



**Technical Report 100**

**Understanding and manipulating stress physiology of  
eucalypt seedlings to improve survival and growth:  
Preliminary report**

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Public

**Understanding and manipulating stress physiology of eucalypt seedlings to  
improve survival and growth: Preliminary report**

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Project B3: Silvicultural Systems

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## **Introduction**

In June 2002, the Australian Research Council (ARC) funded three-year 'Linkage' project 'Understanding and manipulating stress physiology of eucalypt seedlings to improve survival and growth' commenced. This project builds on outcomes from the PhD project 'Cold-induced photoinhibition, pigment chemistry, growth and nutrition of *Eucalyptus nitens* and *E. globulus* seedlings during establishment', CRC-SPF Strategic Initiative Funded (SIF) projects 'Seedling specifications for *E. globulus* planted in mediterranean environments, including SW WA and the Green Triangle' and 'Effects of environment on the leaf chemistry of *Eucalyptus nitens* seedlings and their resistance to mammal browsing' and the Natural Heritage Trust and CRC-SPF funded projects 'Revegetation to combat rural tree decline' and 'Demonstration and Farm Surveys of Eucalypt Seedling Tolerance to Native Animal Browsing'.

The current project investigates the potential effects of nursery management on survival and growth responses of *E. globulus* and *E. nitens* to drought and vertebrate/invertebrate browsing soon after planting. The objectives are as follows:

- 1) To establish field trials to quantify survival and growth of seedlings of varying carbohydrate reserve across a range of drought/browser pressure risk sites;
- 2) A physiological investigation of seedlings exposed to drought and browsing in the field;
- 3) An investigation of seedling carbohydrate stores and remobilisation following leaf area loss in seedlings of varying carbohydrate store level;
- 4) To develop nursery techniques for producing seedlings with characteristics of either acclimation to drought or tolerant to browsing.

This report reviews current knowledge on the manipulation of eucalypt seedling morphology and physiology for improved survival and growth under some abiotic and biotic stresses encountered soon after transplanting from the nursery to the field. Acclimation to the planting environment, photodamage, frost, drought and the effects of seedling size under drought and browsing pressure on survival and growth, are considered.

### ***Acclimation between the nursery and the field***

Tree seedlings acclimate to the growing environment in the nursery. If field conditions are colder than those in the nursery, the transplanted seedling will be stressed as acclimation occurs over several days or weeks. Many plant stresses have a negative effect on photosynthesis. Thus strategies for dealing with light absorption under conditions of decreased photosynthetic activity are crucially important to seedlings suddenly exposed to new environmental conditions. The occurrence of decreased photosynthetic efficiency is termed photoinhibition. This may arise directly due to sudden increases in irradiance, or indirectly through a stress that limits photosynthesis and induces conditions of excess light absorption, e.g. low temperature or drought. Low temperature limits photosynthesis by slowing the rates of photosynthetic enzymes. Drought limits photosynthesis primarily through the induction of stomatal closure and carbon dioxide limitation (Chaves 1991).

Photoinhibition occurs whenever the absorption of light energy exceeds its utilisation in photosynthesis (Huner et al. 1993). Various physiological processes are available

in plants to dissipate this excess energy. Three of these processes are the xanthophyll cycle, the capacity of which is proportional to carotenoid pool size (Adams & Barker 1998), photorespiration, and the water-water cycle (see Niyogi 1999). If their capacity for dissipating excess energy is exceeded, highly reactive and damaging oxygen and chlorophyll radicals are formed (Asada 1992; Foyer et al. 1994). Scavenging compounds and enzyme systems quench these radicals but their capacity can also be exceeded. Damage to chlorophylls, carotenoids and lipid bilayers results in photobleaching or photodamage to plant tissues (Wise & Naylor 1987). If severe, leaf death and abscission, and seedling mortality can result (Close et al. 1999, 2000). Acclimation to abiotic stress involves decreased chlorophyll content, increased xanthophyll content and increased levels of antioxidant scavenging compounds (Close et al. 2001b), thus providing protection against photodamage.

