred. Mortality and growth rates of plantation grown seedlings have been related to many factors, including seedling genotype and quality, planting site and soil moisture (Burdett 1990; Mattsson 1996; Stape et al. 2001; Campbell and Hawkins 2004; Close et al. 2005). Reducing the risk of seedling mortality associated with unfavourable conditions shortly after planting are seen as a management tool to increase the economic viability of plantation forestry, but it is likely the economic costs and benefits of these management interventions will differ with the particular species, site, and time of planting.

Soil moisture is probably the single most important factor in reducing seedling mortality of recently planted seedlings (Grossnickle 2005). In the short term, water available to the seedling consists of that present in the root plug. In the longer term a seedling must absorb moisture from the bulk soil. The ability to do this is dependent on both the ability of roots to grow into the bulk soil and the soil water content (Grossnickle 2005). Deaths following transplanting will be reduced if the planted material is provided with a source of readily available moisture, evaporative loss from leaves is reduced, or root exploration of the bulk soil is stimulated (Grossnickle 2005). One method of extending the time frame for the use of the moisture within the root ball and therefore more time for new root growth to explore the bulk soil is to harden the seedlings to higher sunlight conditions and low soil moisture conditions.

A feasible method of drought hardening seedlings is to utilise reduced irrigation or partial droughting of the seedlings to pre-condition the seedlings to the drought they will experience shortly after transplanting into the field. The reason for the effectiveness of drought hardening is that plants are usually more able to survive periods of low moisture such as conditions experienced shortly after transplanting into field plantations if they have experienced a previous period of low moisture stress. For example tomato plants allowed to wilt for 2–3 days before irrigation survived better after transplanting (Ojemakinde and Onwueme 1980), while drought hardening was promoted in apricot plants receiving irrigated daily at 25% of controls (Ruiz-Sanchez et al. 1998). The reason for the effectiveness of drought hardening may be related to changes associated with limiting water loss or with enhancing water uptake. Droughted plants can limit water loss in several ways including reducing transpiration (e.g. Thomas and Eamus 1999; Searson et al. 2004; Villar-Salvador et al. 2004). Additionally, leaf thickness may increase which provides more leaf volume for carbon assimilation and production of newly produced carbohydrates necessary for root growth and less surface area for water loss (e.g. Ngugi et al. 2003; Searson et al. 2004). Furthermore changes to the allocation of biomass within the seedlings to produce less shoots and leaves can increase the ratio of root:shoot and decrease the leaf area:root mass ratio. However, root production may not necessarily promoted by drought hardening (e.g. Guarnaschelli et al. 2003; Ngugi et al. 2003), but root production following planting can be enhanced by the prior drought hardening procedure. For example *Pinus radiata* D. Don seedlings irrigated bi-weekly for 4 weeks prior to planting had more post planting root growth than seedlings irrigated daily (Rook 1973) and Lotus plants receiving the least moisture during growth showed greater and more rapid root growth than less stressed plants, especially when soil moisture was low (Franco et al. 2001).

An alternate management practice to drought hardening is to manually reduce the seedlings' leaf area just prior to field deployment. This practice, know as top-clipping, modifies the seedling by quickly reducing the evaporative area while maintaining an intact and efficient root system in order to maintain a more favourable water status. These physiological adaptations are associated with increased survival (Close et al. 2005). Uncontrolled field observations have indicated the practice of top-clipping may reduce apical dominance and increase the propensity to form double leaders in the tree. This in turn reduces stem quality and plantation profitability.

One of the favoured plantation forestry species in the coastal region of northern NSW, Australia is *Eucalyptus pilularis* Sm. as it has desirable growth and superior wood properties but a shortfall for its establishment is that seedling mortality shortly after planting can be high. The climate of the region where *E. pilularis* plantations occur has a mean annual rainfall ranging from 1,000 to 1,600 mm with a predominantly summer rainfall pattern when higher temperatures are also prevalent (Bureau of Meteorology 2009). Plantations are typically established during these months of higher rainfall. Deaths of *E. pilularis* by transplant shock were associated with dehydration following planting and occurred predominantly within 2–4 weeks of planting (Thomas 2008) thus ensuring seedlings are supplied with adequate moisture or limiting water loss from seedlings during this period will greatly reduce deaths. The effectiveness of nursery drought hardening and top-clipping in reducing seedling mortality will be examined in this experiment. Tree form and stem quality will be examined under longer term growth conditions to determine residual effects of these altered management practices. In addition the effect of plant type on mortality will be compared. Advances in forestry technology means that planting material can be deployed as vegetative cuttings as an alternative to seedlings. This technology is typically utilized to deploy improved genetic material. Recent advances have increased the production of *E. pilularis* vegetative cuttings, but the hardiness of this material is unknown, thus survival and growth of vegetative cuttings were compared to the routinely used seedlings.

Materials and methods

The experiments aimed at producing drought hardened seedlings and testing the effectiveness of the hardening procedure on survival. Seedlings were drought-hardened during the final weeks of nursery growth and the responses to the drought-hardening were examined while the seedlings were in the nursery and after subsequent planting. In effect there were three experiments; i.e. (1) response of seedlings during nursery production to the drought-hardening procedure; (2) testing the survival of drought-hardened seedlings to survival of unhardened and manually top-clipped seedlings in a glasshouse were irrigation could be controlled (i.e. no irrigation); and (3) testing the survival and growth of drought-hardened seedlings and vegetative cuttings to that of unhardened and manually top-clipped seedlings in the field (Table 1).

