Stress Studies in *Eucalyptus*

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ABSTRACT

Eucalypts are endemic to Australia where they are the dominant species in more than 40 million ha of natural forest and grow across a large range of climatic environments and soil types. Due to its adaptability, extremely fast growth rate and excellent wood and fiber properties, the genus became the most widely planted forest tree (18 million ha) at a world scale mainly for pulpwood and timber. *Eucalyptus globulus* is the most planted species for temperate zone plantations (e.g. Portugal, Spain, Chile and Australia) while *E. grandis* and *E. urophylla* are extensively grown in tropical and subtropical regions (e.g. Brazil, South-Africa, Congo). Adaptation to aridity is considered a major factor in the evolution of the genus and resulting geographic distribution of the species. Apart from drought other environmental stresses such as extreme temperatures, salt or excess light limit productivity which is often less than its very high potential. Moreover frost, drought and competition are the major causes of mortality in juvenile plantations. Among biotic stresses the genus *Eucalyptus* has to deal with a diverse range of invertebrate and mammalian herbivores that consume foliage and fungal diseases also cause great losses very detrimental to economically important *Eucalyptus* species. This review describes the main studies conducted in *Eucalyptus* on these different stresses and specific problems occurring in natural forests or in the plantations spread out around the world. For each type of stress we describe the impact on the trees, the corresponding tolerance mechanisms developed by the plant and the methods designed for protection or genetic improvement.

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INTRODUCTION

Eucalyptus is the most valuable and most widely planted genus of forest trees worldwide. Eucalypt plantation establishment in tropical, subtropical and more recently temperate areas is favoured because these trees are fast growing, easily cultivated, adaptable and suitable for industrial plantations, agroforestry and community forestry.

Eucalypts are native to Australia, New Guinea and neighboring islands (**Fig. 1**). With the exception of extreme

Invited Review

Fig. 1 The "Cazneaux Tree". Ancient (clearly stressed) *Eucalyptus camaldulensis* Dehnh. (river redgum) near Wilpena Pound, Flinders Ranges, South Australia. Photo: R. Wenzl-Bossinger. The photographer Harold Cazneaux took a picture of this exact tree with the walls of Wilpena Pound as background, in May 1937. He regarded it as his most Australian picture and called it the Spirit of Endurance. It won many awards in Australian and International exhibitions. In May 1941 Cazneaux wrote: "This giant gum tree stands in solitary grandeur on a lonely plateau in the arid Flinders Ranges, South Australia, where it has grown up from a sapling through the years, and long before the shade from its giant limbs ever gave shelter from heat to white men. The passing of the years has left it scarred and marked by the elements - storm, fire, water, - unconquered, it speaks to us from a Spirit of Endurance. Although aged, its widespread limbs speak of a vitality that will carry on for many more years. One day, when the sun shone hot and strong, I stood before this giant in silent wonder and admiration. The hot wind stirred its leafy boughs, and some of the living elements of this tree passed to me in understanding and friendliness expressing The Spirit of Australia."

Fig. 2 Eucalypt forest in the Grampians (Gariwerd) National Park, Victoria, Australia. Photo: R. Wenzl-Bossinger.

low temperatures, most climates of the world are represented over this geographic range. They occur naturally from sea level to the alpine tree line, in high-rainfall and temperate zones as well as in semi arid and hot arid regions (Specht 1970; Eldridge *et al.* 1993) (**Figs. 2-4**). With about 700 species, the genus *Eucalyptus* contains a remarkably wide range of species reflecting its adaptation to a wide variety of sites. Adaptations to aridity, and consequently to

Fig. 3 Eucalypt natural stand of *Eucalyptus grandis* at South Forster, NSW, Australia. Photo: G. Moran.

drought, heat and salinity are considered major factors in the evolution of the genus and the resulting geographic distribution of species. However, a number of eucalypt species are currently showing decline in their natural range, probably related to climate change and increased drought (Potts B., personal communication). In contrast to the relatively high tolerance of many species to arid conditions, because of Australia's generally warm climate, the genus *Eucalyptus* is not adapted to intense frost. In particular, a lack of endodormancy and leaf persistence over winter make this tree very exposed to freezing injuries (**Fig. 5**). Only a few species from alpine habitats in southeast Australia are cold-hardy and only at a moderate level.

Introduction of eucalypt species into very different regions of the world was based on their distribution on the Australian continent. In 2000, more than 10 million ha of eucalypts were planted in Asia (mainly in India), around five million ha in South America (mainly in Brazil) and around two million ha in Africa (FAO 2004). Spread of eucalypt plantations in Europe and North America is more limited, mainly due to frost sensitivity. Currently, only a relatively small percentage of the numerous eucalypt species are commercially planted, the inclusion of more species into breeding programs will however expand with time. Four species, *E. camaldulensis*, *E. grandis*, *E. urophylla*, *E. globulus* and their hybrids account for 80% of plantations worldwide. *Eucalyptus grandis* is the most widely used species in tropical and subtropical areas. Particularly with *E. urophylla* as a hybrid, it is extensively grown in Brazil, South Africa, Congo and China (**Figs. 6, 7**). *Eucalyptus globulus* is the preferred species for temperate zone plantations in Portugal, Spain, Chile and Australia. *Eucalyptus camaldulensis* is the most adapted to arid and semi-arid lands and is also one of the most variable species (**Fig. 8**). It is planted

Fig. 4 Forest of *Eucalyptus marginata* and *Eucalyptus calophylla* near Dwellinup, Western Australia. Photo: G. Moran.

Fig. 6 Forest of eucalypts, Colombo/PR, Brazil. Photo: C. Marque.

Fig. 5 Juvenile leaves of the cold-sensitive *E. camaldulensis* under frost, Lectoure, 32, France. Photo: M. Navarro.

Fig. 7 Plantation of *E. grandis* x *E. urophylla*, Zulu Land, South Africa. Photo: C. Marque.

Fig. 8 Healthy (unstressed) *Eucalyptus camaldulensis* Dehnh. (river redgum) Australia. Photo: R. Wenzl-Bossinger.

in southern Europe and North Africa. Cold and drought are the two main stress factors limiting eucalypt plantations and species selection in Australia and around the world.

The physiology of many eucalypts is mostly directed towards productivity or growth at the expense of particular adaptive traits. Its fast growth rate is mainly based on a very high potential productivity combined with opportunistic development. Light-use efficiency values of eucalypts are high when compared with a wide range of temperate and tropical tree species. For example, the maximum value for young *E. grandis* trees growing in plantations in Brazil appears to be the highest recorded for any woody vegetation (Stape 2002) (**Fig. 9**). In contrast, for many natural communities and at sites where water and nutrient supplies are limiting, or where marked temperature extremes exist, productivity is much lower. Opportunistic growth means that growth periods are not interrupted by notable endodormancy nor leaf drop. Growth rates correlate strongly with

mean temperatures, however wide deviations occur in late summer and early autumn, possibly attributable to reduced water availability. Consequently, stresses limiting productivity in order of importance are cold, secondarily drought and then biotic or other abiotic stresses.

Introducing a eucalypt species into a new geographic

Fig. 9 Plantation of *E. grandis* x *E. urophylla*, Aracruz/ES, Brazil.
Photo: C. Marque.

region presents the challenge of finding a good compromise between adaptation to specific environmental conditions and productivity. The most tolerant species (e.g. *E. pauciflora* to cold) are often less productive and vice versa. One example was the introduction into South Africa of *E. grandis*, the most productive eucalypt species at optimum growth temperatures. The experience indicated that mean temperature of the coldest months should be no lower than 11°C and mean daily minimum temperature of the coldest months should be no lower than 4°C for optimum growth of the species. At sites with lower minima, other eucalypt species will be more productive (Eldridge *et al*. 1993).

In addition to assisting in the genetic improvement of eucalypts for adaptive traits, stress studies of a more fundamental nature are employing these trees as models for studies of tolerance mechanisms in long lived plants. Many species are exposed all year round to a variety of stresses. Since they grow across a wide range of climatic environments and soil types, they are likely to have developed unique and complex adaptive strategies. Moreover, with one of the smallest genomes among woody plants (500 Mbp), eucalypts are suitable models for many genomics and genetics studies. The *Eucalyptus* genome sequence is expected by 2010, the second tree genome sequence (after poplar) to be completed thanks to a large international cooperative effort (www.eucagen.org).

Until recently exotic eucalypts were renowned and admired for their strong resistance to pests and diseases where they were newly introduced. Actually, they rather performed well in the absence of their normal indigenous suite of herbivores. In Australia, herbivorous species including marsupials and insects were always the major problems particularly in young plantations. In addition, over recent decades, plantations of cloned forests in the tropics resulted in an enormous reduction in the genetic variability and an apparent increase in fragility and sensitivity to different biotic stresses. Moreover, the worldwide deployment of eucalypt species has created a mosaic of suitable host types through which pests can spread. Some eucalypt-specific pathogens were introduced into new geographic zones, together with their hosts. Among the numerous examples are *Phoracantha semipunctata*, and the redgum lerp psyllid, which appeared in California respectively in 1980 and 1998 and became a major pest (Paine *et al*. 2000, 2006), *Thaumastocoris* in southern Africa or *Ophelimus eucalypti* in southern Europe and Africa. This introduction of insects and fungi from Australia is still on the increase e.g. in New Zealand (the number of introduced spp has increased from 26 since 2001). Finally, of particular concern, are pathogens which do not currently occur in Australia and appear to have adapted to eucalypts. These host jumps are common for fungal pathogens, for example eucalypt rust is not known on eucalypt species in their native habitat. It apparently originated from native Myrtaceae in South America, is highly infective on some eucalypt species and has become one of the

most serious forestry diseases in Brazil. In the same way, various species of *Paropsisterna* which are pathogens of native Myrtaceae, are also devastating diseases of introduced eucalypt species in South East Asia, South America and Africa (Slippers *et al*. 2005).

This review will deal with the major stresses reported in the eucalypt literature, their economic impact on growth and survival of eucalypts in their native range of distribution as well as their productivity where they are grown commercially. Although other stresses have significant impact on eucalypt growth and survival (e.g. soil nutrient deficiency, water logging and interspecific competition), only the major ones would be considered, drought, frost, salt, herbivory and pathogens.

DROUGHT STRESS

The literature on plant responses to drought is vast and recent progress, at the molecular and whole-plant levels, has been comprehensively reviewed by Chaves *et al*. (2003). In their constant struggle between managing water loss and fixing carbon, plants respond differently to gradual and slow water depletion over weeks to months than to sudden water shortage within hours or days.

Passive avoidance, which is a consequence of reduced nutrient availability resulting from limited water availability upon soil drying, affects plant growth (carbon fixation) and function (McDonald and Davies 1996). Tolerance to low tissue water potential can involve osmotic adjustment (Morgan 1984), more rigid cell walls, or smaller cells (Wilson *et al*. 1980). The effects of drought and salinity on photosynthesis and respiration have recently been reviewed by Flexas *et al*. (2006). These authors suggest that metabolic responses to severe drought occur indirectly as a consequence of oxidative stress, rather than as a direct response to water shortage.

Of particular relevance for perennial plants is that mechanisms used during seedling development might differ substantially from those employed by mature plants.

The ecological range of the genus *Eucalyptus* stretches from regions with an annual rainfall of 250 mm in southern and 350 mm in northern Australia to the wet sclerophyll forests of eastern and south western Australia where rainfall exceeds 1500mm/year (Merchant *et al*. 2006). Species of the subgenus *Symphyomyrtus* are widely distributed across the continent, particularly in more arid habitats and regions subject to prolonged periods of water deficit. It was suggested that the distribution of eucalypt species is particularly dependent on the availability of water (Adams 1996). However, while many species of the genus are known for their tolerance of arid conditions, there has been little research into attributes that might confer such tolerance. Transpiration losses globally from eucalypts were found to be similar to those of other forest trees, except in situations where some species of eucalypts that do not have stomatal regulation are planted in soils where the roots have free access to ground water and evaporative demand is high (Calder 1992).

Seedling responses to drought

Studies investigating eucalypt seedling responses to drought stress measured seedling growth, dry matter allocation (Ngugi *et al*. 2003a), transpiration efficiency, water use efficiency and carbon isotope composition (δ13C) (Ngugi *et al*. 2003b). Stomatal closure, reduction of leaf area and change in leaf orientation were identified as the main tolerance mechanisms in seedlings (Gindaba *et al*. 2004). A decrease in soil water availability was found to cause a consistent increase in δ13C in all provenances tested (Ngugi *et al*. 2003b). This stands in contrast to results by Schulze *et al*. (2006) which investigated specific leaf area and isotope ratio (δ13C) in leaves and samples of recent wood where δ13C decreased with decreasing annual rainfall; however, in lower rainfall areas (<300 mm) no statistically significant

relationship was found. Rainfall affected δ13C indirectly through soil type (a surrogate for water-holding capacity) across the rainfall gradient. Schulze *et al.* (2006) concluded that species-specific traits are important in understanding the response of *Eucalyptus* to rainfall and that the diversity of the genus may reflect its response to the large climatic gradient in Australia and to the large annual and inter-annual variations in rainfall at any one location. Ngugi *et al.* (2003c) found significant differences also between prove-nances in osmotic potential at full turgor and water potential at the turgor loss point. In seedlings of *E. spathulata* subjec-ted to water deficit stress, the reduced osmotic potential was found to be correlated with increasing concentrations of a range of constitutive solutes (Merchant and Adams 2005). It was also argued that increased concentrations of the cyclic polyol quercitol, acting as osmolytes, were the likely means of balancing accumulation of inorganic ions.

Upstream of these cellular changes induced by drought stress are alterations of gene expression and synthesis of the corresponding proteins. In order to investigate the initial response of *E. grandis* to water stress deficit Labate CA and co-workers (Andrade *et al.* 2007) recently analysed the changes in the root proteome for six month-old plantlets. After seven days of drought 66 proteins showed significant changes in spot volumes with 49 having increased abun-dance and 17 reducing their expression level. The functio-nal categories of these proteins were identified as defence, energy, metabolism, cell structure and signal transduction.

Rooted cuttings as an experimental system

Eucalyptus globulus rooted cuttings showed significantly reduced evapotranspiration, leaf growth and maximum per-meability when subjected to drought (Pita *et al.* 2003). These authors showed that the most drought resistant clone displayed higher drought-induced reductions in maximum permeability than plants from other clones, had signifi-cantly smaller leaves and maintained higher values of pre-dawn leaf water potential as soil water content declined.