A fourth means of photoprotection is anthocyanin. This pigment is synthesised immediately below the epidermis in *Eucalyptus globulus* and *E. nitens* (Close 2001; Close et al. 2000; 2001b), *Pinus sylvestris* (Nozzolillo 1989) and *Pinus banksiana* (Krol et al. 1995) seedlings. It is hypothesised that anthocyanin alleviates photoinhibition by absorption of light between 400 and 590 nm (Massacci et al. 1998; Close et al. 2001b).

The acclimation condition at planting will depend to a large extent on the difference between the nursery and planting environments. Some acclimation is always required as seedlings are grown at high densities and self shade in the nursery, resulting in lower leaf pairs being shade adapted. For example, nursery-grown seedlings of eastern hemlock (*Tsuga canadensis*) had survival of 58 and 100% after transplanting from shaded to fully exposed or shaded planting sites, respectively (Mohammed & Parker 1999).

The potential capacity of a seedling to acclimate to field conditions is also important (Close et al. 2001c). Different species and sub-populations within species have different capacities to acclimate to a large increase in irradiance (Battaglia et al. 1996; Tognetti et al. 1998). For example, *E. nitens* seedlings have higher concentrations of anthocyanin and carotenoids than *E. globulus* seedlings raised under identical conditions (Close et al. 2000; 2002) and therefore require less acclimation after planting and exposure to high light. Similar differences exist amongst conifers. Sun scald or photodamage is a significant cause of mortality in seedlings of *Tsuga canadensis*, *T. heterophylla* and *Abies amabilis* (Tucker & Emmingham 1977; Tucker et al. 1987) following transplanting. However, rapid acclimation of pre-existing foliage to the higher light environments associated with field conditions has been reported for *Picea abies* (Spunda et al. 1993) and *P. glauca* (Leiffers et al. 1993). Acclimation to the prevailing light condition may be discriminated at species and subspecies level through the activity of the xanthophyll cycle (Adams et al. 1994).

### **Management options**

One method of minimising the period and extent of acclimation after planting is to carefully match species to site characteristics. This practice has been the focus of much research (Saunders et al. 1984; Booth et al. 1988, 1989; Booth & Pryor 1991) and has been adopted by the forestry industry. The importance of the state of acclimation of a seedling at planting is also recognised in the forest industry. Outdoor

nursery areas in conjunction with the withholding of nutrients and/or water are commonly utilised to ‘harden off’ seedlings (Colombo 1986; Gebre & Kuhns 1991; Anderson & Helms 1994). The withholding of nutrients is usually an effective means of hardening for low field temperatures. For example, the risk of cold-induced photodamage was minimized when seedlings raised in a mild environment were deprived of N in the nursery as this induced acclimation to excess light conditions (Close et al. 2000). However, if exposure to low temperature before planting has occurred, N deprivation may not be required as seedlings naturally harden to cold-induced photoinhibition (Close et al. 2001b, c).

### ***Frost***

Tree seedlings planted into cold environments are susceptible to frost damage for a number of reasons. Firstly, the seedlings may not be acclimated to the low temperatures experienced after planting. Secondly, young, recently developed seedling foliage may have a high water content and relatively large-celled leaves with a low osmotic concentration, characteristics that are associated with a high susceptibility to frost. Thirdly, cold air stratification and pooling of cold air expose seedlings to extremely low temperatures. Fourthly, cleared forest sites attain minimum temperatures below adjacent forested areas due to the loss of infra-red radiation to clear night skies (Nunez & Bowman 1986). In addition, the leaf temperature of exposed leaves at night may be lower than the air temperature due to radiative cooling (Jordan & Smith 1995). Nevertheless, tree seedlings often adjust to the prevailing site conditions. This is because tree seedlings acclimate, or ‘harden’, to frost. Unusually early or late frosts can cause serious frost injury as seedlings may not be hardened. Further, hardening occurs within certain genetic constraints. For example, *E. globulus* has a lower frost tolerance than *E. nitens* under identical environmental conditions and this is a factor in its planting distribution (Hallam et al. 1989). *E. nitens* populations from distinct geographic origins also differ in their susceptibility to frost (Raymond et al. 1992a, b).