Production of drought hardened seedlings

Experimental design

Eucalyptus pilularis seedlings aged 25 weeks produced by Forests NSW Grafton Nursery (Lat. 29.63°S, Long. 152.96°E) were used. Seeds were sown into 512 germination trays

Table 1 List of experimental treatments imposed on *E. pilularis* seedlings to produce drought hardened seedlings in experiment 1; and tested in the glasshouse and in the field in experiments 2 and 3

Experiment	Treatments	Description
Experiment 1. Production of hardened seedlings in the nursery	Control	Normal irrigation (no hardening)
	Mild	50% Reduction of irrigation on 1–2 days per week
	Severe	100% Reduction of irrigation on 1–2 days per week
	Control-no irrigation	100% Reduction of irrigation on 1 day per week. Treatment to compare physiological adaptation of hardening
Experiment 2 and 3. Survival of drought hardened seedlings in 2. The glasshouse, and 3. the field	Control	Normal irrigation (no hardening) produced in experiment 1
	Mild	50% Reduction of irrigation on 1–2 days per week produced in experiment 1
	Severe	100% Reduction of irrigation on 1–2 days per week produced in experiment 1
	Top-clipped	Manual removal of upper third to half of the stem and leaves from control seedlings produced in experiment 1
	Cuttings	Vegetative cuttings (no hardening)

which consist of the respective number of small, wedge shaped cells (plugs) of pyramidal shape (1.3 cm square at surface, 0.7 cm square at bottom and 3 cm deep). The potting media in the 512 trays consisted of 90% composted pine bark (0–6 mm diameter) and 10% coir fibre. Seeds were covered with 2 mm vermiculite and placed on raised benches and received daily irrigation and weekly fertilization using 3 g l^{-1} liquid fertilizer 20.6:8.5:16.0 (N:P:K) at a rate of 5 l m^{-2}. Seedlings remained here for 10 weeks until they had developed 1–2 pairs of leaves above the cotyledons and were considered ready for ‘transplanting’. Transplanting involved replanting seedlings into larger volume cells called V93 Hiko’s. These trays consist of 40 individual cells ~8.7 cm deep with a diameter of 4.1 cm and volume of 93 cm^3. Soil media used in these Hiko trays were identical to that used in 512 trays. Seedlings were then managed under standard conditions receiving regular irrigation (dependant on stage of growth but ensuring plants did not show visible signs of water stress such as wilting) with regular (usually weekly or bi-weekly) fertilization with a ‘balanced’ foliar fertilizer [15:2.2:12.4 (N:P:K)] applied through an irrigation system until they were aged 25 weeks. Seedlings were ~25 cm in height with 2.5 mm diameter at this age.

Four experimental treatments were applied. Three treatments consisted of (1) normal irrigation (control), (2) reducing irrigation by 50% for 1 day per week in the first 2 weeks and by 50% on two non-consecutive days for the final 2 weeks (mild), and (3) reducing irrigation by 100% for 1 day per week in the first 2 weeks and by 100% on two non-consecutive days for the final 2 weeks (severe). These treatments were imposed for the final 4 weeks of production from mid October to mid November. There were four replicated blocks and each treatment plot consisted of eight Hiko trays (maximum of 320 seedlings). The groups of seedlings were placed on nursery production benches to allow ease of moving the seedlings of the mild and severe treatments away from irrigation booms. The benches were placed on the western edge of the production area to minimise the variation in light regime between benches in a randomised complete block design.

The fourth treatment was imposed at the time physiological responses to the hardening procedure were measured 4 weeks after initial treatments were imposed. This treatment consisted of not irrigating a subset of the control treatment (control-no irrigation treatment) only for the day on which physiological measurements were taken. This treatment was used to compare seedlings that had no history of reduced irrigation with the mild and severe treatments. Two Hiko trays per plot of each control treatment were randomly selected to receive no irrigation on these days.

All irrigation was applied using an automatic boom fitted with Lurmark F110 nozzels. A rain-gauge was placed on one nursery bench in each treatment to record the amount of daily irrigation or rainfall that the seedlings received. Air temperature and humidity in the nursery production area were monitored with a microprocessor data logger (TGP-1500, Tiny-tag Plus, Gemini Data loggers, Chichester, UK) attached to one rain-gauge stand.

Survival and growth of the seedlings during the drought hardening procedure

Thirty seedlings collected at random from the seedling population were harvested on the day the seedlings were randomised into treatments, and a further 32 seedlings per treatment (eight randomly selected seedlings per treatment plot) were harvested at the termination of the drought hardening procedure. At harvest leaf area of the youngest fully expanded leaf was determined using a LI-3100 Area Meter (LI-COR, Inc. Lincoln, NE, USA). Leaves on the main stem were counted and removed prior to determining leaf area using a LI-3100 Area Meter (LI-COR, Inc. Lincoln, NE, USA). Leaf samples were then oven dried at 70°C and dry weights determined. Specific leaf area (m^2 kg^{-1}), a measure of leaf thickness was calculated from these data. The height from soil level to stem apex was measured and the stem collar diameter was measured at soil level measured using electronic calipers. The seedlings sturdiness quotient was measured as seedling height *10/stem collar diameter. The stem was removed prior to washing soil from the roots. Roots and stems were then oven dried at 70°C and dry weights determined. The root:shoot ratio (g g^{-1}) and leaf area:root mass ratio (cm^2 g^{-1}) were calculated from these data. The quality index developed by Dickson et al. (1960) was calculated as total biomass/(height/collar diameter) + shoot biomass/root biomass.