Drought responses in mature trees

In recent years studies on eucalypts have concentrated on measuring the effects of drought stress on plant growth and survival and mapping the mechanisms employed for drought avoidance. Studies on mature eucalypt trees monitored im-pacts on wood density (Searson *et al.* 2004), root distribu-tion (Moroni *et al.* 2003), stem mortality due to cavitation (Rice *et al.* 2004), morphological responses including leaf orientation and decrease in growth, reduced leaf area ratio, specific leaf area, leaf-to-root area ratio. They indicate grea-ter carbon allocation to the root system in response to drought, in a drought-resistant clone, which also possessed higher root hydraulic conductance and higher leaf-specific hydraulic stomatal conductance (Costa e Silva *et al.* 2004). Physiological responses were also measured, including sto-matal conductance and net photosynthesis (Ngugi *et al.* 2004), dry matter accumulation and allocation, leaf guard cell length, minimum daily leaf water potential, carbon iso-tope composition (described as water-use efficiency) and abscisic acid (ABA) concentration (Li and Wang 2003). One species that responds to reduced water availability by altering growth characteristics is *E. camaldulensis* which was identified as a dehydration postponement species because it uses mechanisms that delay growth while main-taining turgor and water uptake (Lemcoff *et al.* 2002) (**Fig. 10**). Measurements of carbon isotope fractionation at sites along rainfall gradients and estimation of the relationships between leaf area, sapwood cross-sectional area and con-ductance demonstrated homeostatic adjustment of hydraulic properties in relation to growing conditions (Whitehead and Beadle 2004). Metabolic responses in leaves and roots in-cluded changes in concentrations of soluble and insoluble sugars, proline, total protein and several antioxidant en-zymes together with drought-induced reduction in growth
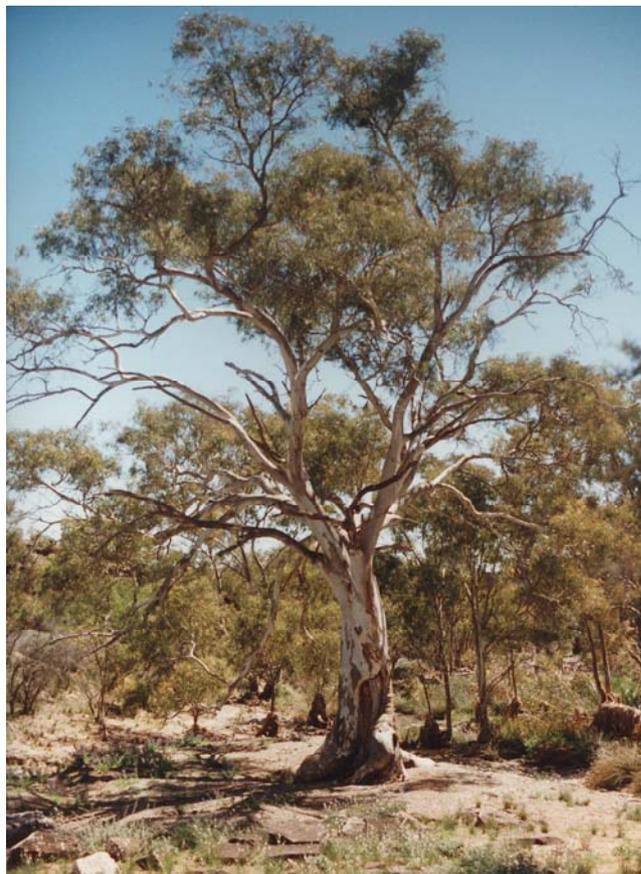

Fig. 10 *Eucalyptus camaldulensis* **Dehnh.** (river redgum) growing in a dry creek bed in the Flinders Ranges, South Australia. River redgums are regarded as one of the species most tolerant to saline conditions. Its ability to also withstand extreme temperatures as well as drought and periods of water logging has enabled this tree to survive in this inhospitable environ-ment for many decades. Photo: R. Wenzl-Bossinger.

and decreased osmotic potential (Shvaleva *et al.* 2005). In these experiments glutathione reductase activity increased significantly in response to water deficit, suggesting that this enzyme plays a protective role in roots during drought stress by catalyzing the catabolism of reactive oxygen spe-cies (Shvaleva *et al.* 2005).

A major response in *Eucalyptus*: osmotic adjustment

Eucalypts respond to drought stresses by lowering cell water potential which assists in maintaining cell water con-tent and consequently turgor (Meyers and Neales 1986; Lemcoff *et al.* 1994; Stoneman *et al.* 1994; White *et al.* 2000). Osmotic adjustment (Meyers and Neales 1986; Lemcoff *et al.* 1994) and inherent osmotic potential (White *et al.* 2000) however, vary greatly between different euca-lypt species. Eucalypts from low rainfall areas generally have lower leaf water potential at the point of turgor loss and weaker stomatal responses to soil drying (White *et al.* 2003). Cyclitols, cyclic polyols characterized by a six-car-bon ring with associated hydroxyl groups, have been sug-gested as playing significant roles in the regulation of os-motic potential (Paul and Cockburn 1989) and have been isolated from an array of tree tissues (Popp *et al.* 1997). One such osmolyte, quercitol, was found to account for about 3% of dry weight in West Australian mallee eucalypts and 5.5% in several Victorian mallee eucalypts, making sig-nificant contributions to cellular osmotic potential (Adams *et al.* 2005).

Such results warranted more detailed investigations into the presence of compatible solutes in a variety of eucalypt species from different ecosystems ('mesic' and 'xeric') and

different sub-generic taxonomic groups (Merchant *et al*. 2006). These authors found that responses to water deficit followed two distinct patterns: *Eucalyptus* species from 'mesic' environments adjusted concentrations of sucrose in response to water deficit, whereas 'xeric' species increased concentrations of quercitol. In root tissues, only species from xeric environments contained high levels of quercitol and mannitol, increasing under water deficit. Accordingly, the mesic strategy was argued to be beneficial as response to short-lasting drought conditions, because sucrose is easily metabolized, whereas the xeric strategy may relate to an effective acclimation to longer-lasting drought. These physiological response groups also were related to taxonomic groups within the genus (Merchant *et al*. 2006).

COLD STRESS

Extreme air temperatures, heat and freezing, can limit plant productivity and sometimes cause their premature death. High temperatures can have direct damaging effects associated with hot tissue temperatures or an indirect effect through plant water deficit that can arise due to high evaporative demands. Heat stress studies in eucalypts seem to indicate that this is not a major problem at a world scale. Overall, eucalypt species are known to be reasonably tolerant to heat, for instance, the heat tolerance value of *E. camaldulensis* leaves varies from 47.4 to 50°C (Karschon and Pinchas 1971) while seedlings of *E. obliqua* are able to survive a temperature of 80°C (Moore *et al*. 1977). Moreover, *E. grandis* micropropagated shoots exposed at 41°C for three hours were able to recover after 24h whereas they did not completely recover after a cold shock (0°C), (Souza

et al. 2004). In the same work, the metabolism, considering especially soluble proteins and proline, appears to be more affected by low than high temperatures although *E. grandis* was found to respond to both classes of stimulus.

Exposure of plants to subzero temperatures results in extracellular ice formation, efflux of water and cellular dehydration. Growing ice crystals can cause mechanical damage to the cells and freezing temperatures can cause denaturation of proteins and disruption of macro-molecular complexes. Consequently, freezing tolerance is strongly correlated with tolerance to dehydration also caused by drought or high salinity. In fact many protective mechanisms and related gene activity are shared between the three stresses. Finally, low temperatures, especially in combination with high light, can cause excessive production of reactive oxygen species (ROS) and hence tolerance to frost also correlates with effective scavenging systems for ROS to cope with oxidative stress.

Eucalyptus is a genus of evergreen trees without endodormancy. All the species of the genus are opportunistic and, if other factors are not limiting, they continue growth through the winter. Therefore, most species are quite sensitive to frost, with the naked buds and leaves the primary organs subject to freezing damage (**Fig. 11**). The frost tolerance of the Tasmanian subalpine eucalypts appears to be amongst the greatest for any species within the genus. *Eucalyptus gunnii* and *E. pauciflora* are probably the hardiest; however, after hardening they only tolerate -14 to -18°C whereas boreal and temperate trees like birch are able to survive below -70°C under dormancy and full cold acclimation. Therefore the establishment of eucalypt plantations outside of their native area is still limited to the most temperate regions and growth characteristics of the hardiest species make them unsuitable for commercial plantations. *Eucalyptus gunnii* is cultivated in some cool European areas like France as a pure species or for the introgression of frost

Fig. 11 Juvenile leaves of the cold tolerant *E. gunnii* under frost, Lectoure, 32, France. Photo: M. Navarro.

Fig. 12 Young plantations of *E. gunnii*, Longages, 31, France. Photo: C. Marque.

Fig. 13 *E. dalrympleana* trees, Longages, 31, France. Photo: C. Marque.

tolerance into the more fast-growing commercial species (**Figs. 12-14**). Unfortunately, the major productive or commercially interesting species, *E. grandis* and *E. globulus*, are highly frost sensitive (-6 to -8°C). *Eucalyptus globulus* was successfully introduced into the Mediterranean basin (mainly Spain and Portugal) and South America (mainly Chile) but the expansion of the plantations is still limited by the periodic occurrence of low temperatures during winter (Scarascia-Mugnozza *et al*. 1989; Fernandez *et al*. 2006).

Frost tolerance of eucalypts is also an important issue in their native continent where unseasonal or particularly severe frosts cause damage to both natural populations and plantations. Frosts occur over many parts of Australia, generally with increasing severity and frequency at more southerly latitudes and higher altitudes. In Tasmania, one of the coldest regions in Australia, a study of subalpine eucalypts suggests that their natural distributions was determined by rare exceptionally severe frosts (Davidson and Reid 1985). Of the fast growing species, *E. nitens* is considered the most suitable for planting on high altitude sites in Tasmania but significant expansion in the area of these plantations has led to their establishment at altitudes that are close to the upper limit of their planting distributions, resulting in productivity losses.

Due to the underlying commercial implications, *E. nitens*, *E. gunnii* and *E. globulus* constitute the main target species in the studies on cold stress. The main investigations first focussed on genetic analyses and further aimed to develop rapid and early laboratory tests of cold tolerance along with biochemical, physiological and molecular genetic studies. Since there was no comprehensive review on the subject up to date, we will give an overview of research over the last 20 years with a focus on recent molecular genetics work.

Genetic studies

Pryor, one of the pioneers of cold tolerance genetics in *Eucalyptus* considered that four lines of research should be

Fig. 14 A young *E. gunnii* x *E. dalrympleana* tree combining cold tolerance and productivity. Catonvielle, 32, France. Photo: C. Teulières.

explored in breeding eucalypts for freezing tolerance: assessing species, provenances and families within species, natural hybrids and manipulated hybrids (Pryor 1957). All these recommendations were addressed in subsequent decades. Quantitative variation in freezing tolerance was first investigated at the intraspecific level. Although it was sometimes shown to vary with seedling age over the first year of development, as for *E. delegatensis* (Battaglia and Reid 1993), winter frost tolerance was a trait with considerable variation within all the tested species as in *E. nitens* (Griffin and Cotterill 1988) or *E. globulus* (Tibbits *et al*. 2006). This indicates that considerable genetic gains may be made by classical programs of recurrent or mass selection within pure species. Moreover, when winter hardened *E. nitens* seedlings from 198 families, representing five provenances were studied for frost tolerance, provenance effect accounted for between 11% and 29% of the total variation, family effect for 14.22% and seedling effects for 22 to 26% (Raymond *et al*. 1992a). However, in this work, no strong relationship was found between frost tolerance and the altitude of origin. These patterns indicate that genetic variation for increasing frost tolerance may be evident at three levels: provenance, family and within family. Heritability of frost tolerance was also tested in controlled and open-pollinated families of *E. globulus* and *E. nitens* (Volker *et al*. 1994). It proved moderate to high for both species, ranging from 0.23-0.5 for controlled pollinated material and similar values were observed for open-pollinated families suggesting that removal of selfing and other inbreeding effects have occurred.

The use of interspecific hybridization was evaluated, as a means of breeding more resistant strains of eucalypts or of hybridizing fast growing species with highly resistant species. Tibbits and co-workers (Tibbits *et al*. 1991) examined the inheritance of freezing tolerance in interspecific F1 hybrid families of eucalypts corresponding to 27 different species combinations and a range of hardiness levels. The trait was found to be inherited in a predominantly additive manner, although there was a tendency in partial dominance toward the more sensitive species in some combinations (e.g. *E. gunnii* × *E. globulus* or *E. nitens* × *E. globulus*). The full expression of this genetic variation appeared to increase with the hardiness and in some cases appeared to vary with ontogeny. These data validate this breeding strategy for the introgression of frost tolerance into the commercial species since interspecific hybrids can be produced which have significantly greater freezing tolerance to that of the less resistant parent.

The most recent strategy for improving frost tolerance of eucalypts relies on the molecular marker approach. The most relevant result concerns the identification of the two first QTL influencing freezing tolerance in an outbred family of *E. nitens* (Byrne *et al*. 1997). These QTL, based on RFLP markers present on the same linkage group but located 40cM apart, explain between 7.7 and 10.8% of the phenotypic variation. Unfortunately this mapping result, with interesting implications for the improvement of freezing tolerance in eucalypts, remains the only one reported to date. More recently, RAPD and isozyme techniques were assessed with regards to cold hardening on leaves of three eucalypt species (Kuzminski and Malvolti 1992). The tender species, *E. camaldulensis* did not present any modification for all the tested systems. However, the cold resistant *E. gunnii* and *E. dalrympleana* showed different isoforms for six enzymes in the hardened vs. the unhardened tissues suggesting these patterns as potential markers of frost tolerance for these species. When the RAPD technique was used to differentiate 15 *E. globulus* clones (8 resistant and 7 sensitive), one unique polymorphism was observed but without any association with cold tolerance level. Undoubtedly, the identification of molecular markers for frost tolerance and mapping could help in the selection of the hardiest eucalypt genotypes. Finally, association studies using single nucleotide polymorphisms (SNPs) within the transcription factor CBF (CRT/DRE Binding Factor) candidate gene have re-

cently commenced in *E. delegatensis*, *E. regnans* and *E. gunnii* (Reed *et al.* 2007).

Development of screening tests for frost tolerance

The development of reliable laboratory methods for non destructive assessment of eucalypt cold tolerance, that are more reliable than visual methods, was a major goal in breeding programs targeting this trait. Such tests are necessary for ranking genotypes and partitioning of total variation into components due to provenances, families and individuals. The main procedure, called the "electroconductivity" or "ion leakage" method measures leaf damage in terms of the electrical conductivity of leaf discs exposed to freezing temperatures, where the conductivity of the leachate correlates with damage to the membranes. This procedure was first described for *E. pauciflora* seedlings (Harwood 1981) and proved very efficient since it is simple and fast and provides results in good agreement with visual classification and data more amenable to statistical treatment and interpretation. The test, successfully applied to other eucalypt species (Hallam and Tibbits 1988; Leborgne *et al.* 1995) and further improved for better reliability (Raymond *et al.* 1992b) is still extensively used for phenotyping in breeding programs. Another test also based on the evaluation of plasma membrane disturbance by measuring ethylene/ethane ratios was assessed for measuring frost injury in several species (Scarascia-Mugnozza *et al.* 1989; Kuzminski and Scarascia-Mugnozza 1992). Although this ratio provided very precise results and allows a sharp distinction between stressed *vs* irreversibly injured tissues, the procedure is not commonly used to date.

Other factors supposed to be representative of hardening and cold tolerance were evaluated as criteria for early selection. Electrical impedance, relative water content and osmotic potential did not prove reliable enough to be used as accurate predictors for frost tolerance, probably due to differences in the physiological state of the tested plants (Serrano and Pardos 1992).

Methods for assessment of cold tolerance were developed for *E. gunnii* using cell suspension cultures and protoplasts under controlled freezing (Teulières *et al.* 1989). In this case, damage was evaluated by the cell viability decrease measured through TTC (Triphenyl Tetrazolium Chloride) reduction of cells or FDA (Fluorescein Diacetate) staining for protoplasts. For both cellular systems, tolerance was correlated with the degree of frost tolerance of the starting clones. These tests constitute useful tools for investigating the cold tolerance mechanisms at the cellular level.