Low, non-freezing temperatures trigger increased frost tolerance or hardiness. The physiology of frost tolerance involves a general relationship where hardiness is correlated with increased concentrations of soluble sugars and other compatible solutes (Ögren et al. 1997; Wanner & Junttila 1999) and increased cellular membrane stability via changed lipid composition (Thomashaw 1999). A recent study has shown that the frost tolerance of *Pinus radiata* (D. Don) increases in a curvilinear fashion as low, but above freezing temperatures decrease. Hardening occurred at temperatures below, and dehardening at temperatures above, 9.5°C. The balance between the two processes determined the development of frost hardiness (Greer et al. 2000).

### **Management options**

Logging minimum temperatures in the nursery can provide an initial indication of potential frost hardiness. Many nurseries are situated at low altitudes on mild sites that maximise the early growth of seedlings. In this case, an intermediate holding area at higher altitude may be beneficial before planting onto cold sites, although in practice this may be difficult to manage. Matching the hardening potential of species to site conditions is essential and widely practiced (Tibbits et al. 1997). Tree guards

can mitigate frost by excluding cold air flow at night and by trapping warm air during the day.

### ***Drought***

Drought-induced stress is the most widely studied and perhaps the most common cause of transplant shock in tree seedlings (Jarvis & Jarvis 1963; Burdett et al. 1983, 1984; Grossnickle 1988). Seedlings often become water stressed soon after planting as the soil volume accessed by roots of a naturally established seedling is over ten-fold that of a transplanted seedling of the same shoot size (Burdett 1990). Drought stress following transplanting may be further exacerbated by poor acclimation to the field environment (Rowe 1964). In the nursery, seedlings are within relatively close proximity to one another, sheltered from wind and watered frequently. Under such conditions leaves of high area:mass ratio and seedlings of high shoot:root ratio are formed which are not well suited to maintaining a favourable water balance in field environments. A low shoot:root leads to a better balance between root water acquisition and shoot water loss following transplanting (Ledig 1983) and a low leaf area:weight minimises stomatal water loss (Stape et al. 2001).

Leaves have physiological mechanisms that provide adaptation to drought. Maintenance of leaf turgor, that is required for many growth-related processes, can be achieved through changes in osmotic potential or tissue elasticity (Tyree & Jarvis 1982). Adjustment in either osmotic potential, cell wall elasticity or a combination of the two occurs in both *E. globulus* and *E. nitens* in response to drought stress (White et al. 1996). Osmotic adjustment, by decreasing the osmotic potential of the cells, increases water retention under dehydrating conditions. This is achieved using organic solutes, termed compatible solutes due to their compatible nature with the structure and function of other cellular macromolecules. Sugars and free amino acids contributed to osmotic adjustment in *P. mariana* seedlings during development of drought tolerance (Tan et al. 1992). Increased cell wall elasticity involves increases in hemicellulose and decreases in lignin and cell wall pectin in *P. glauca* seedlings subjected to drought conditions (Zwiazek 1991).

### **Management options**

A watering regime slightly restricting water availability to seedlings may induce morphological and physiological characteristics conferring drought tolerance. An induced water limitation decreased leaf mass:area ratio which was associated with drought tolerance in *P. sitchensis* (Hellkvist et al. 1974). Droughting in the nursery decreased osmotic potential in seedlings of *Eucalyptus camaldulensis*, *E. tereticornis*, *E. viminalis* and *E. grandis* (Lemcoff et al. 1994) and decreased osmotic potential and increased cell wall elasticity in *P. mariana* seedlings that led to the development of drought tolerance after planting (Colombo 1986). However, this may be difficult to manage given the rapid drying of potting mixes and the problem of subsequent re-wetting.

### **Seedlings for favourable sites**

Nursery managers use macronutrients to manage seedling growth and to meet a particular seedling specification. Foliar nitrogen has been shown to correlate strongly

with seedling growth after planting (Carlson 1986; Larsen et al. 1988; Thaler & Pages 1996; Close et al. 2001a). Seedlings deficient in N put all their available resources into root growth, to acquire N, at the expense of shoot growth. Recent evidence has indicated the importance of N stored in foliage, and its retranslocation, for new growth (Close 2001; Warren and Adams 2001). Internal recycling of nutrients from foliage has been shown to supply up to 100% of nutrients for new growth soon after planting (Folk & Grossnickle 2000).