Seedling height from the soil level to stem apex was measured on eight seedlings per plot at 2 weekly intervals. Seedling mortality was recorded for all seedlings per plot at 2 weekly intervals.

Physiological measurements of seedlings during the drought hardening procedure

Stomatal conductance of sunlit young fully expanded leaves was measured using a LiCor 1600 steady state porometer (LiCor, NE, USA). Stomatal conductance and transpiration were measured over a diurnal period in the fourth week after treatments were imposed on the 2 days corresponding to the day irrigation was withheld and the following day. On these same days the water potential of the upper shoot was measured (Soil Moisture Equipment corporation, Santa Barbara, CA, USA) from pre-dawn (04:30) over a diurnal period and on the following day.

Measurements of water potential, and stomatal conductance and transpiration were made on two to four replicate seedlings per plot of the control, mild, severe and control-no irrigation treatments.

Survival of drought hardened seedlings under drought conditions in a glasshouse

The second and third experiments comprised a glasshouse component and a field component. Some control seedlings were top-clipped by removing the upper third to half of the stem (top-clipped treatment), a common practice designed to reduce the seedlings leaf area and decrease the leaf area:root mass ratio. The morphology of these seedlings was measured using the techniques described earlier.

In addition to examining the survival and growth of the four treatments of *E. pilularis* seedlings (control, mild, severe and top-clipped), a further treatment of *E. pilularis* vegetative cuttings (cuttings) were assessed. This was the first year these cuttings were produced thus it was decided to examine the survivability of this source of planting stock in comparison to the seedling planting stock. A random sample of 20 vegetative cuttings of *E. pilularis* produced by Forests NSW Tree Improvement group by vegetative reproduction of selected plus trees were harvested using the techniques described earlier.

The survival of seedlings and cuttings was tested in a drought environment of a glasshouse. The glasshouse was situated at Coffs Harbour, NSW, Australia (Lat 30°19′S, Long 153°07′E). Photosythnetic photon flux density (PPFD) measured inside the glasshouse using a LiCor quantum sensor (LiCor, NE, USA) was $\sim$60% of incident sunlight. Four household fans were positioned in the glasshouse and glasshouse air vents were left open to facilitate air flow during the experiment. Air temperature and relative humidity (RH) inside the glasshouse were recorded at seedling height using Gemini data loggers (Tinytag Extra TGX-3580).

Soil collected from a local experimental field site known as Hardacres was used in this experiment. It is a brown earth soil (Milford, 1999) derived from late carboniferous siltstone, mudstone and conglomerate (Gilligan, 1992). The soil has a well structured clay loam texture with field holding capacity of 0.41 g water g^{-1} dry soil. In the first year soil moisture content of the bulk soil were amended to 0.200($\pm$0.002) g g^{-1} at the start of the experiment. Soil moisture during the experiment was monitored using Theta probes (Delta-T type 2, Hoddeston, UK) and readings converted to soil moisture content (Delta-T devices, 1999).

Eight seedlings or eight vegetative cuttings were planted into each planting tub of 35 l capacity (plot) using hand trowels so that the top of the root plug was placed 5 cm below the soil surface. There were four replicated blocks of each treatment arranged in a randomised complete block design.

Seedling health and survival was scored at regular intervals using a four point scale. Seedlings classified as (1) had fewer than 25% of leaves that were drooping; seedlings classified as (2) had between 25 and 50% of leaves that were drooping and some drooping of the apex; those seedlings classified as (3) had more than 50% of leaves that were drooping, some loss of leaf colour or partial dehydration of the leaves and the apex; and seedlings classified as four (dead) had pronounced discolouration and dehydration of the remaining leaves, extensive leaf abscission and dehydration of the stem and apex. The scoring was continued until all plants were classified as dead, a period of 45 days from planting.

Stomatal conductance was measured at 11:00 on two seedlings per plot 9 days after planting. Leaf water potential of the upper shoot was at pre-dawn (05:00) and 12:00 on the same day.

Survival and growth of drought hardened seedlings under field conditions

Eucalyptus pilularis seedlings from the control, mild, severe treatments, as well as seedlings that were top-clipped (top-clipped treatment) and vegetative cuttings (cuttings treatment) were tested at a plantation ~20 km north of Grafton, NSW. The transplants (seedlings or cuttings) were arranged in a complete randomised block design with four blocks. Each plot contained forty seedlings arranged in an arrangement of four rows of 10 transplants planted at 2 m spacing. Rows were 4 m spacing.

The plantation was an ex-pasture site formerly consisting of sclerophyll forest. The experimental site had a NE aspect situated on the mid slope of gentle hills with a slope of 6°. Soil texture was classified as loamy sand (Northcote, 1979). Site preparation consisted of ripping (0.7 m) and forming planting mounds (0.2 m height) with 4 m spacing between mounds. Herbicides (glyphosate 4 l ha^{-1}, simazine 2.5 kg ha^{-1} and metolachlor 1.5 l ha^{-1}) were applied to mounded soil 1 month prior to planting.

Prior to planting transplants were soaked in water until the root plugs were fully hydrated. Transplants were planted so the root plug was 5 cm below soil surface. Soil in the top 10 cm and at 10–50 cm soil depth was collected at the time of planting and periodically when assessing seedling survival and stored in plastic bags for later determination of gravimetric moisture content. Two rain-gauges were placed within the experimental area and rainfall recorded on days when seedling survival was assessed. All transplants were fertilized with 50 g di-ammonium phosphate 1 month after planting.