Physiological, biochemical and biophysical studies

Temperate plants develop a greater ability to withstand freezing in response to a period of low but nonfreezing temperatures through a complex adaptive process of cold acclimation. At the cell level, the most notable effect of cold acclimation include the accumulation of compatible osmolytes in the same way as for drought or salt adaptation and changes in membrane lipid composition (for review see Xin and Browse 2000; Kalberer *et al.* 2006).

Literature on the environmental factors affecting the cold acclimation process in eucalypts is rather poor to date. The temperature regime commonly used for trees in controlled conditions consists of a progressive cooling over 5 to 7 weeks down to 3 or 4°C night temperature for *E. nitens* or *E. globulus* (Tibbits and Reid 1987; Davidson *et al.* 2004) and 0°C for *E. gunnii* (Leborgne *et al.* 1995). Cell suspension cultures of *E. gunnii* were also able to harden when cooled to 10°C (Leborgne *et al.* 1995). While for *E. nitens* both hardening and de-hardening were dependent on the temperature regime irrespective of the photoperiod (Tibbits and Reid 1987), cold acclimation of *E. gunnii* slightly increased under short days at low temperature (El Kayal *et al.* 2006). However, in this latter experiment, a strong predomi-

nance of temperature over light was observed for the cold adaptive response in agreement with previous observations of Paton (Paton 1978) for a number of physiological responses in 12 eucalypt species. Characteristic of eucalypts compared to many perennial woody plants, this low or undetectable effect of photoperiod on cold hardening is consistent with the absence of endodormancy and temperature dependent opportunistic development. Finally, as commonly observed for cold responses, the hormone ABA did not prove to be directly involved in cold acclimation (Leborgne 1994).

Due to its opportunistic developmental features including a lack of dormancy and a persistence of the leaves over winter, species of the genus *Eucalyptus* are likely to be subject to photoinhibition when the temperatures drop. Several papers deal with this question. At low temperature, photoinhibition can be severe and is usually associated with high levels of irradiance. In *E. pauciflora*, cold-induced photoinhibition was shown to limit the distribution of juvenile plants due to the protective dissipation of absorbed light energy rather than damage to photosystem II (Ball *et al.* 1991). Another species well adapted to low temperature, *E. nitens*, is also susceptible to severe levels of photoinhibition if planted out in periods associated with repeated frosts but this phenomenon is no more significant following frost in established saplings (Close and Beadle 2003). In any case, *E. pauciflora* recovers more quickly from photoinhibition than *E. nitens*, consistent with its occurrence in colder habitats (Warren *et al.* 1998). For both species, cold hardening was found to reduce photoinhibition resulting in a higher photosynthetic capacity, lower level of non-photochemical quenching and faster recovery (Warren *et al.* 1998). The decreased susceptibility of photosynthesis accompanying the development of cold tolerance was found to be due to a higher photosynthetic capacity and a reduced demand for non-radiative energy dissipation (Warren *et al.* 1998). More recently, photosynthetic responses to overnight frosts were investigated in *E. nitens* and *E. globulus* (Davidson *et al.* 2004). In the field, consecutive frosts had a cumulative effect, reducing maximum net photosynthesis. This decrease is independent of photoinhibition but is associated with decreased quantum yield and stomatal conductance. In order to separate the effects of light, temperature and CO_2, consequences of CO_2 enrichment on eucalypt freezing injury were evaluated (Barker *et al.* 2005). Elevated CO_2-grown plants had more necrotic leaf area following freezing events than ambient CO_2 plants and also exhibited a lower stomatal conductance.

Most of the physical and biochemical studies of cold tolerance in eucalypts involve correlative analyses of differentially tolerant genotypes or more commonly acclimated vs. non-acclimated plant material. Biochemical studies on eucalypt cold tolerance mainly deal with the accumulation of compatible osmolytes in the cells. For *E. globulus* and hybrids, a significant correlation between cold tolerance (LT50) and leaf osmotic pressure was observed during hardening (Almeida *et al.* 1994). This increase, likely to limit cell dehydration due to extracellular ice formation, was predominantly a result of an accumulation of soluble sugars. Interestingly, following this hardening period, photosynthetic capacity and quantum yield were not significantly affected, indicating that feedback inhibition of photosynthesis did not occur in the leaves despite carbohydrate accumulation. These results stand in contrast to observations in conifers where photosynthesis, and subsequently growth, is completely suppressed during cold hardening (Oquist and Huner 1991). When three subspecies of *E. globulus* were compared, Moraga and co-workers (Moraga *et al.* 2006) also found a correlation between their level of cold tolerance and total sugar content. In contrast, for three eucalypt species (*E. gunnii*, *E. dalrympleana* and *E. globulus*), although a good correlation was observed between carbohydrate content and seasonal temperature fluctuations, no difference could be detected between cold hardy and sensitive species (Kuzminski and Scarascia-Mugnozza

1992). In *E. gunnii*, carbohydrates and especially sucrose, fructose and raffinose also accumulate in leaves during cold acclimation both for trees in the field and young plants in controlled conditions (Leborgne *et al.* 1995a). In this case, the accumulation was correlated both with the level of basic cold tolerance and acclimation, strongly suggesting a role for these sugars in the protection. The two correlations were confirmed in cell suspension cultures from the same species (Leborgne *et al.* 1995b) and interestingly, in this simplified system, the cold hardening could be mimicked by feeding the cells with fructose and raffinose. The potential role of the sugars in frost tolerance of *E. gunnii* was further partly elucidated through these feeding experiments (Travert *et al.* 1997). The positive effect was found to be specific to certain carbohydrates and was not colligative since it was independent of osmolality and total sugar content. Therefore, the accumulation of sugars during the cold hardening of these very cold-hardy eucalypt species does not play a role in limiting cell dehydration but acts locally and specifically to protect macromolecules and membranes.

The plant cell membrane is known as the primary site of cold injury. Through two complementary biophysical approaches on the same *E. gunnii* cell suspension cultures, membrane fluidity was investigated in relation to frost tolerance (Leborgne *et al.* 1992). Both the tonoplast and plasma membrane were found to exhibit a higher fluidity in the frost tolerant cell lines and, in all conditions tested, the tonoplast is more fluid, indicating better tolerance to freezing injury. Altogether, these data and preliminary experiments coupling sugar introduction and fluidity measurements suggest a cryoprotector role for sugars in *E. gunnii* cold tolerance through maintenance of plasma membrane fluidity.

Molecular genetic studies

Cold acclimation is associated with wide and complex changes in gene expression, particularly for cold-responsive (Cor) genes encoding proteins directly involved in cell protection. The CBF transcription factors, which regulate a large pool of genes involved in osmolyte synthesis or other protective mechanisms, have emerged as mediators of cold acclimation in higher plants.

Studies on eucalypts include global transcriptome analyses and in depth characterization of cold regulated genes. As a first step towards the identification of genes involved in cold tolerance, cDNA libraries were constructed including total cDNA libraries from leaves of cold-acclimated *E. nitens* (Fullard and Moran 2003) or *E. gunnii* (Keller 2006) of cold-treated *E. globulus* (Krauskopf *et al.* 2007) and a subtractive cDNA library (acclimated *vs* non-acclimated) from *E. gunnii* cell suspension cultures (El Kayal 2004). The *E. nitens* library was used to identify genes from *E. delegatensis* differentially expressed in a range of natural populations exhibiting distinct cold tolerance (Reed *et al.* 2006). From the 4946 *E. globulus* genes, 6% correspond to the stress response (Gamboa *et al.* 2007). From this library the authors recently isolated one CBF gene and one ICE-like (Inducer of CBF expression) gene (Rasmussen-Poblete *et al.* 2007). When expression profiling was carried out on the subtractive *E. gunnii* library, 52 differentially-regulated EST were identified, most of them also responding to other stimuli like NaCl, mannitol or ABA. These genes belong to functional categories corresponding to signalling and transduction, protection against stresses and metabolism. From the *E. gunnii* cold acclimated leaf library, 11303 EST were annotated and functionally classified (Keller, submitted) while 1995 were analysed for expression profiles (Keller 2006). The library proved enriched for genes known to play a role in cold acclimation such as detoxification enzymes or LEA (Late Embryogenesis Abundant) with a very high representation of dehydrins. The clustering of the genes according to their kinetics of cold regulation resulted in very distinct profiles differing by the earliness and duration of the response. Taken together, these annotations and ex-

pressional data, a pool of potential candidate genes could be defined containing unknown strongly regulated genes, genes encoding effector proteins believed to participate in adaptive mechanisms and finally genes encoding transcription factors likely to control these "protector" genes.

Among this collection two *E. gunnii* genes were further characterized. EgSXD1 encoding the tocopherol cyclase involved in vitamine E synthesis was isolated from the cold down-regulated library (El Kayal *et al.* 2006). Cold-inhibition of EgSXD1, confirmed by real time RT-PCR, leads to a decrease in leaf tocopherol content. This was unexpected given that tocopherols are commonly reported to accumulate after cold or salt stresses and to play an antioxidant role. The alteration in tocopherol content in eucalypts after a cold exposure is suggested to act as a signal because of the modifications of the redox potential in the chloroplasts and would result in the activation of one or several oxidative stress response pathways. These data were the first to suggest the potential involvement of this gene in cold hardening of plants. The second family of genes to be studied in the context of *Eucalyptus* cold tolerance encodes the CBF/DREB1 transcription factors which are well-known for their key role in the cold response of a number of plants. Two CBF homologs EguCBF1a and EguCBF1b were isolated from *E. gunnii* (El Kayal *et al.* 2006). Real-time RT-PCR analyses showed a very strong and fast response to cold. Both genes exhibited several similar features such as the positive effect of dark conditions and cold intensity on cold regulation. However, interestingly, their regulation by cold differed and proved complementary in particular in terms of induction kinetics. The positive effect of short day-length both on freezing tolerance and CBF transcript accumulation suggests a potential involvement of these genes in adaptive responses. In addition to the confirmation of CBF as good candidate genes of cold tolerance in eucalypts, this study highlighted contrasting responses of *Eucalyptus* CBF compared to *Arabidopsis* and other herbaceous plants suggesting significant structural and regulatory differences. These differences may be related to the very characteristic biology and physiology of eucalypts and stresses the necessity of carry out such studies on homologous genes to determine candidate genes for breeding strategies.

Although at a very early stage, these molecular studies of cold tolerance in the genus *Eucalyptus* already indicate how specific and complex this taxa is in its adaptive response. They open exciting prospects for revealing the molecular mechanisms underlying its unique developmental features. Combined with the mapping data of QTL influencing cold tolerance, these genomics achievements and EST collections should also within the next decade result in powerful new tools that can be integrated into breeding programs.

SALINITY STRESS

In many parts of the world, rising groundwater tables and increasing secondary salinization are major land degradation problems (FAO 2005). As a solution to the problems, revegetation of degraded catchments with deep-rooted perennials, including trees tolerant of saline soils, has been advocated (Akilan *et al.* 1997; Niknam and McComb 2000). Salt in soil water inhibits plant growth firstly because it reduces the plant's ability to take up water through a reduction in the water potential gradient (Thomson 1988) leading to reduced leaf and, to a lesser extent, root growth (Munns 1993), similar to osmotic stress caused by drought. Secondly, salt may enter the transpiration stream and eventually injure cells in transpiring leaves (ion-excess). The metabolic toxicity of Na^+ is largely a result of its ability to compete with K^+ for binding sites essential for cellular function, as many enzymes are activated by K^+ and Na^+ cannot substitute for this (Bhandal and Malik 1988). The main mechanisms for salt tolerance are to minimize the amount of salt taken up by the roots and to partition it at the tissue and cellular levels so that it does not build up to toxic

concentrations in the cytosol of transpiring leaves. Our current understanding of how plants deal with and respond to salinity stress has recently been comprehensively presented in an excellent review by Munns (2005). The hierarchical cascade of specific signals that bring about growth responses to salinity and their timing, be it hormonal control, water status or photosynthate availability, are still being debated. Anyway, genes that might be involved in the molecular control of salt tolerance have been categorized into three functional groups (Munns 2005) : (1) Genes that control salt uptake and transport, (2) genes with osmotic or protective function and (3) genes that allow plants to outgrow adverse conditions.

Among the eucalypt species, *E. camaldulensis* has been highly regarded for some time as a tree with useful tolerance of soil salinity. In a study of seedlings of 52 species of eucalypts in saline solution, Blake (Blake 1981) found that this species was the second most tolerant after *E. woodwardii*. Moreover, variation of salt tolerance between provenances of *E. camaldulensis* was found to be considerable ranging from 466 to 636 mM for NaCl concentration causing mortality of seedlings in glass-house trials (seawater is equivalent to *ca.* 550 mM) (Thomson *et al.* 1987). In this work the salinity level in the root zone at the site of origin, was not closely correlated with NaCl tolerance. These data indicate that localized selection for tolerance has taken place for populations growing at more saline sites, but high levels of tolerance also exist in some populations growing in soils of low salt content. As with frost tolerance, the development of non destructive screening methods that allow efficient monitoring of salt tolerance on very large numbers of propagules was an issue for salt stress studies. The use of chlorophyll fluorescence attributes proved useful for the early detection of particularly susceptible or resistant seedlings (Corney *et al.* 2003).

Long term adaptations

A range of physiological mechanisms have evolved in eucalypts to cope with the effects of excessive soil ion concentrations (Bell and Williams 1997). Germination is a crucial stage for establishment of any individual plant, and under saline conditions, it is strongly influenced by osmotic pressure caused by salts in the soil solution (Bell 1999; Madsen and Mulligan 2006), resulting in reduction of the total number of seeds germinating and postponement of initiation of the germination process (Kozlowski 1997). Seeds of *E. occidentalis*, *E. eremophila* and *Melaleuca cardiophylla* withstood the osmotic stress and ion toxicity after imbibition, and germinated in 200 mM NaCl while for most eucalypt species germination was severely limited at these concentrations (van der Moezel and Bell 1987).

Morphological (and anatomical) responses to reduce water loss due to salt stress were reported for specific *E. camaldulensis* hybrids (James and Bell 1995). More salt tolerant hybrids tended to have smaller but thicker leaves with a thicker cuticle, which is typical of plants growing in dry habitats. Cuticle thickness is often related to high light environments, where plant leaves usually have greater density but smaller stomata (Tichá 1985; Igboanugo 1992) enabling plants to be more flexible in regulating water loss (Bolhar-Nordenkampf 1985). Salinity is assumed to reduce the capacity of waterlogged plants to produce adventitious roots while poor substrate aeration is also known to decrease salt tolerance (Bell 1999). *E. camaldulensis* and *Casuarina obesa* have the ability to produce aerenchymous roots under condition of freshwater flooding, but this ability may be lost as salinity levels are increased (van der Moezel *et al.* 1988). A lack of adventitious roots was also noted in saline waterlogged *E. lesouefii* plants (van der Moezel *et al.* 1989). In contrast, a study on four eucalypt species has found the formation of adventitious roots and the swelling of the primary roots under saline-waterlogged treatment (Marcar 1993).