### **Management options**

Sampling for foliar nutrient analysis is widely practiced and indicates the necessity for corrective nutrient application during the seedling production period. Trials of nutrient application methods for nurseries under well-defined environmental conditions (Close et al. unpubl.) and using potting mixes that affect the leaching (Geraldson 1996) and draw-down (Bragg & Whiteley 1995) of nutrients have provided useful information. For example seedlings at nurseries in regions of high temperature and rainfall need higher levels of nutrient application for a given level of uptake as more nutrient remains in solution and leaches out of the potting mix. Seedlings in potting mixes with higher air-filled porosity and higher microbial populations also need higher nutrient application for a given level of uptake as leaching and draw-down are proportionately greater. Also given that foliar N content is proportionate to foliar chlorophyll level and influential on specific leaf area (i.e. leaf thickness), nursery managers can gauge seedling N content by eye.

### ***Carbohydrate reserves***

### **Biomass partitioning**

Low shoot:root ratio is desirable for maximising new growth after planting. However, shoot:root is relatively high after nursery production in containers and this restricts acquisition of sufficient nutrients and water to support shoot growth after planting (Ledig 1983; Reitveld 1989; Close 2001). For example, growth of *P. taeda* seedlings after planting was strongly negatively correlated with shoot:root at planting (Larsen et al. 1988). The physiological basis of this effect has been investigated using just transplanted, non water-stressed *Picea mariana* seedlings. The seedlings had decreased levels of all foliar macro- and some micro-nutrients, total amino acids and sucrose relative to non-transplanted controls (Young et al. 1999). These results are consistent with those in recently transplanted *Eucalyptus* seedlings (Close 2001) and are indicative of re-translocation of mobile nutrients to enable new root growth.

Container depth has been found to better correlate with seedling growth after planting than container volume and many other pre-planting seedling specifications (Close et al. 2001a). Deeper containers do not necessarily decrease shoot:root but affect root architecture by increasing the initiation of primary roots in the container (Nelson 1996). Thus the initial soil mass occupied by roots increases with increasing container depth.

Relatively large seedlings are inherently robust to stressful growing conditions (South 1993; South & Mitchell 1999). Levels of carbohydrate and nutrient reserve are related to seedling size (Ritchie 1982). For example, the larger of two *Pinus banksiana* stock types of otherwise similar genetic origin and age had greater survival and growth

relative to the smaller stock type (Mohammed et al. 1998). In the same study, larger amounts of available, retranslocatable nutrient were associated with higher photosynthetic rates (Mohammed et al. 1998). Adequate carbohydrate reserves are essential also under conditions where photosynthesis is restricted but resources are still required for maintenance respiration and to support new leaf development where stress (e.g. severe drought, frost or browsing) has led to leaf loss after planting.

### **Biomass and drought**

Empirical observations suggest that larger seedlings are more robust to drought in Australian plantings. *P. pinaster* that had been repotted into 1L 'Jumbo' containers (approximately 40 cm tall) had survival of 60-70% after planting into deep sands under dry conditions compared to approximately 30% survival of stock grown in standard sized containers (Arthur Lyons pers. comm.). Further, seedlings of *Eucalyptus perineana*, *E. viminalis*, *E. ovata* and *Bursaria spinosa*, that had been held over in the nursery and repotted into 1L containers, had superior survival and performance compared to smaller stock raised in standard sized containers on a upper slope site with shallow soil (Close and Davidson 2001).

### **Biomass and browsing**

Relatively large 'half/half' stock only suffered 4 % mortality and there was little effect on growth compared to relatively small container stock that suffered ~25 % mortality after planting onto a high browsing pressure site (McArthur et al. 2002). The large half/half stock with high root collar diameter have proved resistant to stem nipping by European rabbits and are more likely to survive severe wallaby browsing relative to container stock (R. Appleton pers. comm.). However they are prone to drought susceptibility and mortality during summer drought (McArthur et al 2002) due to imbalanced root:shoot caused by the root pruning treatment in the field nursery. Future research is investigating the use of containerised stock that has been 'held over' in the nursery and has a relatively high root collar diameter and woody stem (R. Appleton pers. comm.).