Health and survival of seedlings and cuttings was measured using a four point scale (as described earlier) at 3, 7, 14, 21, 28, and 81 days after planting into a field plantation. Survival was determined at later dates from measurements of sapling height recorded at 4, 8, 14, 25, 36 months. The number of double leaders at 1.3 m height was recorded at 14, 25 and 36 months. Form of the entire stem and of the section of stem from 0 to 1.3 m was assessed using a six point scale at 36 months with one representing worst form and six representing a straight stem with minimal defects.

Tree height of the seedlings and cuttings in the field experiment were measured at 14, 25 and 36 months. Diameter over bark at 1.3 m (DBHOB) was measured at 36 months.

Statistical analysis

The number of seedling deaths during the drought hardening procedure in the nursery was analysed using contingency tables. Repeated measures Analysis of variance (ANOVAR) was used to measure the effect of treatment on seedling height growth. Analysis of variance (ANOVA) was used to determine differences in seedling morphology and physiological measurements resulting from the treatments.

In the glasshouse experiments all seedlings and cuttings progressively moved from healthier categories to category 4, which meant the proportion of seedlings that met or exceeded a particular health category could be calculated for each sample date. The influence of treatments on this non-censored data was analysed using log-rank non-parametric survival analysis and Kaplin-Meier estimates generated to show the proportion of failures of a particular health category as a function of days after planting. In effect these represent when the seedling or cuttings' health declined below a particular health category.

It was noticed that seedlings and cuttings in the field experiment could decrease and increase in health categories over time (e.g. 1–2 then return to 1) with only the final 'Dead' category being permanent, indicating the seedling or cutting had not survived. The proportion of seedlings or cuttings meeting or exceeding a particular health category was analysed using repeated measures analysis of variance after arcsin transformation of the data. Differences in survival attributed to the effect of the treatments were examined by analysis of variance throughout the experiment.

The number of leaders at 1.3 m, and the visual stem ratings were analysed using generalised linear mixed model (GLMM) with a binomial distribution and a linked logit transformation. Scores or counts are presented as back-transformed numbers.

Results

Production of drought hardened seedlings

Growth conditions

Average daily irrigation for the 4 week drought hardening procedure was 5.0, 4.6 and 4.2 mm for the control, mild and severe treatments, while on the days the drought hardening procedure was implemented the average daily irrigation was 5 mm for control, 2.5 mm for mild and 0 mm for severe treatments. 45 mm rain fell during the 4 week drought hardening procedure. Average daily maximum temperature was 26°C and daily evaporation was 4.5 mm.

Seedling mortality was not affected by the drought hardening treatment ($P > 0.05$). Number of *E. pilularis* seedling deaths during the 4 week drought hardening period averaged 0.9 per Hiko (of 40 seedlings), equating to 2.5% mortality with a range from 1.3 to 3.5% between treatments.

Seedling height growth was not significantly ($P > 0.05$) reduced by the drought hardening irrigation treatments. Overall *E. pilularis* seedlings grew from 25 to 40 cm during the 4 week drought hardening period.

Stem collar diameter was greater in seedlings receiving normal irrigation, but stem sturdiness quotient was unaffected by the drought hardening procedure (Table 2). Seedlings receiving less irrigation had fewer leaves and lower leaf area. These seedlings also had a higher root:shoot ratio and less leaf area per unit weight of root mass (Table 2). Biomass of leaves, roots, stems and total seedlings were not affected by the drought hardening procedure (data not shown). Severe seedlings had a lower quality index than mild or control seedlings.

Seedling physiology

Leaf water potential declined during the day in all seedlings. The decline was significantly ($P < 0.05$) greater after 12:00 noon in seedlings of that received no irrigation on the day of measurement. These seedlings included seedlings in the severe drought hardening treatment, and the control-no irrigation seedlings that had not received reduced irrigation until the day measurements were taken. As the day progressed the control-no irrigation seedlings became significantly ($P < 0.05$) more moisture stressed than seedlings in the severe treatment. The following morning, before irrigation had been supplied these same seedlings remained more stressed.

Stomatal conductance (and transpiration—data not shown) behaved in a similar manner. Seedlings had similar rates of stomatal conductance in the morning that irrigation was withheld, but the decline in stomatal conductance was significantly ($P < 0.05$) greater in

Table 2 Seedling characteristics of seedlings measured pre-hardened, and after 4 weeks growth in each of control or mild or severe drought hardening procedures

	Pre hardened	Control	Mild	Severe	Top-clipped	Cuttings
Height (cm)	25.4	38.4a	37.8a	35.9a	27.2b	19.8c
Diameter (mm)	2.48	3.0c	2.8c, b	2.7b	3.0c	3.3a
Sturdiness quotient	103	129a	132a	135a	92b	60c
Leaf number	7.5	5.8d	5.2d, c	4.8c	3.1b	7.0a
Leaf area (cm^2)	88	74c	71c	60b	61b	115a
Specific leaf area (m^2 kg^{-1})	18.4	18.1a	16.0a	17.2a	18.1a	13.6b
Root:shoot ratio	0.25	0.27c	0.28c	0.32b	0.38a	0.34b
Leaf area:root mass (cm^2 g^{-1})	536	325a	267b	237b	182b	252b
Dickson quality index	4.2	4.1a	3.7a	3.3b	3.7a	2.8c

Means of treatments that are significantly different ($P < 0.05$) are followed by different letters. Details of control seedling that were top-clipped, and of vegetative cuttings that were used in the survival after planting studies are also included. Means of 32 plants

those seedlings receiving less irrigation. Stomatal conductance of seedlings of the control-no irrigation treatment declined to a significantly ($P < 0.05$) larger extent than other treatments, even the severe drought hardening treatment (Fig. 1).