In addition to these physiological and morphological

adaptive mechanisms, responses of eucalypts to salt stress in relation to growth, nutrient uptake and transport have been analyzed in some detail (Sun and Dickinson 1993; Dunn *et al.* 1994; Grieve and Shannon 1999; Marcar *et al.* 2003). Many eucalypt species have evolved on salt-affected and nutrient-poor soils, and variation in growth between eucalypts grown in saline habitats is believed to represent species-specific salt tolerance mechanisms (Niknam and McComb 2000). Tolerance to saline conditions in eucalypts has generally been measured in terms of their survival and growth responses (recent examples include James *et al.* 2002; Marcar *et al.* 2002; Mahmood *et al.* 2003; Marcar *et al.* 2003; Archibald *et al.* 2006; Madsen and Mulligan 2006), as well as the ability to exclude Na$^+$ and Cl$^-$ both from organelles and plant tissues (Greenway and Munns 1980; Thomson 1988), and improvement in nutrient acquisition, uptake, use and retention (Kreidemann and Cromer 1996; Marshall *et al.* 1997). Recent studies have started to gather information also on eucalypt responses to salinity at the molecular level (Faridah 2004). These studies investigated the significance of Na$^+$/H$^+$ antiporters in trees by assessing gene expression levels in different clones, in response to hydroponic salt treatment, over time and in relation to leaf sodium accumulation. Results indicated that the eucalypt antiporter gene *EcgNaH* (a homologue of *Arabidopsis thaliana At*NHX1) was activated in response to Na$^+$ accumulation and that sodium compartmentalization into vacuoles of leaf cells is in fact an important salt tolerance mechanism in eucalypts (Faridah 2004).

Osmotic adjustment

Osmotic adjustment in eucalypts can be achieved by accumulation of ions (mainly sodium) in leaf tissue (Sands 1981; van der Moezel *et al.* 1988; Morabito *et al.* 1996) but this varies greatly between species. *E. camaldulensis* for example seems to be able to tolerate increased internal concentrations of sodium and/or chloride (Sands 1981) while other species achieve tolerance *via* stomatal closure and salt exclusion (van der Moezel and Bell 1990).

Protection against ion toxicity

Many eucalypt species have evolved on salt-affected and nutrient-poor soils of Australia and may have developed ion exclusion or ion partitioning mechanisms to cope with potentially toxic elements, usually Na$^+$ and Cl$^-$, that may occur in the root zone (Marcar and Temaat 1990; Munns 2002). Metabolic toxicity of Na$^+$ is largely a result of its ability to compete with K$^+$ for binding sites essential for cellular function (Su *et al.* 2002) and adverse effects of Na$^+$ on K$^+$ concentration have been reported for various plant species including eucalypts (Marcar 1993; Morabito *et al.* 1994; Chen *et al.* 1998; Grieve and Shannon 1999). Some contributions to our current knowledge of the effects of salinity on specific enzyme activities and for example chloroplast ultrastructure have in fact been made by research on eucalypts (de Aragão *et al.* 1997; Keiper *et al.* 1998).

To deal with ion toxicity, deposition of salts into bark, ray cells, tracheid walls, and older senescent leaves have been considered as tolerance mechanisms (Andolfatto *et al.* 1994; Niknam and McComb 2000). Removal of salts by shedding older leaves that are primary sites for salt accumulation has been observed in both halophytic (Weber *et al.* 1977) and glycophytic species including eucalypts (Marcar 1993; Munns 2002).

Related to tolerance of toxic Na$^+$ levels, studies on various plant species have provided evidence for active Na$^+$/H$^+$ antiport systems in plants exposed to salinity (Barkla *et al.* 1995; Apse *et al.* 1999; Fukuda *et al.* 1999; Shi *et al.* 2000; Hamada *et al.* 2001). Such systems make use of membrane transport proteins that simultaneously transport in opposite directions two different molecules across a membrane (in contrast to symport systems, which transport two different

molecules in the same direction). Gene transcripts homologous to known Na^+/H^+ antiporter genes have been found to be up-regulated in response to salinity also in eucalypts (Faridah 2004) and their activity increased in the presence of NaCl (Gaxiola *et al.* 1999; Hamada *et al.* 2001; Faridah 2004). Transcripts were present in root, shoot, leaf and flower tissues both in *Arabidopsis* (Apse *et al.* 1999) and eucalypts (Faridah 2004).

Despite obvious complexities in the regulation of overall plant metabolism, it seems that trees tolerate salt stress in similar ways to crops and weedy model plants, e.g. by maintaining low levels of Na^+ in the cell cytosol through Na^+ compartmentalization into the vacuoles and Na^+ exclusion through plasma membranes.

Nutrient uptake

Acquisition of essential mineral nutrients from soils may become difficult for plants when the soil solution contains very low concentrations of available macro and micronutrients, or if the environmental conditions (e.g. salinity, waterlogging, and drought) cause nutrients to become unavailable for uptake. Decreased levels of ions including Ca^{2+}, Mg^{2+}, and P were reported for eucalypt seedlings exposed to high NaCl concentrations (Prat and Fathi-Ettai 1990; Morabito *et al.* 1994; Sun and Dickinson 1995; Grieve and Shannon 1999).

It is reasonable to assume that to maintain P levels, plants affected by salinity may activate phosphate uptake and transport systems by regulating the activity of phosphate transporter genes. Supporting such assumptions, expression of a eucalypt phosphate transporter (*Ecg*PT) was found to be affected by salt treatment suggesting a direct effect of salinity on phosphate uptake (Faridah 2004).

Also, Ca^{2+} plays an important role in altering plant tolerance to salinity, and an increase in Ca^{2+} availability is considered to increase plant tolerance (Thomson 1988). However, patterns of Ca^{2+} content in relation to salinity are unclear. Some studies reported that increasing salinity increased Ca^{2+} content in eucalypt seedlings (Morabito *et al.* 1996), but other studies on the same species showed a reduction of Ca^{2+} with higher salinity levels (Grieve and Shannon 1999).

Other stress due to soil quality

Calcareous soil may be stressful for eucalypts, either in isolation or in combination with the above cited detrimental conditions. Because Australian soils are mainly acidic, very few eucalypt species are adapted to alkaline soils. Most of them develop chlorosis on these soils, associated with decreased levels of chlorophylls a and b and carotenoids (Anderson and Ladiges 1998). However, this stress is of major concern especially in relation to frost tolerance since there are no eucalypt species that combine a high level of tolerance to both cold and alkaline soils. Consequently, very few papers address this subject. These studies mention in particular the efficiency for absorbing Fe and holding it in an available form as a tolerance mechanism (Anderson 1982) or the positive effect of an endomycorrhiza-ectomycorrhiza succession on this tolerance (Lapeyrie and Chilvers 1985). Very recently, a technique for *in vitro* selection of *E. camaldulensis* according to their tolerance to alkaline soil was described (Hinjan *et al.* 2007).

BIOTIC STRESS

Eucalypts occur in a wide range of ecological communities and environments and there are vast numbers of other biological organisms that coexist with them and have coevolved with them. The main biotic groups that interact and can cause biotic stress in eucalypts are mammals, insects, fungi and bacteria. Clearly, there are many differences between eucalypt species in their tolerance to both biotic and abiotic factors. Evidence for genetic variation in resistance to herbivory and disease within eucalypt species is being investigated with emphasis on the nature of the genetic inheritance and the possible mechanism of resistance. Foliar secondary metabolites have been implicated in herbivory resistance in eucalypts and their roles in defence to mammalian and insect attack are also being evaluated. Traditional experimental approaches to herbivory and disease in eucalypts are limited by assessment of phenotypes much more so than for abiotic factors. Other methods including QTL analyses and gene-based studies are being used to progress our understanding of the resistance mechanisms involved. The resistances of eucalypts to vertebrates, invertebrates and fungi in native populations and field trials within Australian plantations are also being studied, as is variation in susceptibility of eucalypt species in exotic plantations to bacterial infection and exotic pathogens.

Plantations and biotic stress

A combination of lack of cold tolerance and susceptibility to *Mycosphaerella* leaf blight has largely stopped planting of *E. globulus* in Tasmania. Establishment of *E. grandis* plantations in Australia has ceased because of extensive mortality and reduced growth from a combination of extensive damage from beetles (Stone *et al.* 1998) and fungi, especially *Kirramyces epicoccoides* (Carnegie 2007). In southern Australia susceptibility of several *Monocalyptus* species to *Phytophthora cinnamomi* has limited their use as plantation species while resistant *Symphyomyrtus* species such as *E. globulus* have been used instead.

Growth can be a key factor in expression of a combination of stress responses in eucalypt plantation species. For species with early rapid growth the advent of abiotic stress such as drought can reduce canopy growth and increase foliar damage from biotic stresses such insect herbivory (Stone *et al.* 2001). Any selection within eucalypts for genetic resistance to stress factors needs to account for appropriate selection for growth as well.

Many eucalypt pathogens have much wider distributions beyond just Australia (Cielsa *et al.* 1996; Old *et al.* 2003). In many cases the pathogens, such as Cryphonectria stem canker in Brazil, can be more important diseases in exotic plantations than in Australia (Gadgil *et al.* 2000). *Mycosphaerella* species have invaded plantation areas such as in South Africa, New Zealand and Chile and limited what can be planted where as in Australia (Cielsa *et al.* 1996; Park *et al.* 2000). There are 26 insect species that have colonised eucalypt plantations in New Zealand and have influenced what species and populations can be established particularly in northern parts of New Zealand (Withers *et al.* 2001). For these cases selections of resistant species and populations have been required for successful plantations, yet in most cases the genetic basis of the resistance is not known.

There are also exotic species which have invaded overseas eucalypt plantations. Guava rust, *Puccinia psidii*, is a native of South America where it has spread to eucalypt plantations and caused considerable damage to young trees of various species. Many myrtaceous species are susceptible and the impact of its introduction to the Australian landscape could be very serious, especially in north eastern Australia (see Glen *et al.* 2007 for recent review). Bacterial wilt, *Ralstonia solanacearum*, although present in Australia but as a non significant disease, causes significant disease in South America and China. With both guava rust and bacterial wilt it is likely that eucalypt hosts have been exposed to new pathogens or at least new biotypes of the pathogen and hence no long term coevolution has occurred (Slippers *et al.* 2005). It is of note that molecular analysis suggests the presence a major gene for resistance to guava rust in eucalypts (Grattapaglia 2004). Will major resistance genes be found in mirror situations such as recent exotic plantations of *E. globulus* in Western Australia in the presence of apparent native populations of the canker pathogen *Botryosphaeria australis* (Burgess *et al.* 2006)?

Vertebrates and herbivory of eucalypts

Leaves of eucalypts are a source of food for a variety of herbivores primarily arboreal marsupials including koalas, possums and gliders (**Fig. 15**). Young trees and seedlings can also be a source of food for wallabies, the Tasmanian pademelon and kangaroos. In forests and woodlands the occurrence of these herbivores can be very patchy (Braithwaite 1983; Martin 1985) complicating assessment of herbivory relationships between animals and eucalypts. The challenge is to demonstrate that there is variation in eucalypt resistance to vertebrate herbivory, that it is heritable and to determine the molecular basis of the resistance.

From both studies of mammalian herbivory in natural populations and feeding trials with captive marsupials it is evident that there is variation within and between species in susceptibility to foliovores (Lawler *et al*. 1998; McArthur *et al*. 2000; O'Reilly-Wapstra *et al*. 2002). Lawler *et al*. (1998) found variation between trees in foliage of *E. ovata* and *E. viminalis* eaten by the common ringtail possum and the koala and that this variation was associated with amounts of FPCs (formylated phloroglucinol compounds) in the trees. The FPCs are a group of secondary compounds that were recently characterised in eucalypts (Eschler *et al*. 2000). Similarly in *E. polyanthemos* and *E. sideroxylon* greater feeding on leaves by common ringtail possums was found to be associated with lower levels of the FPC, sideroxylonal (Lawler *et al*. 2000). In *E. polyanthemos* there was patchiness in foliar concentrations of sideroxylonal between trees within a population suggesting a mechanism by which eucalypt species resist to the biotic stress of mammalian herbivory. In a field trial of *E. globulus* there were significant genetic differences in browsing damage of coppice shoots by marsupials among populations and between open pollinated families. Populations from north-east Tasmania were least resistant to browsing although the heritability of variation in browsing damage was low (O'Reilly-Wapstra *et al*. 2002). In koalas, foraging (as measured by tree visitation rates) was influenced by FPC concentrations with trees foraged less if they had high levels of deterrent FPCs (Moore and Foley 2005). Tree size and nitrogen levels also influence the amount of foraging. Similarly, in a population of *E. melliodora* near Canberra, Australia the concentrations of foliar sideroxylonal and 1, 8-cineole were collinear and there was significant fine-scale spatial genetic structure for both chemical traits (Wallis *et al*. 2002; Andrew *et al*. 2007a). The variation between trees in the predicted foraging levels of brushtail possums largely followed that of sideroxylonal.

These studies demonstrate that it is very difficult to quantify directly phenotypic variation of mammalian herbivory of eucalypts either in natural populations or even field trials. The majority of the evidence for variation within eucalypt species in mammalian herbivory has come from experiments with a limited number of captive animals and only a comparatively small number of individual trees.

Fig. 15 The "eucalypvory" koala, Canberra/ACT, Australia. Photo: C. Teulières.

Only limited progress in understanding genetics and selection for mammalian herbivory tolerance in eucalypts will be possible through quantitative genetic approaches since sufficient phenotypic data is not easily obtainable.

On the other hand the differential feeding of marsupials is at least in part explained by leaf concentrations of FPCs (Wallis *et al*. 2002; Moore *et al*. 2004). These compounds and other plant secondary compounds such as terpenes can be measured comparatively rapidly in large samples of trees within a eucalypt species. Other suites of chemicals in the leaves or other biochemical mechanisms may also be involved. As greater understanding of the biosynthetic pathways and the genes controlling these pathways emerge, molecular genetic approaches may help elucidate the selective agents responsible for resistance of eucalypts to stress of mammalian herbivory.

Invertebrates and herbivory of eucalypts

Eucalypts dominate most of the forest communities in Australia and provide food resources for thousands of native insect species which have coevolved with them and are dependent on them (Majer *et al*. 1997). The most noticeable are the leaf eaters, especially beetles, stick insects and lepidopteran larvae (Ohmart and Edwards 1991). Other groups include sap-feeding insects, gall insects, wood, bark and seed feeders. However, only a comparatively small number of these species have been recorded as pests in eucalypt plantations in Australia (Carne and Taylor 1978; Strauss 2001).

Early studies by Pryor (1953) indicated variation in resistance to leaf-eating scarab beetles within and between some eucalypt species. Since then considerable variation in resistance or susceptibility has been documented within and between a number of mainly commercially important species. In *E. delegatensis* the level of defoliation from insects in provenance field trials was greater for Tasmanian populations than mainland populations (Ohmart *et al*. 1984). Significant levels of genetic variation were found in progeny trials of both *E. regnans* and *E. nitens* for levels of defoliation by the beetle, *Chrysophtharta bimaculata* (average h^2 = 0.3 and 0.4 respectively; Raymond 1995). In *E. globulus*, variation in resistance to several insect herbivores has been found in a number of field trials (Farrow *et al*. 1994; Floyd *et al*. 2002; Jones *et al*. 2002a, 2002b; Rapley *et al*. 2004). In general families and populations from the Victorian part of the geographic range were more susceptible to insect herbivory than material from Tasmania and the Bass Strait islands. A number of insect species have been studied including *Mnesampela privata*, *Phylacteophaga froggatti*, *Paropsisterna agricola*, *Perga affinis* ssp. *insularis* and several *Anoplognathus* spp. For instance, sawfly susceptibility in *E. globulus* was greatest on average in populations from the eastern Otways region in Victoria. The heritability of sawfly damage from this study was high (h^2 = 0.43) (Jones *et al*. 2002a). In *E. grandis* there is significant variation between populations in susceptibility to defoliation by the beetle *Paropsis atomaria* (average h^2 = 0.31, Henery 2006). Similarly in the ironbark, *E. tricarpa*, the level of defoliation was found to vary widely between populations and between families within some populations (Andrew *et al*. 2007b).