Nutrient loading of hardy nursery material immediately before planting, either by placing mini-osmocote at the base of seedling plugs in the nursery with *E. nitens* or by a 2-3 liquid fertiliser rate application for *E. globulus*, has resulted in significantly greater growth soon after planting that allows seedlings to rapidly out-grow browsing wallabies (R. Appleton pers. comm.). *E. nitens* with mini-osmocote on the bottom of containers had better survival than controls after summer drought, possibly due to the roots 'chasing' nutrients that leached down the soil profile (R. Appleton pers. comm.).

*E. globulus* that were 0.5-1.0 m in height were planted onto a site of high browsing pressure in 2000 in order to investigate their robustness to browsing (H. Cusak pers. comm.). It was thought that the increased woodiness would be more resistant to damage, and that the seedlings would be able to re-sprout leaves after browsing. The seedlings were planted in early winter and little mortality was observed, although the seedlings did not appear to put on much growth for some time. A noticeable disadvantage was that the top-heavy seedlings were prone to windthrow that was exacerbated when leaves sprouted only from the very top of the stem – giving a standard rose-like appearance (H. Cusak pers. comm.).

## Management options

Increasing seedling size ensures an adequate carbohydrate and nutrient reserve after planting (South & Mitchell 1999) and can increase cost:benefit more than site preparation and establishment practices such as insecticides, herbicides and fertilisers (South et al. 2001). However, this should not be driven by an increased shoot:root ratio which can be detrimental to successful establishment (Larsen et al. 1988; Zwolinski and Bayley 2001). Seedling production in larger plugs (i.e. >100 cf. 50 cm<sup>3</sup>) with adequate, but not excessive, fertilization, and minimising the holding period in the nursery to keep down shoot:root may be desirable. Avoidance of shallow containers will ensure root architecture does not limit seedling growth after planting.

### *Seedling resistance to browsing*

Browsing of *E. globulus* and *E. nitens* seedlings by swamp wallabies, European rabbits (Clunie and Becker 1991), red-bellied pademelons and common brushtail possums (Bulinski 1999; Bulinski and McArthur 1999) is a significant cost to the forestry industry. Browsing often occurs soon after seedlings are planted (Bulinski 1999). It has been shown that nursery management can reduce the palatability of seedlings to herbivores (Marks and Moore 1998; McArthur et al. 2002a, b). When nutrients are limiting seedlings produce greater levels of foliar tannins. Tannins act as digestibility reducers or as toxins to herbivores (Hagerman et al. 1992, Foley et al. 1999). Investigation of seedling nutrient application treatments in feeding trials has shown up to 2-fold greater intake of high N (> 1.8% DM) seedlings c.f. low N (0.6 % DM) seedlings by pademelons and possums under controlled environment experiments (McArthur et al 2002a, b). However, seedlings with intermediate foliar N content (0.80 – 1.0 % DM) were not browsed significantly more than seedlings of low N content (0.6 %DM). Investigation indicated that browsers responded to leaf nutrient content and leaf toughness (McArthur et al. 2002a). These results have translated to field planted *E. globulus* and *E. nitens* seedlings (McArthur, Pietrzykowski and Close unpub results). Although preliminary, low N, high tannin seedlings were relatively resistant to browsing compared with high N, low tannin seedlings, although no difference was observed at a site with very high browsing pressure by pademelons and wallaby. An alternative is the use of browsing repellents. Research has indicated that Neem seed extracts containing azadirachtin, a repellent with a pungent odour imitating the smell of urine of predators had a modest effect, but that wallaby-repellent (WR-1) – a spray on grit – was highly effective on foliage to which it was applied (Witt 2002). Spraying of WR-1 in the nursery has proved feasible, although two applications may be necessary on *E. nitens* for sufficient adherence (C. Martin pers. comm.).

## Management options

Avoiding high rates of nutrient application to *E. nitens* is warranted if browsing after planting is a concern. Nutrient application resulting in foliar N concentration of 0.8-1.0% DM may represent the best trade-off between seedling resistance to browsing and growth potential after planting. In-nursery application of WR-1 is feasible and may be cost effective.



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