SELECTION FOR EXTREME FROST RESISTANCE IN *EUCALYPTUS*

Bertrand Cauvin [1] and Brad Potts [2]

[1] AFOCEL,
Association Forêt Cellulose,
98 route de Tournefeuille,
31270, CUGNAUX (France).

[2] Department of Plant Science,
University of Tasmania,
GPO Box 252C,
Hobart, 7001,
Tasmania, Australia.

Summary

The climatic conditions in France necessitates selection of eucalypt ortets with the highest level of tolerance to different types of frost. A series of tests utilising artificial and natural frosts have been developed for early selection of the most resistance genotypes and subsequent confirmation of the selections. A selection of more than 300 clones has demonstrated the high frost resistance of *E. gunnii* and closely related species and constitutes the new genetic base of AFOCEL.

Key words: *Eucalyptus gunnii*, frost resistance, early selection

Introduction

AFOCEL commenced a breeding programme for *Eucalyptus* in 1972 after several tentative species introductions had been undertaken by the Administration Forestière Française. Introductions of *Eucalyptus* into France had been stopped previously because of the exceptionally cold winter of 1956. Interest in eucalypt plantations for pulpwood production increased following the success of trials established near Toulouse, in the south of France (Marquestuat *et al.* 1978, Marien and Cauvin 1984) and was encouraged by more than a century of growth of eucalypts as ornamentals near the Mediterranean coast. The good growth rates and survival of species such as *Eucalyptus dalrympleana*, *E. viminalis*, *E. macarthurii* and *E. nitens* as well as the slower growing, but more frost resistant species such as *E. gunnii* resulted in the development of an intensive eucalypt breeding programme (Cauvin 1984; Cauvin *et al.* 1987) and approximately 400 hectares of industrial plantings of clonal selections had been established prior to the devastating frost of 1985 (Figure 1).

Up until the winter of 1985, considered as extreme as that of 1956, AFOCEL had proposed a group of clones for the industrial plantations which principally comprised *E. gunnii* x *dalrympleana* hybrids. These clones represented what, at the time, was considered an acceptable compromise between vigour and frost resistance given the low probability of another extreme winter. However, since the frost of 1985 which killed even the more frost resistant species such as *E. gunnii* to ground level (Potts and Potts 1986), industrial plantings were stopped and AFOCEL has since concentrated on selection of eucalypt clones for extreme frost resistance. AFOCEL is selecting close to the natural limits of the frost resistance of the genus and the challenge is to develop plants which are resistant to relatively infrequent, unpredictable extreme frost temperatures, yet suitably productive to remain economically competitive with more traditional species.

Frosts in the south of France can be characterised by two important parameters, viz. the minimum temperature reached (i.e. -19°C screen temperature at Toulouse in 1985) and the rate of change of temperature. The latter parameter is particularly important in autumn where precocious, "out-of-season" frosts may occur (e.g. -12°C on 21/11/1988 with a maximum temperature of 20°C 48 hours earlier). Indeed, the high temperatures during the growing season in France, particularly when coupled with high soil moisture in spring and autumn, appear to result in dehardening to a greater degree, and for longer periods, than in the native habitats of the frost resistant species (e.g. mountain tops). Both the precocious and extreme low temperature winter frosts have caused considerable damage in plantations in France but at the same time have allowed good selection for this trait. A third parameter, the duration of the frost must also be considered. While not as yet fully understood, this parameter may be important even when negative temperatures are not particularly extreme.

Since 1985 selection for frost resistance has been achieved by -

(i) developing a scale of frost resistance based on visual damage scores (1 undamaged to 9 killed; see Cauvin 1988),
(ii) a radical change in the species and genetic base being utilised,
and (iii) the development of two methods of testing frost resistance, *viz.* artificial tests (electroconductivity, frost chamber) or field trials arranged to exploit natural frost gradients (altitudinal or longitudinal transects).

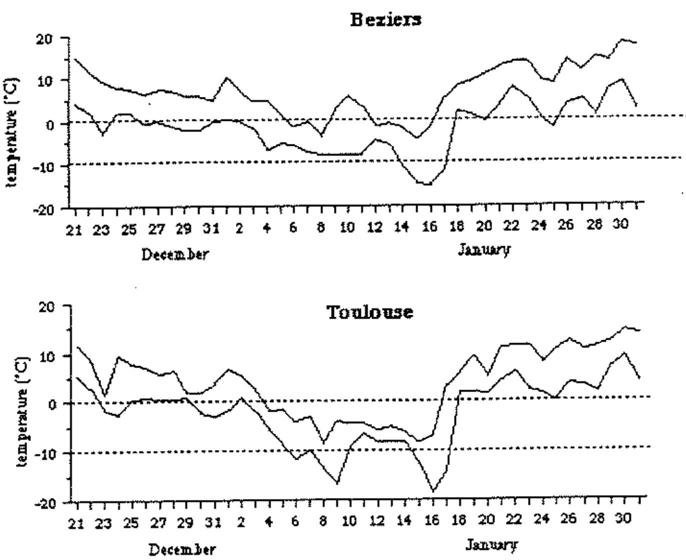

Figure 1. Minimum and maximum daily screen temperatures (°C) during the extreme 1984/1985 winter in France. Temperatures are shown for Beziers near the Mediterranean coast where frost resistant species such as *Eucalyptus gunnii* were not severely damaged in plantations and further inland at Toulouse where virtually all eucalypts were killed to ground level, although many resprouted from lignotubers the following spring. This frost was followed by another severe frost in 1987 when temperatures near Toulouse again dropped to -19°C.