Survival of drought hardened seedlings under drought conditions in a glasshouse

Seedlings remained healthier for longer and survived for longer if they had been drought hardened (Fig. 2). The increase in survival was extended with more pronounced drought hardening procedures. Top-clipped seedlings performed as well as drought hardened (mild or severe) seedlings. The vegetative cuttings declined in health faster and died more quickly than seedlings. However, the difference between survival of vegetative cuttings and control seedlings was minimal. For example it took ~23 days for 50% of cuttings to die and 25 days for 50% of control seedlings to die. In comparison it took 33, 35 and 34 days for 50% of mild, severe and top-clipped seedlings to die (Fig. 2).

Soil moisture declined to 0.15(±0.07) g g^{-1} measured 8 days after planting, to 0.10(±0.03) g g^{-1} measured 20 days after planting, and to 0.56(±0.04) g g^{-1} measured 30 days after planting. Soil moisture did not differ significantly ($P > 0.05$) between treatments.

Both leaf water potential of seedlings measured pre dawn and at noon, and noon measurements of stomatal conductance measured 9 days after trans-planting the seedlings in the glasshouse were lower in the control treatment than either the two drought hardened (mild and severe) treatments and the top-clipped treatment (Fig. 3). Stomatal conductance of seedlings in the severe treatment were similar (not significantly different) to those of the top-clipped treatment. The vegetative cuttings of the cuttings treatment had marginally lower (not significantly different) stomatal conductance and leaf water potential to the seedlings of the control treatment.

Survival and growth of drought hardened seedlings under field conditions

Survival during the first 15 weeks from planting was not significantly ($P > 0.05$) different between treatments, survival ranging from 85% in the severe treatment, to 94% in the mild

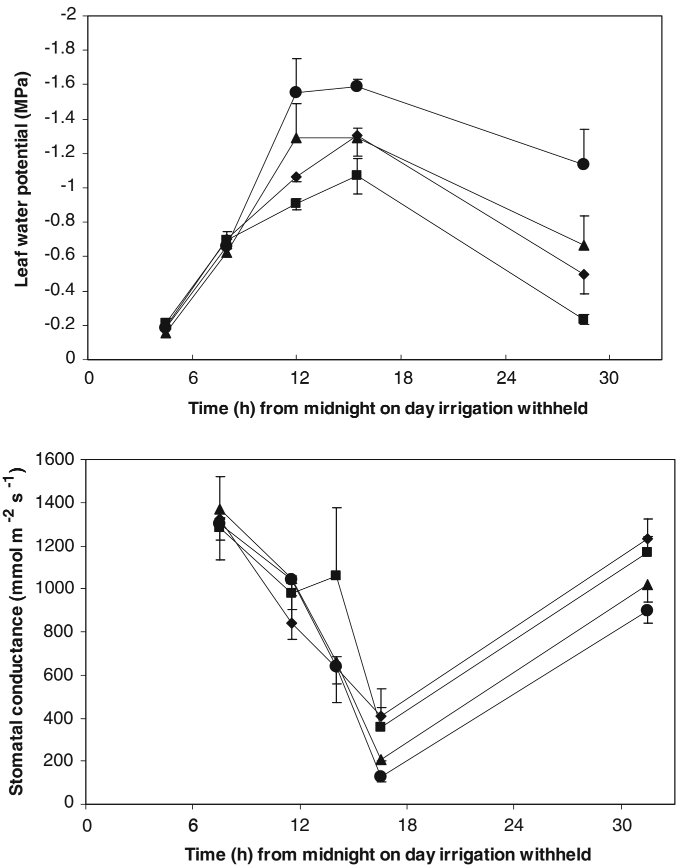

Fig. 1 Leaf water potential and stomatal conductance of *E. pilularis* seedlings in the control (■), mild (◆), and severe (▲) treatments on the day irrigation was withheld and the following morning. A subset of control seedlings receiving no irrigation for the first time, i.e. control-no irrigation (●) are also shown. Means and standard error are shown

and the control treatments. Survival of seedlings in the top-clipped treatment was also 91% survival at 15 weeks, while survival of Cuttings was 94%. Survival measured at 14 months, 2 and 3 years had declined by ~1–2% each year in all treatments, but the was no significant treatment effect on survival.

The high survival may have been associated with high rainfall shortly after planting. 12 mm rain fell within 4 days of planting, and a total of 41 mm within 28 days of planting. Overall in excess of 200 mm rain fell within 15 weeks of planting. Soil moisture varied little over this period and typically ranged from 0.1 to 0.14 g g^{-1} in the top 0–10 cm, and from 0.15 to 0.18 g g^{-1} in the 10–50 cm soil depth. There was no significant ($P > 0.05$) difference in soil moisture between treatments.

There were no carry-over effects of the drought hardening treatment on tree growth with no statistical differences in height or DBHOB between hardening treatments. Top-clipped seedlings did not produce more double leaders measured at 1.3 m at age 25 or 36 months after planting. Similarly tree form measured over the lower 1.3 m or over the entire stem was the same for seedlings regardless of drought hardening procedures or top-clipping.