These studies have all been possible because of replicated field trials and opportunistic assessment of defoliation from insect outbreaks. It also highlights a major experimental limitation of studies of biotic stresses in trees, particularly eucalypts. The lack of control of herbivorous species such as mammals and insects in adequate sample sizes in appropriate experiments has limited the ability to determine the genetic basis of herbivory in eucalypts.

A number of characters in eucalypts could be responsible for constitutive defences against insect herbivory including leaf characteristics, heterophylly of juvenile and mature leaves, presence of oil glands and secondary compounds (Ohmart and Edwards 1991). There is little evi-

dence that tannin and phenol concentrations influence patterns of insect herbivory. For oils such as terpenes their role in defence or resistance of eucalypts is unclear. Some field studies have found no relation between leaf oil concentrations and insect damage (Morrow and Fox 1980; Patterson *et al.* 1996) but other studies of natural populations of several species have found a correlation between 1,8-cineole oil levels and levels of defoliation (Edwards *et al.* 1993; Stone and Bacon 1994). Moreover in some mosaic trees of *E. melliodora* there is variation between branches in defoliation by Christmas beetles (*Anoplognathus montanus*), which has been associated with different oil profiles (Edwards *et al.* 1990).

The plant secondary compounds, the FPCs, occur in species of the major subgenera in eucalypts except the subgenus *Monocalyptus* (Eschler 2000). In *E. tricarpa*, trees with low FPC concentrations were preferentially defoliated by Christmas beetles compared to trees with higher concentrations (Andrew *et al.* 2007b). However, in *E. grandis* there was no apparent relationship between FPC concentrations and defoliation by the beetle, *Paropsis atomaria* (Henery 2006). Generally in eucalypts there has been found to be a significant positive correlation between cineole and FPCs (Moore *et al.* 2004; Andrew *et al.* 2005) and it is thought that FPCs act as cues to foliovores so they can avoid high levels of terpenes.

Fungi and susceptibility of eucalypts

A comprehensive account of the diseases and pathogens of eucalypts was published in 2000 (Keane *et al.* 2000). Many hundreds of fungal species associate with eucalypt species and many cause substantial biotic stress in natural populations of eucalypts and some severe damage in plantations. Fungal pathogens can be associated with various parts of the eucalypt tree including woody root rots such as *Armillaria* (Kile 2000), soil borne species such as *Phytophthora* (Shearer and Smith 2000), cankers of stems (Old and Davison 2000) and many foliar specific species like *Mycosphaerella* sp. (Park *et al.* 2000). Consideration will be limited to evidence of genetic variation in susceptibility of eucalypt species to fungal species of major significance in Australian plantations.

Mycosphaerella cryptica and *M. nubilosa* are the most significant foliar diseases of *E. globulus* in Australia and worldwide. Genetic variation in susceptibility of *E. globulus* to the *Mycosphaerella* disease on juvenile foliage was found at the family and population level (Carnegie *et al.* 1994) and heritability estimates range from 0.12 to 0.6 (Dungey *et al.* 1997; Milgate *et al.* 2005). There is evidence of variation in resistance to *M. cryptica* between blue gum species with *E. bicostata* significantly more susceptible than the other four blue gums assessed. Also, heritability of the disease was low on adult foliage compared to that of the juvenile foliage (Carnegie *et al.* 2005). It has been suggested that variation in tolerance to *Mycosphaerella* disease is due to differences in cellular leaf structure (Smith 2006). Two major QTL with high LOD and genome-wide significance for *Mycosphaerella cryptica* leaf disease resistance were recently identified in *E. globulus* (Vaillancourt *et al.* 2007 IUFRO). The resistance appears to be oligogenic and controlled by Mendelian genes where resistance is dominant, consistent with a gene-for-gene model. This hypothesis is compatible with the moderate to high heritability for *Mycosphaerella* leaf blight (Milgate *et al.* 2005a) and the specialised associations that appear to exist between *M. cryptica* and *E. globulus* (Milgate *et al.* 2005b). In *E. pilularis*, a major plantation species in NSW, significant genetic variation in both *Mycosphaerella* and *Aulographina eucalypti* leaf diseases was observed within and between populations in field trials (Carnegie *et al.* 2004).

Species of the genus *Corymbia* in southern Queensland and northern NSW are susceptible to Quambalaria shoot blight (*Quambalaria pitereka*) the most significant disease of eucalypt plantations in subtropical eastern Australia (Car-

negie 2007). Dickenson *et al.* (2004) showed substantial variation in susceptibility between populations and species in field trials. Possibly more resistant populations were from higher rainfall regimes (due presumably to higher selection pressures from the fungus). Substantial estimates of heritability of resistance ($h^2 = 0.44$) for *E. variegata* suggested significant additive genetic variance in a field population (King 2004). Of note there was no significant spatial structure with regard to resistant and susceptible trees in this population.

Phytophthora cinnamomi is a soil-borne pathogen that causes the most damage to native eucalypt communities of south-west and south-east Australia and is considered an exotic species of south-east Asian tropical origin (Shearer and Smith 2000). There is a clear difference in susceptibility between eucalypt groups with species of the subgenus *Monocalyptus* more susceptible generally than species of the subgenus *Symphyomyrtus* and the genus *Corymbia*. Damage in Australian plantations has been minor since species used, such as *E. globulus,* are resistant to *P. cinnamomi*. There is variation in resistance within eucalypt species in field trials (Shearer and Smith 2000). Genetic variation has been shown in *E. marginata* from field trials and also seedling trials in the glasshouse (Stukely and Crane1994) and initial QTL locations have been characterized (Byrne *et al.* 1997).

In the studies of fungal stress of eucalypts described above, variation in susceptibility shows a quantitative distribution, and with appropriate experimental material significant heritability estimates were demonstrated. Eucalypt resistance to native pathogens appears to be polygenic with no evidence of major gene effects.

Bacterial diseases of eucalypts

The only reported bacterial disease of importance to eucalypts is bacterial wilt caused by *Ralstonia solanacearum* for which several biotypes are known (Wardlaw *et al.* 2000). This soil borne disease was first reported in Brazil and then China and more recently in South Africa (Coutino *et al.* 2000). The pathogen infects roots of seedlings and young trees and the disease can cause considerable mortality in plantations (Ran *et al.* 2005). Resistance has been demonstrated within and between some species (Dianese *et al.* 1990; Ran *et al.* 2005) but comprehensive assessment of resistance of material from across the geographic range of any species is lacking. The genetic basis of resistance to bacterial wilt was studied in eucalypt hybrid crosses (Gan *et al.* 2005) involving three female parents of *E. urophylla*. Resistance to bacterial wilt was only moderately heritable (h^2 from 0.11 to 0.38) but with limited parents the generality of this result for eucalypts is not established. A single dominant resistance gene has been found in a number of plants such as in *A. thaliana* (Ho and Yang 1999). With this primarily exotic disease of eucalypt plantations the detection or otherwise of dominant major genes may be dependent on a combination of more accurate assessment of the disease phenotype and molecular genetic approaches.

Genetics of resistance to herbivory and disease

There is considerable variation between eucalypt species in their tolerance to herbivorous and pathogenic species. Within a number of species variation in tolerance to insect or fungal species has been shown to have a quantitative genetic component. In theory, selection of genetically resistant material in breeding programs should be possible, but a major limitation is that measurement of the biotic tolerance is field-based and largely uncontrollable. However, progress may be possible through studies of the physiological, chemical or molecular mechanisms underlying the resistance. The secondary compounds, terpenes and FPCs, have been implicated in eucalypt defence to some animal herbivores. The leaf content of terpenes and FPCs are quantitative traits that are known to be highly heritable in eucalypts (Doran

and Matheson 1994; Andrew *et al.* 2005; Henery 2006; Andrew *et al.* 2007b). QTL analysis was carried out in a three generation pedigree of *E. nitens* to dissect further the genetic mechanisms responsible for these foliar compounds (Henery *et al.* 2007). A large number of significant QTL loci were located across 9 linkage groups on the genetic map. On linkage groups 7, 8 and 9 there was significant clustering of QTL loci. A surprising result was the co-location of major QTL for terpenes and FPCs, which originate from different biosynthetic pathways. Biochemical pathways for terpenes (Huber and Bohlmann 2004) and availability of genes from genomes of several model plant species is enabling candidate genes to be co-located on the eucalypt genetic map. Association studies with the candidate genes are currently in progress to test what genes are responsible for the qualitative and quantitative variation in terpenes and FPCs in eucalypts and will further clarify the molecular bases of the tolerance to biotic stresses in eucalypts.

It is apparent that there are a number of features common to evolutionary groups of eucalypts (e.g. susceptibility of *Monocalyptus* species to *P. cinnamomi*, absence of FPCs in *Monocalyptus* species, insect susceptibility of different taxonomic groups) that could be relevant clues to the coevolution of eucalypts and other biological organisms in forest communities. Comparative molecular phylogenetic and population genomic approaches to these co-adaptions (Wade 2007) could help elucidate the genetic resistance mechanisms to biotic stress in eucalypts. A caveat to much of the work described in this review is that except for a few species, such as *P. cinnamomi* (Shearer and Smith 2000), little is known about the variation in herbivorous capacity of vertebrates and invertebrates and pathogenicity of the fungal species coevolving with eucalypts.

CONCLUSION

With many species adapted to arid conditions, eucalypts are considered drought, salt and heat tolerant compared to other plant species. Therefore they have attracted interest as a model species to study the corresponding mechanisms of tolerance. Studies on abiotic stresses in eucalypts have mainly been physiological with the aim of understanding how eucalypts adapt to drought, with less emphasis on the genetic basis of drought tolerance. The response of eucalypts to drought differentially expresses in young seedlings and mature trees, but in both cases, the passive avoidance results in reduced growth. Physiological investigations in seedlings reveal the importance of stomatal closure and increased solute contents suggesting that osmotic adjustment is one of the main responses to drought in eucalypts. In mature trees, drought affects root distribution and wood density, associated with a decrease of growth and productivity.

In contrast, most eucalypt species are known to be frost sensitive and a major commercial focus is improving freezing tolerance of key eucalypt species so as to extend the available areas for plantations. Previous efforts in studying cold stress have largely concentrated on the design of accurate phenotyping methods and more generally on genetic improvement strategies. They have relied on the knowledge of the specific stress-induced damage and the corresponding adaptive mechanisms. In parallel with the genetic studies, physiological and biochemical analyses confirmed the very specific behaviour of eucalypts over winter, being able to cold-acclimate without a strong dependency on light and without stopping growth. Collections of frost tolerance candidate genes were recently made available both in Australian and European laboratories. Interestingly, the first gene studies already suggest an advanced adaptive capacity for the most tolerant species with promising prospects for designing markers for selection and following the introgression of the trait. In addition, most of the biochemical and molecular results reported in this review highlight how *Eucalyptus* is an interesting and suitable model studying cold adaptive mechanisms in plants. Due to its unique develop-

mental characteristics (opportunistic), acclimation mechanisms can be distinguished from dormancy. In addition, protective mechanisms as well as their regulation are expected to be more complex in these species compared to classical annual herbaceous models or deciduous trees.

Interactions between different stresses occur by causing common or additive injuries, by leading to common adaptive responses or through interconnected pathways by crosstalk. Like other plant species, a common response of eucalypts to salt, drought or cold stress was to limit the effects of cell dehydration and oxidization which impair plant growth and productivity. If this tolerance to abiotic stresses shares some physiological mechanisms, the molecular basis of protection appears to be different. First, the few data concerning signalling reveal that the hormone ABA seems to be involved in the drought response while it does not mediate cold acclimation. In addition, sugar accumulation looks like a general strategy in eucalypts to better tolerate dehydration unless the accumulated sugars are distinct in response to water deficit and frost. Interestingly, the role of carbohydrate accumulation was suggested to be different according to the stimulus with an osmotic adjustment during drought and a specific non colligative protection of membranes during cold. However, the induction of genes encoding enzymes with an antioxidant activity seems to be a common response of eucalypts to cold and drought stresses. The mechanism of osmotic adjustment is again different for salt stress compared to drought since in this case ions are accumulated instead of sugars. Finally, mechanisms specific for salt stress were evidenced with the ion transporters acting to prevent toxicity.

Many herbivores and pathogens contribute to biotic stress of eucalypts in native environments. With appropriate experimental material significant genetic components of eucalypt tolerance have been found, for a number of herbivores and pathogens. In these cases the genetic basis of the tolerance is clearly polygenic. For some mammals and Christmas beetles the tolerance of eucalypts appears to be due to high levels of FPCs which act as cues to foliovores so they can avoid high levels of terpenes. Whether these or other secondary metabolites are used as avoidance mechanisms for other herbivores is not known. The mechanisms of resistance to eucalypt pathogens are generally little known since the biology and identification of many of the pathogens is in its infancy. Especially for herbivores, a major limitation has been accurate measurement of variation in resistance of eucalypts.

Commonly, plants subjected to one stress are considered more fragile and subsequently more sensitive to other stresses. For instance, Coleopteran and Lepidopteran stem borers will become key damaging agents of eucalypt plantations which are stressed from abiotic factors. However, cross tolerances also can be observed. For instance, a water deficit during summer proved to lead to better frost tolerance of *E. gunnii* due to acclimation to cell dehydration (unpublished data). Although untested, a moderate drought is also likely to increase salt tolerance since salt, drought and frost all induce cell dehydration. At the opposite extreme, drought associated with cold, occurring in winter on hardened eucalypts could have cumulative negative effects on growth. In addition, the most cold tolerant eucalypt species, such as *E. gunnii*, are not amongst the most salt or drought tolerant. Similarly *E. camaldulensis*, which occurs mostly in the arid regions is not cold tolerant. These observations highlight the fact that responses of eucalypts to abiotic stresses depend on the stress, for the gene regulation pathway as well as for the molecules involved in protection mechanisms. These environmental stresses have in common a negative impact on eucalypt growth, as does biotic stress or salinity. However, the response to cold and drought stresses, acting in particular on photosynthetic rate, has a direct limiting effect on eucalypt productivity. A correlation was established between production and environmental conditions, the most significant being the number of days with rain (positive effect) and the number of days with frost

(negatively correlated) (Pereira 1992). However, local variation in soils and geology may also influence this productivity. The complexity of environmental impacts on eucalypt growth was recently investigated (Costa e Silva *et al.* 2006) through studies on genotype by environment interactions for growth of *E. globulus* in Australia. During summer growth, subraces appear differentially adaptable to environmental conditions promoting water and light stresses and, to a lesser extent, temperature stresses. In addition, differential susceptibility to biotic factors has also contributed to the growth results.

Finally, the understanding of stress tolerance mechanisms is in its infancy in eucalypts and the emerging molecular data and tools should contribute strongly to providing new insights in the next few years. With the urgency of global climate change, the data on stress tolerance will become more and more useful for industrial eucalypt plantations as well as for understanding factors determining distributions of natural populations of eucalypt species.