Selection strategy

Breeding has concentrated on the Tasmanian sub-alpine species *E. gunnii*. Species trials prior to 1985 have shown it to be one of the most frost resistant species in the genus and the most suitable for breeding in France (Marquestuat *et al.* 1978; Marien 1980; Davidson and Reid 1985; Table 1). This species is closely related to *E. archeri*, which is sometimes treated as a subspecies of *E. gunnii* (e.g. Pryor and Johnston 1971, c.f. Chippendale 1988) and this species was also included in the base population. However, other potential species such as *E. parvifolia*, *E. glaucescens* and *E. rodwayi* are continually being scanned. The strategy used for selecting frost resistant clones is summerised in Figure 2.

The first stage in the selection strategy has been to intensively sample the range of variation in natural populations in order to establish a broad base population. AFOCEL's initial base population collection of *E. gunnii* and related species from Tasmania (Marien 1985) included 132 families from 25 provenances from the *E. gunnii-archeri* complex and encompassed virtually the full geographic and ecological range of both *E. gunnii and E. archeri* (see Potts and Reid 1985a,b). Consistent family and provenance rankings and high frequencies of clonal selections from specific provenances have resulted in the identification of the *E. gunnii* provenances around Great Lake on the Central Plateau of Tasmania as being the most frost resistant within *E. gunnii* (Potts 1985; Cauvin 1988; Table 2). In 1987, more detailed sampling of the area around Great Lake was undertaken with seed collected from a minimum of 10 trees from 7 of the most resistant subprovenances. Based on the combined results of trials established from the 1984 and 1987 collections, backward-selection of elite ortets in native stands has been undertaken in order to provide elite seed for more intensive screening and pollen for controlled crossing. Forward-selection of individual ortets from field trials or by artificial tests has proceeded simultaneously from the material established in France. While there is variation between clones, in general *E. gunnii* is relatively easily propagated from hardwood cuttings (see Cauvin 1982, Cauvin and Marien 1979; Potts and Potts 1986). The second stage thus consists of cloning selections for confirmation of the frost resistance of the selected ortets using the same sceening procedures as used previously.

In the final stage of selection clonal trials are established to study the growth, form and stability of the clones. In general, the use of the more frost resistant species has resulted in less satisfactory productivity, especially where the clones are derived from collections of small populations or isolated trees in natural stands (e.g. isolated trees in frost hollows). Such selections may result from self-fertilisation or crossing between closely related individuals and inbreeding has been shown to cause strong inbreeding depression in growth of *Eucalyptus* (van Wyk 1980; Cauvin *et al.* 1987; Griffin and Cotterill 1989). However it may be possible to remove this effect by crossing between selections from widely separate provenances having the same level of frost resistance (assuming a high level of additivity for frost resistance). This poor vigour represents the most important problem encountered with *E. gunnii*. The variability in this species may be better exploited by first selecting for growth traits and then selecting for frost resistance by using the electro-conductivity method to screen individual adult trees.

Table 1. Mean frost damage recorded in 1987 (age 1 year) of five Tasmanian sub-alpine species in the base population trial at Lamasquere (1 undamaged to 9 dead; see Cauvin 1988). Lines indicate means which are not significantly different at the 5% probability level on the basis of Tukey's Studentised Range test.

SPECIES	Mean	Number of families
E. rodwayi	9.0	8
E. urnigera	8.9	24
E. subcrenulata	8.6	12
E. archeri	8.2	41
E. gunnii	7.7	135

