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Genetic Variability and Leaf Waxes
of some
Eucalyptus Species with Horticultural Potential

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Abstract

Optimum pruning height for cut foliage production was initially investigated for 3-year-old trees of *Eucalyptus globulus* Labill. Trees cut at a height of 1.0 m above ground level had most stems resprouting from the trunk, but a pruning height of 0.5 m produced the longest stems. Seventeen species of *Eucalyptus* L'Hér. were subsequently subjected to trials to investigate their suitability for floriculture and the effect of pruning for cut foliage production. There was variation in leaf colour within and between species, and in the time to phase change and flowering. There were significant differences at 16 months between species for tree height, trunk diameter and lignotuber diameter with *E. globulus* having the largest dimensions. Following pruning at 19 months, there was an initial significant interaction between species and pruning height in relation to tree height, height increment, and trunk and lignotuber diameter. After one year there were significant differences between species, in the length of stems, number of stems and total weight of stems, with *E. globulus* producing the highest number and weight of stems. There was a positive correlation of trunk diameter and lignotuber diameter at 3 and 6 months since pruning, with the number of cut foliage stems produced at 12 months. For *E. gunnii* J.D.Hook. pruning to 1.0 m at 25 months produced most stems at 6 months after pruning.

Postharvest trials were conducted to assess the vase life of cut stems, and the effect of pulsing and simulated transportation on vase life. Holding solutions containing 1% or 2% sucrose and 8-hydroxyquinoline citrate (8-HQC) at 200 mg.L⁻¹ significantly increased vase life of *E. globulus* and *E. cinerea* F.Muell. ex Benth. over the control, but pulsing *E. cinerea* in 1%, 5%, or 10% sucrose plus 8-HQC for 2 hours at 24 °C or 24 hours at 3 °C had no effect. In simulated transport trials, pulsing overnight in 1% or 5% sucrose plus 8-HQC at 3 °C followed by 1 week dry storage at 3 °C had no effect on the vase life of cut stems of *E. sideroxylon* Cunn. ex Wools., *E. platypus* Hook., *E. spathulata* Hook., *E. cladocalyx* F.Muell. x *E. platypus*, or *E. spathulata* x *E. sargentii* Maiden, but a 5% sucrose pulse plus 8-HQC significantly increased the vase life of *E. spathulata* x *E. platypus*. A long pulse at low temperature (24 hours/3 °C) followed by

1 week dry storage was more effective than a short pulse at high temperature (2 hours/24 °C) for *E. albida* Maiden & Blakely stems and no sucrose was more effective than 1% or 5%. Thus, a 2% sucrose holding solution extended vase life of *E. globulus* and *E. cinerea*. There was no advantage of sucrose pulsing to extend vase life, or to improve vase life following dry storage, except for the hybrid *E. spathulata* x *E. platypus*.

Eighteen species of *Eucalyptus* were studied for changes in wax morphology of juvenile foliage with leaf age using Environmental Scanning Electron Microscopy. Three species were studied for wax regeneration following removal from the adaxial surface of day 16 and day 30 leaves (16 and 30 days following lamina separation, respectively) while still attached to the tree. For each leaf age, four leaves per species were sampled at 0, 1, 3, 9 and 15 days after wax removal. All species had tube wax on juvenile leaves but there were differences between the species, in the length and conformation of tubes and percentage surface area covered by wax. Tube length was greatest in day 30 leaves, but tube diameter was narrower than day 0 leaves (unfolding leaves). There were significant differences in wax structure between the proximal and distal ends of day 0 leaves of ten species, and tubes were observed crystallising from amorphous wax deposits in the proximal area only. Significant wax regeneration occurred more rapidly on day 16 leaves than day 30 leaves. Wax morphology was consistent across six species of the series *Viminalis*. Thirteen species of *Eucalyptus* are recommended for the cut foliage industry.

Variation in amount and composition of leaf epicuticular wax among 17 species of *Eucalyptus* was characterised by gas chromatography (GC), thin-layer chromatography (TLC), and gas chromatography-mass spectrometry (GC-MS). Across species, wax yield ranged from 0.7 to 4.5 mg/cm². The major wax constituent in all species except *E. delegatensis* R. Baker, was β -diketones (24.7–83.0%), followed by wax esters (6.4–26.5%), n-alkanes (3.5–26.5%), fatty acids (3–15.9%), n-alcohols (0.6–11.1%) and aldehydes (0–9.2%). Volatile organic compounds emitted from developing *Eucalyptus* leaves have been studied. Headspace samples were collected from six species in a plantation from leaves *in situ*, using solid phase microextraction (SPME),

and were analysed by GC and GC-MS. Leaf oils were extracted and the relative amounts of volatile compounds in the extract and headspace were assessed. The major volatile constituents were α -pinene (11.4 – 57.8%, headspace; 15.3 – 32.2%, oil extract) and 1,8-cineole (3.2 – 88.6%, headspace; 20.8 – 54.7%, oil extract). Other terpenoids present in the headspace in significant quantities were alloaromadendrene, viridiflorene, thujene, α -phellandrene, γ -terpinene and limonene. In the oil extract an unidentified compound at R_t 21.2 min. (GC), alloaromadendrene, α -phellandrene, phenol derivative, viridiflorene and limonene were present in significant quantities.

Eucalyptus gunnii leaves can appear as green or glaucous phenotypes with the latter more desirable for floriculture. The epicuticular wax from these two types were compared morphologically using environmental scanning electron microscopy (ESEM), chemically using gas chromatography, and molecular markers were found to distinguish the two types using RAPDs and bulked segregant analysis. Both phenotypes had tube wax which in the glaucous type were significantly longer and thicker and the surface area was covered more densely than on the green type. When compared chemically, the glaucous wax contained a higher percentage of alkanes, alcohols and free fatty acids, but lower percentages of β -diketones and esters than the wax from the green type. The glaucous type had a greater yield of wax per unit area of leaf than the green type. Seven molecular markers were found which would distinguish the green and glaucous bulks. No individual marker could totally distinguish all green individuals from all glaucous individuals but this could be achieved using combinations of markers. These markers may facilitate the management of *E. gunnii* breeding and selection for the cut foliage industry, by providing an initial screen for glaucousness.