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REFERENCES

Adams M (1996) Distribution of eucalypts in Australian landscapes: landforms, soils, fire and nutrition. In: Attiwill P (Ed) *Nutrition of Eucalypts*, CSIRO, Collingwood, Australia, pp 61-76

Adams MA, Richter A, Hill AK, Colmer TD (2005) Salt tolerance in *Eucalyptus* spp.: Identity and response of putative osmolytes. *Plant Cell and Environment* **28**, 772-787

Akilan K, Farrell RCC, Bell DT, Marshall JK (1997) Responses of clonal river red gum (*Eucalyptus camaldulensis*) to waterlogging by fresh and salt water. *Australian Journal of Experimental Agriculture* **37**, 243-248

Almeida M, Chaves M, Silva J (1994) Cold acclimation in *Eucalyptus* hybrids. *Tree Physiology* **14**, 921-932

Anderson C (1982) The effect of high pH and P on the development of lime-chlorosis in two seedling populations of *Eucalyptus obliqua* L'Herit. *Plant and Soil* **69**, 199-212

Anderson C, Ladiges P (1998) A comparison of three populations of *Eucalyptus obliqua* L'Herit. Growing on acid and calcareous soils in Southern Victoria. *Australian Journal of Botany* **26**, 93-109

Andolfatto P, Bornhouser A, Bohnert HJ, Thomas JC (1994) Transformed hairy roots of *Mesbryanthemum crystallinum*: gene expression patterns upon salt stress. *Physiologia Plantarum* **90**, 708-714

Andrade A, Bonatto J, Salvato F, Camargo E, Boaretto L, Labate C (2007) Changes in the root proteome of *Eucalyptus grandis* in response to water stress. IUFRO *Tree Biotechnology*, Ponta Delgada, Azores, Portugal, pp SII 5p

Andrew RL, Peakall R, Wallis IR, Foley WJ (2007a) Spatial distribution of defense chemicals and markers: implications for the maintenance of variation. *Ecology* **88**, 716-728

Andrew RL, Peakall R, Wallis IR, Wood JT, Knight EJ, Foley WJ (2005) Marker-based quantitative genetics in the wild? The heritability and genetic correlation of chemical defences in *Eucalyptus*. *Genetics* **171**, 1989-1998

Andrew RL, Wallis IR, Harwood CE, Henson M, Foley WJ (2007b) Heritable variation in the foliar secondary metabolite sideroxylonal confers cross-resistance in *Eucalyptus*. *Oecologia* **153**, 891-901

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na$^+$/H$^+$ antiport in *Arabidopsis*. *Science* **285**, 1256-1258

Archibald RD, Harper RJ, Fox JED, Silberstein RP (2006) Tree performance and root-zone salt accumulation in three dryland Australian plantations. *Agroforestry Systems* **66**, 191-204

Ball M, Hodges V, Laughlin G (1991) Cold-induced photoinhibition limits regeneration of snow gum at tree-line. *Functional Ecology* **5**, 663-668

Barker D, Loveys B, Egerton J, Gorton H, Williams W, Ball M (2005) CO_2 enrichment predisposes foliage of a eucalypt to freezing injury and reduces spring growth. *Plant Cell and Environment* **28**, 1506-1515

Barkla BJ, Zingarelli L, Blumwald E, Smith JAC (1995) Tonoplast Na$^+$/H$^+$ antiport activity and its energization by the vacuolar H$^+$-ATPase in the halophytic plant *Mesembryanthemum crystallinum* L. *Plant Physiology* **109**, 549-556

Battaglia M, Reid J (1993) Ontogenetic variation in frost resistance of *Eucalyptus delegatensis* R.T. Baker. *Australian Journal of Botany* **41**, 137-141

Bell DT (1999) Australian trees for the rehabilitation of waterlogged and salinity-damaged landscapes. *Australian Journal of Botany* **47**, 697-716

Bell DT, Williams JE (1997) Eucalypt ecophysiology. In: Williams J, Woinarsky J (Eds), *Eucalypt Ecology – Individuals to Ecosystems*, Cambridge University Press, Cambridge, UK, pp 168-196

Bhandal IS, Malik CP (1988) Potassium estimation, uptake and its role in the physiology and metabolism of flowering plants. *International Review of Cytology* **110**, 205-254

Blake T (1981) Salt tolerance of eucalypt species grown in saline solution culture. *Australian Forest Research* **11**, 179-183

Bohnert HJ, Golldack D, Ishitani M, Kamasani UR, Rammesmayer G, Shen B, Sheveleva E, Jensen RG (1996) Salt tolerance engineering – which are the essential mechanisms? In: Singhal GS *et al.* (Eds) *Concepts in Photobiology, Photosynthesis and Photomorphogenesis*, Narosa Publishing House, New Delhi, pp 649-667

Bolhar-Nordenkampf HR (1987) Shoot morphology and leaf anatomy in relation to photosynthesis. In: Coombs J, Hall DO, Long SP, Scurlock JMO (Eds) *Techniques in Bioproductivity and Photosynthesis* (2nd Edn) Pergamon Press, Oxford, UK, pp 107-117

Bossinger G, Leitch MA (2000) Isolation of cambium-specific genes from *Eucalyptus globulus* Labill. In: Savidge R, Barnett J, Napier R (Eds) *Cell and Molecular Biology of Wood Formation*, BIOS Scientific, Oxford, UK, pp 203-207

Braithwaite LW (1983) Studies on the arboreal marsupial fauna of eucalypt forests being harvested for pulpwood at Eden, NSW 1. The species and distributions of animals. *Australian Wildlife Research* **10**, 219-229

Burgess TI, Sakalidis ML, Hardy GE (2006) Gene flow of the canker pathogen *Botryosphaeria australis* between *Eucalyptus globulus* plantations and native eucalypt forests in Western Australia. *Austral Ecology* **31**, 559-566

Byrne M, Moran GF, Stukely M, Emebiri LC, Williams ER (1997) Identification of QTL for resistance to *Phytophthora cinnamomi* in *Eucalyptus marginata*. *Australasian Plant Pathology Conference*, Perth 1st-4th Oct., 1997, 111 pp

Byrne M, Murrell JC, Owen JV, Williams ER, Moran GF (1997) Mapping of quantitative trait loci influencing frost tolerance in *Eucalyptus nitens*. *Theoretical and Applied Genetics* **95**, 975-979

Calder I (1992) *Water use of Eucalyptus: A review*. In: Calder IR HR, Adlard PG (Eds) *Growth and Water Use of Forest Plantations*, Wiley, New York, pp 167-179

Carne PB, Taylor KL (1978) Insect pests. In: Hillis WE, Brown AG (Eds) *Eucalypts for Wood Production*, CSIRO, Australia, pp 155-168

Carnegie AJ (2007) Forest health conditions in New South Wales, Australia 1996-2005. II Fungal damage recorded in eucalypt plantations during forest health surveys and their management. *Australasian Plant Pathology* **36**, 225-239

Carnegie AJ, Ades PK (2005) Variation in *Eucalyptus globules* Labill and *E. nitens* dean and Maidne in susceptibility of adult foliage to disease caused by *Mycosphaerella cryptica* (Cooke) Hansf. *Silvae Genetica* **54**, 174-184

Carnegie AJ, Johnson IG, Henson M (2004) Variation among provenances and families of blackbutt (*Eualyptus pilularis*) in early growth and susceptibility to damage from leaf spot fungi. *Canadian Journal of Forest Research* **34**, 2314-2326

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought – from genes to the whole plant. *Functional Plant Biology* **30**, 239-264

Chen DM, Keiper FJ, de Filippis LF (1998) Physiological changes accompanying the induction of salt tolerance in *Eucalyptus microcorys* shoots in tissue culture. *Journal of Plant Physiology* **152**, 555-563

Ciesla WM, Diekmann M, Putter CAJ (1996) *Eucalyptus* spp. FAO/IPGRI technical guidelines for the safe movement of germplasm No. 17 FAO, Rome, 66 pp

Close D, Beadle C (2003) Chilling-dependant photoinhibition, nutrition and growth analysis of *Eucalyptus nitens* seedlings during establishment. *Tree Physiology* **23**, 217-226

Corney H, Sasse J, Ades P (2003) Assessment of salt tolerance in eucalypts using chlorophyll fluorescence attribute. *New Forests* **26**, 233-246

Corney HJ, Sasse JM, Ades PK (2003) Assessment of salt tolerance in eucalypts using chlorophyll fluorescence attributes. *New Forests* **26**, 233-246

Costa e Silva F, Shvaleva A, Maroco JP, Almeida MH, Chaves MM, Pereira JS (2004) Responses to water stress in two *Eucalyptus globulus* clones differing in drought tolerance. *Tree Physiology* **24**, 1165-1172

Costa e Silva J, Potts B, Dutkowski G (2006) Genotype by environment interaction for growth of *Eucalyptus globulus* in Australia. *Tree Genetics and Genomes* **2**, 61-75

Coutino TA, Roux J, Riedel KH, Terblanche J, Wingfield MJ (2000) First report of bacterial wilt caused by *Ralstonia solanacearum* on eucalypts in South Africa. *European Journal of Forest Pathology* **30**, 205-210

Davidson N, Battaglia M, Close D (2004) Photosynthetic response to overnight frost in *Eucalyptus nitens* and *E. globulus*. *Trees* **18**, 246-252

Davidson N, Reid J (1985) Frost as a factor influencing growth and distribution of subalpine eucalypts. *Australian Journal of Botany* **33**, 657-667

de Aragão MEF, Jolivet Y, Lima MD, de Melo DF, Dizengremel P (1997)

NaCl-induced changes of NAD(P) malic enzyme activities in *Eucalyptus citriodora* leaves. *Trees – Structure and Function* **12**, 66-72

Dianese JC, Dristig MCG, Cruz AP (1990) Susceptibility to wilt associated with *Pseudomonas solanacearum* among six species of *Eucalyptus* growing in equatorial Brazil. *Australasian Plant Pathology* **19**, 71-76

Dickenson GR, Lee DJ, Huth JR (2004) Early plantation growth and tolerance to ramularia shoot blight of provenances of thre spotted gum taxa on a range of sites in Queensland. *Australian Forestry* **67**, 122

Doran JC, Matheson AC (1994) Genetic parameters and expected gains from selection for monoterpene yields in Petford *Eucalyptus camaldulensis*. *New Forests* **8**, 155-167

Dungey HS, Potts BM, Carnegie AJ, Ades PK (1997) *Mycosphaerella* leaf disease: genetic variation in damage to *Eucalyptus nitens, Eucalyptus globulus*, and their F$_1$ hybrid. *Canadian Journal of Forest Research* **27**, 750-759

Dunn GM, Taylor DW, Nester MR, Beetson TB (1994) Performance of 12 selected Australian tree species on a saline site in southeast Queensland. *Forest Ecology and Management* **70**, 255-264

Edwards PB, Wanjura WJ, Brown WV (1993) Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. *Oecologia* **95**, 551-557

Edwards PB, Wanjura WJ, Brown WV, Dearn JM (1990) Mosaic resistance in plants. *Nature* **347**, 434

El Kayal W (2004) Réponse aux stress abiotiques chez *Eucalyptus gunnii*: analyse globale du transcriptome sur filtres haute-densité et caractérisation du gène Sxd1 (synthèse de la vitamine E). *UMR 5546*, Université Paul Sabatier, Toulouse, 110 pp

El Kayal W, Keller G, Debayles C, Kumar R, Weier D, Teulières C, Marque C (2006) Regulation of tocopherol biosynthesis through transcriptional control of tocopherol cyclase during cold hardening in *Eucalyptus gunnii*. *Physiologia Plantarum* **126**, 212-223

El Kayal W, Navarro M, Marque G, Keller G, Marque C, Teulières C (2006) Expression profile of CBF-like transcriptional factor genes from *Eucalyptus* in response to cold. *Jounal of Experimental Botany* **57**, 2455-2469

Eldridge K, Davidson J, Harwood C, van Wyk G (1993) *Eucalypt Domestication and Breeding*, Oxford University Press Inc., New York, 288 pp

Eschler BM, Pass DM, Willis R, Foley WJ (2000) Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. *Biochemical Systematics and Ecology* **28**, 813-824

FAO (2004) Preliminary review of biotechnology in forestry, including genetic modification. *Forest Genetic Resources Working Papers FGR/59E*, Forest Resources Development Service, Forest Resources Division. Rome, Italy, Available online: http://www.fao.org/docrep/005/y2316e/y2316e0a.htm

FAO (2005) Global Network on Integrated Soil management for Sustainable Use of Salt-affected Soils. FAO Land and Plant Nutrition Management Service, Rome, Italy. Available online: http://www.fao.org/agr/agl/agll/spush

Faridah E (2004) Molecular and physiological studies of salt tolerance in eucalypts. PhD Thesis, The University of Melbourne, 212 pp

Farrow RA, Floyd RB, Neumann FG (1994) Inter-provenance variation in resistance of *Eucalyptus globulus* juvenile foliage to insect feeding. *Australian Forestry* **57**, 65-68

Fernandez M, Valenzuela S, Balocchi C (2006) RAPD and freezing resistance in *Eucalyptus globulus*. *Electronic Journal of Biotechnology* **9**, 303-309

Flexas J, Bota J, Galmes J, Medrano H, Ribas-Carbo M (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum* **127**, 343-352

Floyd RB, Farrow RA, Matsuki M (2002) Variation in insect damage and growth in *Eucalyptus globulus*. *Agricultural and Forest Entomology* **4**, 109-115

Fukuda A, Nakamura A, Tanaka Y (1999) Molecular cloning and expression of the Na$^+$/H$^+$ exchanger gene in *Oryza sativa*. *Biochimica et Biophysica Acta* **1446**, 149-155

Fullard K, Moran GF (2003) Identification of frost tolerance genes in a *Eucalyptus* hybrid cross. *IUFRO Tree Biotechnology*, Umea Plant Science Centre, Umea, Sweden, pp S6 18p

Gadgil PD, Wardlaw TJ, Ferreira FA, Sharma JK, Dick MA, Wingfield MJ, Crous PW (2000) Management of diseases in eucalypt plantations. In: Keane PJ, Kile GA, Podger FD, Brown BN (Eds) *Diseases and Pathogens of Eucalypts*, CSIRO, Melbourne, pp 519-529

Gamboa MC, Rasmussen-Poblete S, Valenzuela PD, Krauskopf E (2007) Isolation and characterization of a cDNA encoding a CBF transcription factor from *E. globulus*. *Plant Physiology and Biochemistry* **45**, 1-5

Gan S, Li M, Li F, Wu K, Wu J, Bai J (2004) Genetic analysis of growth and susceptibility to bacterial wilt (*Ralstonia solanacearum*) in *Eucalyptus* by interspecific factorial crossing. *Silvae Genetica* **53**, 254-258

Gaxiola RA, Rao R, Sherman A, Grisafi P, Alper SL, Fink GR (1999) The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. *Genetics* **96**, 1480-1485

Gindaba J, Rozanov A, Negash L (2004) Response of seedlings of two *Eucalyptus* and three deciduous tree species from Ethiopia to severe water stress. *Forest Ecology and Management* **201**, 121-131

Glen M, Alfenas AC, Zauza EAV, Wingfield MJ, Mohammed C (2007) *Puccinia psidii:* a threat to the Australian environment and economy – a review. *Australasian Plant Pathology* **36**, 1-16