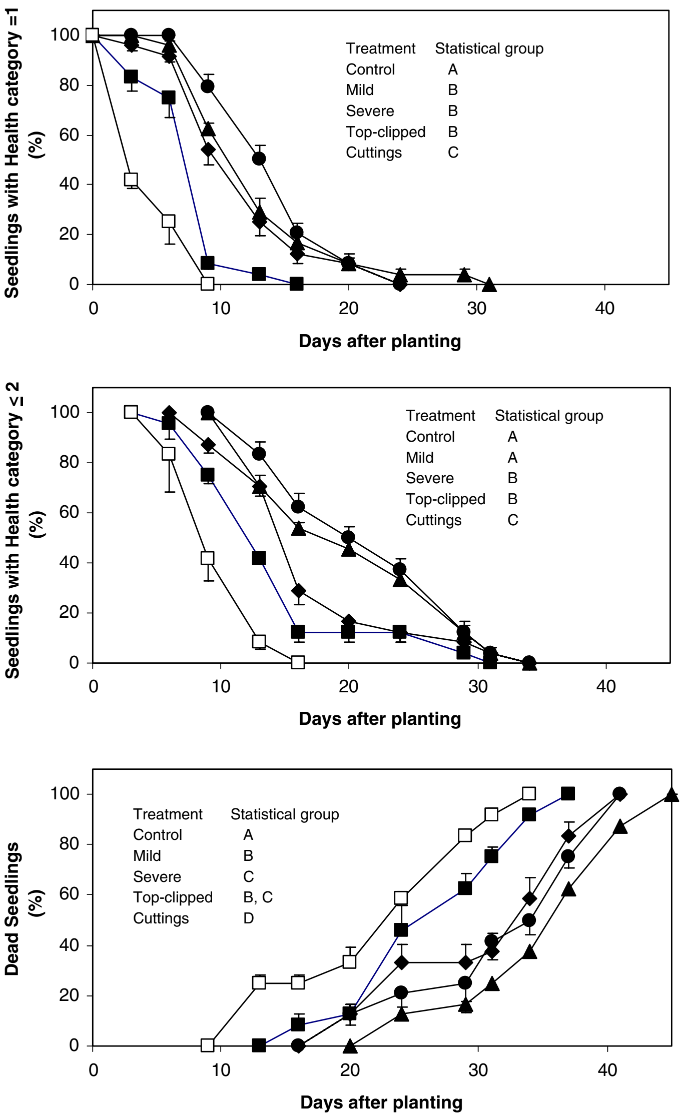

Fig. 2 Percentage of seedlings considered to have a health category of 1, ≤ 2 or 4 (i.e. dead) as a function of time after planting. Drought hardening procedures of control (■), mild (◆), severe (▲), top-clipped control plants (●), and vegetative cuttings (□). Means and SE are shown. Treatments with responses measured by log-rank analysis which are not statistically different are grouped by a *similar letter*

One highlight of the research was related to the dramatic improvement in stem form of vegetative cuttings compared to seedlings. Stem form was significantly higher at scores of 4.5 for both the lower 1.3 m and the entire stem compared to scores <3.3 for both characteristics in all seedlings with no significant effect of drought hardening or top-clipping. However, this can not be related directly to an effect of propagation material as the vegetative cuttings were of material highly selected for genetically superior stem form.

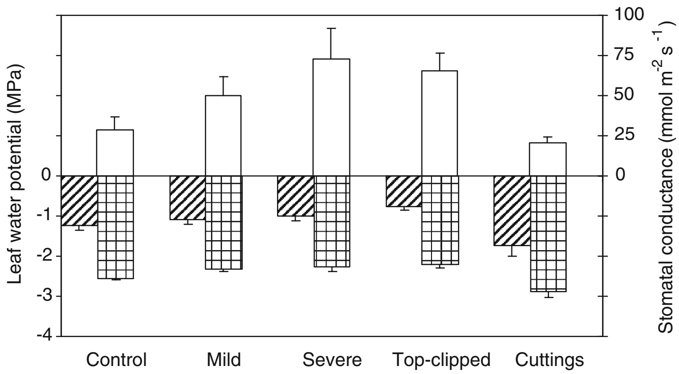

Fig. 3 Leaf water potential measured predawn (*diagonal*) and at noon (*cross Hatch*); and stomatal conductance (*open*) of the five treatments measured 9 days after planting in a glasshouse. Means and standard errors of eight seedlings

Discussion

Drought hardening reduced mortality rate of *E. pilularis* seedlings when planted into the drought conditions of the glasshouse. In comparison survival in the field environment was not affected by the drought hardening procedures. The field environment was probably less limiting as high rainfall occurred shortly after planting. Seedling survival will be similar when adequate post-planting conditions are met, while in harsh conditions survival can be directly affected by the physiological status of plants (e.g. Reis and Hall 1986). Others (e.g. Royo et al. (2001)) challenge the worth of drought hardening as survival of drought hardened *Pinus halepensis* Mill. seedlings was related to rainfall rather than the drought hardening procedure. Additionally, Stape et al. (2001) conclude post-planting soil moisture is the most important environmental factor for plant survival, while the findings of Close and Davidson (2002) that sites that received post-planting water achieved 71% higher survival than sites that did not also highlight the importance of adequate soil moisture or rainfall on limiting seedling mortality. In this experiment, the similar survival of drought hardened and unhardened seedlings in the field experiment also highlight the importance of rainfall shortly after planting on reducing mortality, while the results from the glasshouse experiment highlight the advantage of hardening on delaying seedling mortality when rainfall is limiting. Rainfall along the sub-tropics of the north coast of NSW, Australia, where *E. pilularis* is the premier hardwood plantation species, experience a predominantly summer rainfall pattern interspersed with short durations of hot dry conditions. Mean annual rainfall of *E. pilularis* plantations can vary from 1,600 mm to as low as 1,000 mm, with close to 70% of the rainfall occurring between December and March when higher temperatures are also prevalent. There is a limited period considered ideal for planting as planting early in the ‘planting season’ when temperatures are cooler correspond with drier soil and low rainfall, while planting later in the ‘planting season’ when soil moisture has increased following summer rainfall events leaves too little time for growth of the seedlings to attain sufficient height to overcome frost events. The benefits of hardening would be expected to be most clearly observed in early season plantings.

It is possible the drought hardening procedures undertaken in the nursery were inadequate, although the results from the glasshouse experiment suggest that in this instance seedlings were drought hardened. In our experience drought hardening is not always possible. This was highlighted by a repeat of this experiment in a subsequent year that proved ineffective as persistent rainfall during the final stages of nursery growth which did not allow the drought hardening procedure to be completed as the seedling potting media became water-logged (unpublished data). In that instance drought hardened *E. pilularis* seedlings showed negligible improvements in survival while drought hardening another species, *Corymbia citriodora* subsp. *variegata* (F. Muell.) A. R. Bean & M. W. McDonald, produced seedlings with only a small (7%) reduction in mortality following transplanting into a field situation.

Stem diameter is reported a useful predictor of field survival (McGrath and Duryea 1994; Mattsson 1996) but others (Guarnaschelli et al. 2003) and this experiment showed drought hardened seedlings can have smaller stem diameters yet better survival. Likewise root:shoot ratio is reported to be of value in predicting field performance (Mattsson 1996), but drought hardening may increase (Rhizopoulou and Davies 1993 cited in Stape et al. 2001) or have no affect on this ratio (Guarnaschelli et al. 2003; Villar-Salvador et al. 2004), and furthermore these same drought hardening procedures may or may not enhance survival. We observed root:shoot ratio increased and leaf area decreased with drought hardening and were associated with enhanced survival. These morphological responses would be expected to lead to a better balance between root water acquisition and shoot water loss following transplanting, and hence promote survival. Manual top-clipping of the upper foliage obtained these same morphological changes in seedlings, and similarly promoted survival. This showed that survival was influenced by leaf area, and that removal of younger leaves by top-clipping, or senescence of older leaves by drought hardening obtained similar responses. In a practical sense this suggests several management options during nursery production i.e. drought harden during the final stages of growth, or manual top-clipping can achieve the same result.

Drought hardening also modified the stomatal behaviour of the seedlings and allowed maintenance of photosynthesis under moisture stress. The pattern of reduced stomatal conductance and lower leaf water potential in drought plants was typical (e.g. Turner and Begg 1981; Thomas and Turner 1998) and most clearly observed in seedlings of the severe treatment which were able to maintain stomatal conductance and leaf water potential than seedlings experiencing their first period of no irrigation (i.e. control-no irrigation treatment). Leaf water potential of these seedlings of the severe treatment was probably maintained due to osmotic adjustment (Jones 1992). The finding that these physiological adaptations of higher stomatal conductance and less negative leaf water potential continued after transplanting suggests the drought hardened seedlings were more able to regulate water conservation, maintain stomatal aperture which would in turn promote photosynthesis. This would then provide carbohydrate for continued seedling root growth for exploration and consequently extraction of water from the bulk soil (Burdett 1990; Maillard et al. 2004).

Eucalyptus pilularis seedlings and cuttings had similar water relations and gas exchange in response to drought but cuttings died faster than seedlings in this experiment, although a subsequent experiment showed cuttings and seedlings had similar abilities to survive when planted into drought conditions (D. Thomas and G. Heagney, ForestsNSW, unpublished data). Sasse and Sands (1996) showed drought hardened *E. globulus* cuttings died faster than drought conditioned seedlings, and concluded the root systems of cuttings which differ developmentally, architecturally and anatomically from the root systems of seedlings may play a role in the different responses to drought. This suggests further research should be undertaken to develop protocols for drought hardening vegetative cuttings of *E. pilularis*.

Conclusion

Plantation establishment is a costly process requiring the optimization of many processes. For drought hardening to be beneficial to successful plantation establishment it must not only enhance survival of field planted seedlings, but the process of hardening must not be so severe as to cause death of the seedlings while the seedlings remain in the nursery. The procedures used in this experiment to drought harden *E. pilularis* seedlings was not so severe that seedlings died while implementing the drought hardening procedure. Limiting deaths during the drought hardening procedure can be managed by a suitable and flexible irrigation protocol, although many protocols may be suitable. For example drought hardening of *E. camaldulensis* was achieved by a variety of irrigation protocols of differing severity yet none were so severe as to increase mortality (Chaudhry et al. 1995). For drought hardening of seedlings to become routine management practice it must also be shown to be beneficial it must be beneficial to survival of transplanted seedlings. The hardening procedure did enhance survival, but rainfall after planting had a larger impact on survival than the hardening process. It would be expected that hardened plants would show enhanced survival when planting conditions are more adverse. Thirdly, the hardening process must have no negative impacts of longer term growth. No long term effects of drought hardening or top-clipping on growth in height or diameter of field grown plants were observed. Additionally neither drought hardening nor top-clipping increased the propensity to form double leaders in stems, and stem form was not affected by these treatments, thus detrimental effects of drought hardening were negligible. This is an important result as stem form is a major determinant of log quality and hence economics of plantation forestry.