Grattapaglia D (2004) Resistance to rust (*Puccinia psidii* Winter) in *Eucalyptus*: mode of inheritance and mapping of a major gene with RAPD markers. *Theoretical and Applied Genetics* **108**, 175-180

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology* **31**, 149-190

Grieve CM, Shannon MC (1999) Ion accumulation and distribution in shoot components of salt-stressed *Eucalyptus* clones. *Journal of the American Society for Horticultural Science* **124**, 559-563

Griffin A, Cotterill P (1988) Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucalyptus regnans* and some implications for breeding strategy. *Silvae Genetica* **37**, 127-131

Hallam P, Tibbits W (1988) Determination of frost hardiness of *Eucalyptus* using the electrical conductivity of diffusate in conjunction with a freezing chamber. *Canadian Journal of Forest Research* **18**, 595-600

Hamada A, Shono M, Xia T, Ohta M, Hayashi Y, Tanaka A, Hayakawa T (2001) Isolation and characterization of a Na$^+$/H$^+$ antiporter gene from halophyte *Atriplex gmelini*. *Plant Molecular Biology* **46**, 35-42

Harwood C (1981) Frost resistance of subalpine *Eucalyptus* species. II. Experiments using the resistance index method of damage assessment. *Australian Journal of Botany* **29**, 209-218

Henery ML (2006) Foliar secondary metabolites in *Eucalyptus* and their role in resistance to defoliating insects. Australian National University 162 pp

Henery ML, Moran GF, Wallis IR, Foley WJ (2007) Identification of quantitative trait loci influencing foliar concentrations of terpenes and formylated phloroglucinol compounds in *Eucalyptus nitens*. *New Phytologist* **176**, 82-95

Hinjan S, Chanprame S, Srijantr P, Chanprame S (2007) In *vitro* selection technique of *Eucalyptus camaldulensis* Dehnh. resistance to calcareous soil. *IUFRO Tree Biotechnology*, Ponta Delgada, Azores, Portugal, pp SIX 32 p

Huber DPW, Bohlmann J (2004) Terpene synthases and the mediation of plant-insect ecological interactions by terpenoids: a mini review. In: Cronk QCB, Whitton J, Ree RH, Taylor IEP (Eds) *Plant Adaptation: Molecular Genetics and Ecology*, NRC Research Press, Ottawa, pp 70-81

Igboanugo ABI (1992) Characteristics of leaf structure and photosynthetic apparatus within the crown of systematically shaded *Quercus petraea* and *Nothofagus procera* seedlings. *Biologia Plantarum* **34**, 401-407

James SA, Bell DT (1995) Morphology and anatomy of juvenile leaves of geographically widespread clones of *Eucalyptus camaldulensis*. *Australian Journal of Botany* **43**, 415-433

Jones GJ, Potts BM, Clarke AR (2002a) Susceptibility of *Eucalyptus globulus* ssp. *globulus* to sawfly (*Perga affinis* ssp. *insularis*) attack and its potential impact on plantation productivity. *Forest Ecology and Management* **160**, 189-199

Jones TH, Potts BM, Vaillancourt RE, Davies NW (2002b) Genetic resistance of *Eucalyptus globul us* to autumn gum moth defoliation and the role of cuticular waxes. *Canadian Journal of Forest Research* **32**, 1961-1969

Junghans DT, Alfenas AC, Brommonschenkel SH, Oda S, Mello EJ, Kalberer S, Wisniewski M, Arora R (2006) Deacclimation and reacclimation of cold-hardy plants: current understanding and emerging concept. *Plant Science* **171**, 3-16

Karschon R, Pinchas L (1971) Variation in heat resistance of ecotypes of *Eucalyptus camaldulensis* Dehn. and their significance. *Australian Journal of Botany* **19**, 261-272

Keane PJ, Kile GA, Podger FD, Brown BN (Eds) (2000) *Disease and Pathogens of Eucalypts*, CSIRO, Melbourne, 565 pp

Keiper FJ, Chen DM, de Filippis LF (1998) Respiratory, photosynthetic and ultrastructural changes accompanying salt adaptation in culture of *Eucalyptus microcorys*. *Journal of Plant Physiology* **152**, 564-573

Keller G (2006) Analyse du transcriptome de l'*Eucalyptus* pendant l'acclimation au froid; recherche de gènes candidats de la tolérance au gel. *Unité Mixte de Recherche 5546*, Paul Sabatier Toulouse III, Toulouse, 100 pp

Kile GA (2000) Woody root rots of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN (Eds) *Diseases and Pathogens of Eucalypts*, CSIRO, Melbourne, pp 293-306

King R (2000) Spatial structure and population genetic variation in a eucalypts species complex. PhD Thesis, Griffith University, Queensland, 118 pp

Krauskopf E, Rasmussen-Poblete S, Valenzuela P, Gamboa M (2007) A first glance at the *Eucalyptus globulus* cold activated regulatory pathway. *IUFRO Tree Biotechnology*, Ponta Delgada, Azores, Portugal, pp SII 33p

Kreidemann PE, Cromer RN (1996) The nutritional physiology of the eucalypts – nutrition and growth. In: Adams MA (Ed) *Nutrition of Eucalypts*, CSIRO Publishing, Collingwood, Victoria, Australia, pp 109-121

Kuzminski E, Malvolti M (1992) Isoenzymatic phenotypes related to cold hardening in *Eucalyptus* clones. Pereira H, Pereira H (Eds) *Eucalyptus for Biomass Production*, Commission of European Communities, Lisbon, Portugal, pp 181-189

Kuzminski E, Scarascia-Mugnozza G (1992) Biochemical modifications during cold hardening of *Eucalyptus* clones. Pereira H, Pereira H (Eds) *Eucalyptus for Biomass Production*, Commission of European Communities, Lisbon, Portugal, pp 143-160

Lapeyrie F, Chilvers G (1985) An endomycorrhiza-ectomycorrhiza succession associated with enhanced growth of *Eucalyptus dumosa* seedlings planted in a calcareous soil. *New Phytologist* **100**, 93-104

Lawler IR (1988) Intraspecific variation in *Eucalyptus* secondary metabolites

determines food intake by foliovorous marsupials. *Oecologia* **116**, 160-169

Lawler IR, Foley WJ, Eschler BM (2000) Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81**, 1327-1338

Leborgne N (1994) Approches biochimiques des mécanismes de résistance au froid chez *Eucalyptus gunnii* Hook. *Biologie cellulaire et moléculaire végétale*, Université Paul Sabatier, Toulouse, 165 pp

Leborgne N, Dupou-Cézanne L, Teulières C, Canut H, Tocanne JF, Boudet AM (1992) Lateral and rotational mobilities of lipids in specific cellular membranes of *Eucalyptus gunnii* cultivars exhibiting different freezing tolerance. *Plant Physiology* **100**, 246-254

Leborgne N, Teulières C, Cauvin B, Travert S, Boudet AM (1995a) Carbohydrate content of *Eucalyptus gunnii* leaves along an annual cycle in the field and during frost-hardening in controlled conditions. *Trees* **10**, 86-93

Leborgne N, Teulières C, Travert S, Rols MP, Teissie J, Boudet AM (1995b) Introduction of specific carbohydrates into *Eucalyptus gunnii* cells increases their freezing tolerance. *European Journal of Biochemistry* **229**, 710-717

Lemcoff JH, Guarnaschelli AB, Garau AM, Bascialli ME, Ghersa CM (1994) Osmotic adjustment and its use as a selection criterion in *Eucalyptus* seedlings. *Canadian Journal of Forest Research – Revue Canadienne de Recherche Forestiere* **24**, 2404-2408

Lemcoff JH, Guarnaschelli AB, Garau AM, Prystupa P (2002) Elastic and osmotic adjustments in rooted cuttings of several clones of *Eucalyptus camaldulensis* Dehnh. from southeastern Australia after a drought. *Flora* **197**, 134-142

Li CY, Wang KY (2003) Differences in drought responses of three contrasting *Eucalyptus microtheca* F. Muell. populations. *Forest Ecology and Management* **179**, 377-385

Madsen PA, Mulligan DR (2006) Effect of NaCl on emergence and growth of a range of provenances of *Eucalyptus citriodora*, *Eucalyptus populnea*, *Eucalyptus camaldulensis* and *Acacia salicina*. *Forest Ecology and Management* **228**, 152-159

Mahmood K, Marcar NE, Naqvi MH, Arnold RJ, Crawford DF, Iqbal S, Aken KM (2003) Genetic variation in *Eucalyptus camaldulensis* Dehnh. for growth and stem straightness in a provenance-family trial on saltland in Pakistan. *Forest Ecology and Management* **176**, 405-416

Majer JD, Recher HF, Wellington AB, Woinarski JCZ, Yen AL (1997) Invertebrates of eucalypt formations. In: Williams JE, Woinarski JCZ (Eds) *Eucalypt Ecology: Individuals to Ecosystems*, Cambridge University Press, Cambridge, pp 278-303

Marcar NE (1993) Waterlogging modifies growth, water use and ion concentrations in seedlings of salt-treated *Eucalyptus camaldulensis*, *E. tereticornis*, *E. robusta* and *E. globulus*. *Australian Journal of Plant Physiology* **20**, 1-13

Marcar NE, Ansari R, Khanzada AN, Khan MA, Crawford DF (2003a) Performance of several tree species on a saline site in southern Pakistan. *Journal of Tropical Forest Science* **15**, 457-468

Marcar NE, Crawford DF, Hossain A, Nicholson AT (2003b) Survival and growth of tree species and provenances in response to salinity on a discharge site. *Australian Journal of Experimental Agriculture* **43**, 1293-1302

Marcar NE, Crawford DF, Saunders A, Matheson AC, Arnold RA (2002a) Genetic variation among and within provenances and families of *Eucalyptus grandis* W. Hill and *E. globulus* Labill. subsp. *globulus* seedlings in response to salinity and waterlogging. *Forest Ecology and Management* **162**, 231-249

Marcar NE, Criddle RS, Guo JM, Zohar Y (2002b) Analysis of respiratory metabolism correlates well with the response of *Eucalyptus camaldulensis* seedlings to NaCl and high pH. *Functional Plant Biology* **29**, 925-932

Marcar NE, Termaat A (1990) Effects of root-zone solutes in *Eucalyptus camaldulensis* and *Eucalyptus* bicostata seedlings: responses to Na$^+$, Mg^{2+} and Cl$^-$. *Plant and Soil* **125**, 245-254

Marcar NE, Zohar Y, Guo JM, Crawford DF (2002c) Effect of NaCl and high pH on seedling growth of 15 *Eucalyptus camaldulensis* Dehnh. provenances. *New Forests* **23**, 193-206

Marshall JK, Morgan AL, Farrel RC, Bell DT (1997) Water uptake by two river red gum (*Eucalyptus camaldulensis*) clones in a discharge site plantation in the Western Australian wheatbelt. *Journal of Hydrology* **200**, 136-148

Martin RW (1985) Overbrowsing and decline of a population of the koala, *Phascolarctos cinerus*, in Victoria I. Food preference and food tree defoliation. *Australian Wildlife Research* **12**, 355-365

McArthur C, Goodwin A, Turner S (2000) Preferences, selection and damage to seedling under changing availability by two marsupial herbivores. *Forest Ecology and Management* **139**, 157-173

McDonald AJS, Davies WJ (1996) Keeping in touch: responses of the whole plant to deficits in water and nitrogen supply. *Advances in Botanical Research* **22**, 229-300

Merchant A, Adams M (2005) Stable osmotica in *Eucalyptus spathulata* – responses to salt and water deficit stress. *Functional Plant Biology* **32**, 797-805

Merchant A, Tausz M, Arndt SK, Adams MA (2006) Cyclitols and carbohydrates in leaves and roots of 13 *Eucalyptus* species suggest contrasting physiological responses to water deficit. *Plant Cell and Environment* **29**, 2017-2029

Milgate AW, Potts BM, Joyce K, Mohammed C, Vaillancourt RE (2005) Genetic variation in *Eucalyptus globulus* for susceptibility to *Mycosphaerella nubilosa* and its association with tree growth. *Australasian Plant Pathology* **34**, 11-18

Moore BD, Foley WJ (2005) Tree use by koalas in a chemically complex landscape. *Nature* **435**, 488-490

Moore BD, Wallis IR, Marsh KJ, Foley WJ (2004b) The role of nutrition in the conservation of the marsupial foliovores of eucalypt forests. In: Lunney D (Ed) *Conservation of Australia's Forest Fauna*, Royal Zoological Society NSW, Mosman, Australia, pp 549-575

Moore BD, Wallis IR, Pala-Paul J, Brophy JJ, Willis RH, Foley WJ (2004a) Antiherbivore chemistry of *Eucalyptus* – Cues and deterrents for marsupial folivores. *Journal of Chemical Ecology* **30**, 1743-1769

Moore G, Rowan K, Blake T (1977) Effects of heat on the physiology of seedlings of *Eucalyptus obliqua*. *Functional Plant Biology* **4**, 283-288

Morabito D, Jolivet Y, Prat D, Dizengremel P (1996) Differences in the physiological responses of two clones of *Eucalyptus microtheca* selected for their salt tolerance. *Plant Science* **114**, 129-139

Morabito D, Mills D, Prat D, Dizengremel P (1994) Response of clones of *Eucalyptus microtheca* to NaCl *in vitro*. *Tree Physiology* **14**, 201-210

Moraga P, Escobar R, Valenzuela S (2006) Resistance to freezing in three *Eucalyptus globulus* Labill subspecies *Electronic Journal of Biotechnology* **9**, 310-314

Morgan JM (1984) Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **35**, 299-319

Moroni MT, Worledge D, Beadle CL (2003) Root distribution of *Eucalyptus nitens* and *E. globulus* in irrigated and droughted soil. *Forest Ecology and Management* **177**, 399-407

Morrow PA, Fox LR (1980) Effects of variation in *Eucalyptus* essential oil yield on insect growth and grazing damage. *Oecologia* **45**, 209-219

Munns R (1993) Physiological processes limiting plant growth in saline soil: some dogmas and hypotheses. *Plant Cell and Environment* **16**, 15-24

Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell and Environment* **25**, 239-250

Munns R (2005) Genes and salt tolerance: bringing them together. *New Phytologist* **167**, 645-663

Myers BA, Neales TF (1986) Osmotic adjustment, induced by drought, in seedlings of 3 *Eucalyptus* species. *Australian Journal of Plant Physiology* **13**, 597-603

Ngugi MR, Doley D, Hunt MA, Dart P, Ryan P (2003a) Leaf water relations of *Eucalyptus cloeziana* and *Eucalyptus argophloia* in response to water deficit. *Tree Physiology* **23**, 335-343

Ngugi MR, Doley D, Hunt MA, Ryan P, Dart P (2004a) Physiological responses to water stress in *Eucalyptus cloeziana* and *E. argophloia* seedlings. *Trees – Structure and Function* **18**, 381-389

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

Ngugi MR, Hunt MA, Doley D, Ryan P, Dart P (2004b) Selection of species and provenances for low-rainfall areas: physiological responses of *Eucalyptus cloeziana* and *Eucalyptus argophloia* to seasonal conditions in subtropical Queensland. *Forest Ecology and Management* **193**, 141-156