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References

Burdett AN (1990) Physiological processes in plantation establishment and development of specifications for forest planting stock. Can J For Res 20:415–427

Bureau of Meteorology (2009) Climate statistics for Australian locations. http://www.bom.gov.au/climate/

Campbell KA, Hawkins CDB (2004) Effect of seed source and nursery culture on paper birch (*Betula papyrifera*) uprooting resistance and field performance. For Ecol Manag 196:425–433

Chaudhry AK, Hafeez SM, Ghauri MJ (1995) Optimum watering level for *Eucalyptus camaldulensis* plants in the nursery stage. Pak J For 42:41–44

Close DC, Davidson NJ (2002) Revegetation to combat tree decline in the Midlands and Derwent valley lowlands of Tasmania: practices for improved plant establishment. Ecol Manag Restor 4:29–36

Close DC, Beadle CL, Brown PH (2005) The physiological basis of containerised tree seedling ‘transplant shock’: a review. Aust For 68:112–120

Dickson A, Leaf AL, Hosner JF (1960) Quality appraisal of white spruce and white pine seedling stock in nurseries. For Chron 36:10–13

Franco JA, Banon S, Fernandez JA, Leskovar DI (2001) Effect of nursery regimes and establishment irrigation on root development of *Lotus creticus* seedlings following transplanting. J Hort Sci Biotech 76:174–179

Grossnickle SC (2005) Importance of root growth in overcoming planting stress. New For 30:273–294

Guarnaschelli AB, Lemcoff JH, Prystupa P, Basci S (2003) Responses to drought preconditioning in *Eucalyptus globulus* Labill. Provenances. Trees 17:501–509

Jones HG (1992) Plants and microclimate: a qualitative approach to environmental plant physiology, 2nd edn. Cambridge University Press, Cambridge

Maillard P, Garriou D, Deleens E, Gross P, Guehl JM (2004) The effects of lifting on mobilisation and new assimilation of C and N during regrowth of transplanted Corsican pine seedlings. A dual 13C and 15N labelling approach. Ann For Sci 61:795–805

Mattsson A (1996) Predicting field performance using seedling quality assessment. New For 13:223–248

McGrath DA, Duryea ML (1994) Initial moisture stress, budbreak and two-year field performance of three morphological grades of slash pine seedlings. New For 8:335–350

Ngugi MR, Hunt MA, Doley D, Ryan P, Dart P (2003) Dry matter production and allocation in *Eucalyptus cloeziana* and *Eucalyptus argophloia* seedlings in response to soil water deficits. New For 26:187–200

Ojemakinde OA, Onwueme IC (1980) Heat and drought hardening of tomato (*Lycopersicon esculentum*) plants as an aid to field survival. Q J Int Agric 19:25–28

Reis GG, Hall AE (1986) Resistencia a desidratacao de *Eucalyptus camaldulensis* Dehn. com restricao do sistema radicular. Arvore 10:168–180

Rhizopoulou S, Davies WJ (1993) Leaf and growth dynamics in *Eucalyptus globulus* seedlings grown in dry soil. Trees 8:1–8

Rook DA (1973) Conditioning Radiata Pine seedlings to transplanting, by restricted watering. NZ J For Sci 3:54–69

Royo A, Gil L, Pardos JA (2001) Effect of water stress conditioning on morphology, physiology and field performance of *Pinus halepensis* Mill. seedlings. New For 21:127–140

Ruiz-Sanchez MC, Domingo R, Torrecillas A, Perez-Pastor A (1998) Water stress preconditioning to improve drought resistance in young apricot plants. Plant Sci 156:245–251

Sasse J, Sands R (1996) Comparative responses of cuttings and seedlings of *Eucalyptus globulus* to water stress. Tree Physiol 16:287–294

Searson MJ, Thomas DS, Montagu KD, Conroy JP (2004) Leaf water use efficiency differs between *Eucalyptus* seedlings from contrasting rainfall environments. Funct Plant Biol 31:441–450

Stape JL, Gonclaves JLM, Goncalves AN (2001) Relationships between nursery practices and field performance for *Eucalyptus* plantations in Brazil. New For 22:19–41

Thomas DS (2008) Hydrogel applied to the root plug of subtropical eucalypt seedlings halves transplant death following planting. For Ecol Manag 255:1305–1314

Thomas DS, Eamus D (1999) The influence of predawn leaf water potential on stomatal responses to atmospheric water content at constant C_i and on stem hydraulic conductance and foliar ABA concentrations. J Exp Bot 50:243–251

Thomas DS, Turner DW (1998) Leaf gas exchange of droughted and irrigated banana cv Williams (*Musa* spp.) growing in hot, arid conditions. J Hort Sci Biotech 73:419–429

Turner NC, Begg JE (1981) Plant-water relations and adaption to stress. Plant Soil 58:97–131

Villar-Salvador P, Planelles R, Oliet J, Penuelas-Rubira JL, Jacobs DF, Gonzales M (2004) Drought tolerance and transplanting performance of holm oak (*Quercus ilex*) seedlings after drought hardening in the nursery. Tree Physiol 24:1147–1155