Ngugi MR, Hunt MA, Doley D, Ryan P, Dart PJ (2003c) Effects of soil water availability on water use efficiency of *Eucalyptus cloeziana* and *Eucalyptus argophloia* plants. *Australian Journal of Botany* **51**, 159-166

Niknam SR, McComb J (2000) Salt tolerance screening of selected Australian woody species – a review. *Forest Ecology and Management* **139**, 1-19

Ohmart CP, Edwards PB (1991) Insect herbivory on *Eucalyptus*. *Annual Review of Entomology* **36**, 637-657

Ohmart CP, Thomas JR, Stewart LG (1984) Differential defoliation by insects among provenances of *Eucalyptus delegatensis*. *Journal of Australian Entomological Society* **23**, 105-111

Old KM, Davison EM (2000) Canker disease of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN (Eds) *Diseases and Pathogens of Eucalypts*, CSIRO, Melbourne, pp 241-253

Old KM, Wingfield MJ, Yuan ZQ (2003) *A Manual of Diseases of Eucalypts in South-East Asia*, CIFOR, Bogor, Indonesia, 104 pp

Oquist G, Huner N (1991) Effects of cold acclimation on the susceptibility of photosynthesis to photoinhibition in Scots pines and in winter and spring cereals: a fluorescence analysis. *Functional Ecology* **5**, 91-100

O'Reilly-Wapstra JM, McArthur C, Potts BM (2005) Genetic variation in resistance of *Eucalyptus globulus* to marsupial browsers. *Oecologia* **130**, 289-296

Paine T, Dreistadt S, Garrison R, Gill R (2006) *Eucalyptus* redgum lerp psyllid. *Pest Notes N° 7460, University of California*, 1-4

Paine T, Millar J, Dreistadt S (2000) *Eucalyptus* longhorned borers. *Pest Notes N° 7425, University of California*, 1-4

Park RF, Keane PJ, Wingfield MJ, Crous PW (2000) Fungal diseases of *Eucalyptus* foliage. In: Keane PJ, Kile GA, Podger FD, Brown BN (Eds) *Diseases and Pathogens of Eucalypts*, CSIRO, Melbourne, pp 153-240

Paton D (1978) *Eucalyptus* physiology. I. Photoperiodic responses. *Australian Journal of Botany* **26**, 633-642

Patterson KC, Clarke AR, Raymond CA, Zalucki MP (1996) Performance of first instar *Chrysophtharta bimaculata* larvae (Coleoptera: Chrysomelidae) on nine families of *Eucalyptus regnans* (Myrtaceae). *Chemoecology* **7**, 94-106

Paul MJ, Cockburn W (1989) Pinitol, a compatible solute in *Mesembryanthemum crystallinum* L? *Journal of Experimental Botany* **40**, 1093-1098

Pereira J (1992) Introduction. In: Pereira J, Pereira H (Eds) *Eucalyptus for Biomass Production*, Commission of European Communities, Lisbon, Portugal, pp 5-10

Pita P, Gasco A, Pardos JA (2003) Xylem cavitation, leaf growth and leaf water potential in *Eucalyptus globulus* clones under well-watered and drought conditions. *Functional Plant Biology* **30**, 891-899

Popp M, Lied W, Bierbaum U, Gross M, Grosse-Schulte T, Hams S, Oldenettel J, Schuler S, Wiese J (1997) Cyclitols – stable osmotica in trees. In: Rennenberg H, Eschrich W, Ziegler H (Eds) *Trees – Contributions to Modern Tree Physiology*, Blackhuys Publishers, Leiden, the Netherlands, pp 257-270

Prat D, Fathi-Ettai RA (1990) Variation in organic and mineral components in young *Eucalyptus* seedlings under saline stress. *Physiologia Plantarum* **79**, 479-486

Pryor L (1957) Selecting and breeding for cold resistance in *Eucalyptus*. *Silvae Genetica* **6**, 98-109

Pryor LD (1953) Variable resistance to leaf-eating insects in some eucalypts. *Proceedings of the Linnean Society of New South Wales* **77**, 364-368

Ran LX, Liu CY, Wu GJ, van Loon LC, Bakker PAHM (2005) Suppression of bacterial wilt in *Eucalyptus urophylla* by *Pseudomonas* spp. in China. *Biological Control* **32**, 111-120

Rapley LP, Allen GR, Potts BM (2004) Genetic variation in *Eucalyptus globulus* in relation to susceptibility from attack by the southern eucalypt leaf beetle, *Chrysophtharta agricola. Australian Journal of Botany* **52**, 747-756

Rasmussen-Poblete S, Gamboa M, Valenzuela P, Krauskopf E (2007) Identification and characterization of the *Eucalyptus globulus* ICE transcription factor. *IUFRO Tree Biotechnology*, Ponta Delgada, Azores, Portugal, pp SII 34p

Raymond C, Owen J, Rvenwood I (1992a) Genetic variation for frost tolerance in breeding population of *Eucalyptus nitens. Silvae Genetica* **41**, 355-372

Raymond C, Owen JV, Eldridge K, Harwood C (1992b) Screening *Eucalyptus* for frost tolerance in breeding programs. *Canadian Journal of Forest Research* **22**, 1271-1277

Raymond CA (1995) Genetic variation in *Eucalyptus regnans* and *Eucalyptus nitens* for levels of observed defoliation caused by the *Eucalyptus* leaf beetle, *Chrysophtharta bimaculata* Oliver, in Tasmania. *Forest Ecology and Management* **72**, 21-29

Reed A, Marque G, Marque C, Potts B, Ades P, Bossinger G, Teulières C (2007) The CBF transcription factor as a candidate gene for association studies of cold tolerance in *Eucalyptus*. *IUFRO Tree Biotechnology*, Ponta Delgada, Azores, Portugal, pp SII 4p

Reed A, Southerton S, Ades P, Blomstedt C, Bossinger G (2006) Cold acclimation in *Eucalyptus delegatensis*: expression profiling in natural populations. *Tree Biotechnology* **S9**, 16 pp

Rice KJ, Matzner SL, Byer W, Brown JR (2004) Patterns of tree dieback in Queensland, Australia: the importance of drought stress and the role of resistance to cavitation. *Oecologia* **139**, 190-198

Sands R (1981) Salt resistance in *Eucalyptus camaldulensis* Dehn. from three different seed sources. *Australian Forest Research* **11**, 93-100

Scarascia-Mugnozza G, Valentiny R, Kuzminski E, Giordano E (1989) Freezing mechanisms, acclimation processes and cold injury in *Eucalyptus* species planted in the Mediterranean region. *Forest Ecology and Management* **29**, 81-94

Schulze ED, Turner NC, Nicolle D, Schumacher J (2006) Leaf and wood carbon isotope ratios, specific leaf areas and wood growth of *Eucalyptus* species across a rainfall gradient in Australia. *Tree Physiology* **26**, 479-492

Searson MJ, Thomas DS, Montagu KD, Conroy JP (2004) Wood density and anatomy of water-limited eucalypts. *Tree Physiology* **24**, 1295-1302

Serrano L, Pardos J (1992) Hardening and cold resistance in *Eucalyptus globulus*: water relations and bioelectrical tests of frost injury. In: Pereira J, Pereira H (Ed) *Eucalyptus for Biomass Production*, Commission of European Communities, Lisbon, Portugal, pp 134-142

Shearer BL, Smith IW (2000) Diseases of eucalypts casued by soilborne species of *Phytophthora* and *Pythium*. In: Keane PJ, Kile GA, Podger FD, Brown BN (Eds) *Diseases and Pathogens of Eucalypts*, CSIRO, Melbourne, pp 259-293

Shi H, Ishitani M, Kim C, Zhu J-K (2000) The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na^+/H^+ antiporter. *Proceedings of the National Academy of Sciences USA* **97**, 6896-6901

Shvaleva AL, Costa e Silva F, Breia E, Jouve L, Hausman JF, Almeida MH, Maroco JP, Rodrigues ML, Pereira JS, Chaves MM (2006) Metabolic responses to water deficit in two *Eucalyptus globulus* clones with contrasting drought sensitivity. *Tree Physiology* **26**, 239-248

Slippers B, Stenlid J, Wingfield MJ (2005) Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology and Evolution* **20**, 420-421

Smith AH, Pinkard EA, Hunter GC, Wingfield MJ, Mohammed CL (2006) Anatomical variation and defence responses of juvenile *Eucalyptus nitens* leaves to *Mycosphaerella* disease. *Australasian Plant Pathology* **35**, 725-731

Souza G, Cardoso V, Gonçalves A (2004) Proline content and protein patterns in *Eucalyptus grandis* shoots submitted to high and low temperature shocks.

Brazilian Archives of Biology and Technology **47**, 1-8

Specht R (1970) *Vegetation* In: Leeper G (Ed) *The Australian Environment*, CSIRO and Melbourne University Press, Carlton, Victoria, pp 44-67

Stape J (2002) Production ecology of clonal *Eucalyptus* plantations in northeastern Brazil. Colorado State University, Fort Collins, USA, 84 pp

Stone C (2001) Reducing the impact of insect herbivory in eucalypt plantations though management of extrinsic influences on tree vigour. *Australian Ecology* **26**, 482-488

Stone C, Bacon PE (1994) Relationships among moisture stress, insect herbivory, foliar cineole content and the growth of river red gum *Eucalyptus camaldulensis. Journal of Applied Ecology* **31**, 604-612

Stone C, Simpson JA, Gittens R (1998) Differential impact of insect herbivores and their fungal pathogens on the *Eucalypt* subgenera *Symphyomyrtus* and *Monocalyptus* and the genus *Corymbia. Australian Journal of Botany* **46**, 723-734

Stoneman GL, Turner NC, Dell B (1994) Leaf growth, photosynthesis and tissue water relations of greenhouse-grown *Eucalyptus marginata* seedlings in response to water deficits. *Tree Physiology* **14**, 633-646

Strauss SY (2001) Benefits and risks of biotic exchange between *Eucalyptus* plantations and native Australian forests. *Australian Ecology* **26**, 447-457

Stukely MJ, Crane CE (1994) Genetically base resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi. Phytopathology* **84**, 650-656

Su H, Golldack D, Zhao C, Bohnert HJ (2002) The expression of HAK-type K^+ transporters is regulated in response to salinity stress in common ice plant. *Plant Physiology* **129**, 1482-1493

Sun D, Dickinson G (1993) Responses to salt stress of 16 *Eucalyptus* species, *Grevillea robusta, Lophostemon confertus* and *Pinus caribaea* var Hondurensis. *Forest Ecology and Management* **60**, 1-14

Teulières C, Feuillet C, Boudet A (1989) Differential characteristics of cell suspension cultures initiated from *Eucalyptus gunnii* differing by their frost tolerance. *Plant Cell Reports* **8**, 407-410

Thomson L, Morris J, Halloran G (1987) Salt tolerance in eucalypts. *International Symposium on afforestation of salt-affected soils*, Central Soil Salinity Research Institute, Karnal, India, pp 1-12

Thomson LAJ (1988) Salt tolerance in *Eucalyptus camaldulensis* and related species. PhD Thesis, The University of Melbourne, 307 pp

Tibbits W, Potts B, Savva M (1991) Inheritance of freezing resistance in interspecific F_1 hybrids of *Eucalyptus. Theoretical and Applied Genetics* **83**, 126-135

Tibbits W, Reid J (1987) Frost resistance in *Eucalyptus nitens* (Deane & Maiden) Maiden: Physiological aspects of hardiness *Australian Journal of Botany* **35**, 235-250

Tibbits W, White T, Hodge G, Borralho N (2006) Genetic variation in frost resistance of *Eucalyptus globulus ssp globulus* assessed by artificial freezing in winter. *Australian Journal of Botany* **54**, 521-529

Ticha I (1985) Ontogeny of leaf morphology and anatomy. In: Sestak Z (Ed) *Photosynthesis During Leaf Development*, Junk Publishers, The Netherlands, pp 16-50

Travert S, Valerio L, Fouraste I, Boudet AM, Teulières C (1997) Enrichment in specific soluble sugars of two *Eucalyptus* cell-suspension cultures by various treatments enhances their frost tolerance via a noncolligative mechanism. *Plant Physiology* **114**, 1433-1442

van der Moezel PG, Bell DT (1987a) The effect of salinity on the germination of some Western Australian *Eucalyptus* and Melaleuca species. *Seed Science and Technology* **15**, 239-246

van der Moezel PG, Bell DT (1987b) Comparative seedling salt tolerance of several *Eucalyptus* and *Melaleuca* species from Western-Australia. *Australian Forest Research* **17**, 151-158

van der Moezel PG, Bell DT (1990) Saltland reclamation: selection of superior Australian tree genotypes for discharge sites. *Proceedings of the Ecological Society of Australia* **16**, 545-549

van der Moezel PG, Watson LE, Bell DT (1989) Gas-exchange responses of 2 *Eucalyptus* species to salinity and waterlogging. *Tree Physiology* **5**, 251-257

van der Moezel PG, Watson LE, Pearcepinto GVN, Bell DT (1988) The response of 6 *Eucalyptus* species and *Casuarina obesa* to the combined effect of salinity and waterlogging. *Australian Journal of Plant Physiology* **15**, 465-474

Volker P, Owen JV, Borralho N (1994) Genetic variances and covariances for frost tolerance in *Eucalyptus globulus* and *E. nitens. Silvae Genetica* **43**, 366-372

Wade MJ (2007) The co-evolutionary genetics of ecological communities. *Nature Reviews Genetics* **8**, 185-195

Wallis IR, Watson ML, Foley WJ (2002) Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Australian Journal of Zoology* **50**, 507-519

Wardlaw TJ, Kile GA, Dianese JC (2000) Disease of eucalypts associated with viruses, phytoplasmas, bacteria and nematodes. In: Keane PJ, Kile GA, Podger FD, Brown BN (Eds) *Diseases and Pathogens of Eucalypts*, CSIRO, Melbourne, pp 339-352

Warren C, Hovenden M, Davidson N, Beadle C (1998) Cold hardening reduces photoinhibition of *Eucalyptus nitens* and *E. pauciflora* at frost temperatures *Oecologia* **113**, 350-359

Weber DJ, Rasmussen HP, Hess WM (1977) Electron microprobe analysis of salt distribution in the halophytes *Salicornia pacifica* var *utahensis*. *Canadian Journal of Botany* **55**, 1516-1523

White DA, Battaglia M, Macfarlane C, Mummery D, McGrath JF, Beadle CL (2003) Selecting species for recharge management in Mediterranean south western Australia - some ecophysiological considerations. *Plant and Soil* **257**, 283-293

White DA, Turner NC, Galbraith JH (2000) Leaf water relations and stomatal behavior of four allopatric *Eucalyptus* species planted in Mediterranean southwestern Australia. *Tree Physiology* **20**, 1157-1165

Whitehead D, Beadle CL (2004) Physiological regulation of productivity and water use in *Eucalyptus*: a review. *Forest Ecology and Management* **193**, 113-140

Wilson JR, Ludlow MM, Fisher MJ, Schulze ED (1980) Adaptation to water stress of the leaf water relations of four tropical forage species. *Australian Journal of Plant Physiology* **7**, 207-220

Withers TM (2001) Colonization of eucalypts in New Zealand by Australian insects. *Austral Ecology* **26**, 467-476

Xin Z, Browse J (2000) Cold comfort farm: the acclimation of plants to freezing temperatures. *Plant Cell and Environment* **23**, 893-902