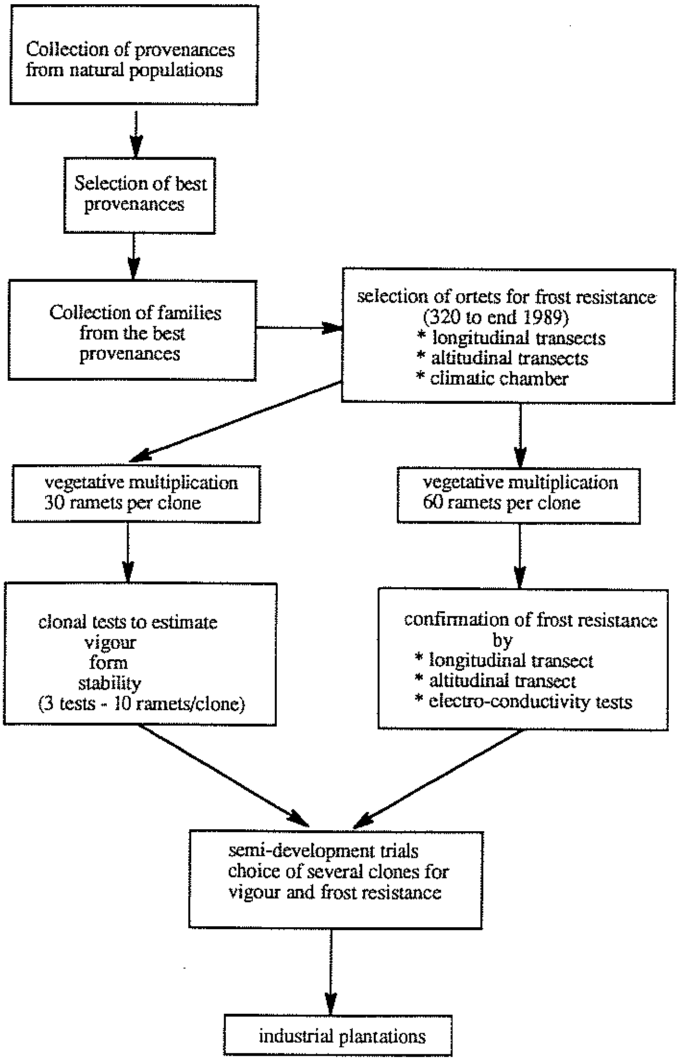

Figure 2. Simplified strategy for the selection of clones having good frost resistance and productivity, indicating the genetic base and the different tests for selection and confirmation.

Table 2. Mean frost damage recorded in 1987 (age 1 year) of 19 provenances of *Eucalyptus gunnii /archeri* in the base population trial at Lamasquere (1 undamaged to 9 dead; see Cauvin 1988). Lines indicate means which are not significantly different at the 5% probability level on the basis of Tukey's Studentised Range test. Provenance details are given in Marien (1985).

Provenance	Species	N	Mean
Hunterston	gunnii	24	8.73
Mt. Victoria	gunnii	18	8.66
Mt. Barrow	archeri	20	8.57
Snow Hill	gunnii	23	8.54
Mt. Maurice	archeri	25	8.54
Projection Bluff	archeri	24	8.49
Snug Plains	gunnii	29	8.38
Macclesfield	gunnii	10	8.38
Pine Tier	gunnii	33	8.16
Jacks Marsh	gunnii	23	7.96
Lake Webster	gunnii	22	7.87
Ben Lomond	archeri	25	7.65
Mt. Rufus	gunnii	24	7.58
Jimmeys Marsh	gunnii	20	7.21
Poatina Highway	gunnii	23	6.99
Poatina Inlet	gunnii	23	6.77
Liawenee	gunnii	13	6.23
Breona	gunnii	19	6.05
Shannon Lagoon	gunnii	33	5.79

Great Lake

Selection methods

Tests utilising natural frosts

A series of specialised trials have been established along altitudinal and longitudinal transects to enable selection from families from either the base population collections or controlled pollinations and to confirm the frost resistance of selected ortets. These tests utilise the natural frost gradients which occur in France with changes in altitude or longitude (Fig. 3). Each year, family trials (for selection) or clones (for confirmation of selected ortets) are planted on sites chosen to encompass the full gradient in natural temperatures (Table 3). The minimum number of sites planted is six, three of which are along the altitudinal transect (600m - 800m - 1100m). These trials are planted at a high density (3m x 1m) as they are not required to remain more than 3 years. The location of these specialised transects is indicated in Figure 3. Other plantations not associated with the specialised transects for frost resistance have also permitted other selections over the 1987 to 1989 winters (provenance and family trials, clonal conservatories and clonal family tests etc.).

The correlation between the frost damage scores of the clones at the various sites is usually quite high ($r = 0.67$ to 0.97), but in cases may be low, and is dependent to a large extent upon obtaining a frost of sufficient intensity to optimally differentiate clones. Figure 4 demonstrates the complexity of evaluating frost resistance even to frosts of the same minimum temperature (-14°C). The clones do not respond in exactly the same manner in each frost. The frost caused more damage in 1988 than in 1989. The onset of the 1988 frost was rapid and plants did not have a long period of hardening as they did in 1989. The physiological state of the plants is an important factor in determining the extend of damage. While clones receiving little damage in 1988 also received little damage in 1989, the reverse is not the case. Precocious frosts appear to be more selective than winter frosts which occur when plants are well hardened. The same phenomenon is observed when frost damage is measured using the electro-conductivity method. The best choice of clones consists of retaining those which are consistently good performers from one winter to another (situated on the lower right of the regression) and have better performance than the current best clone (Figure 4).

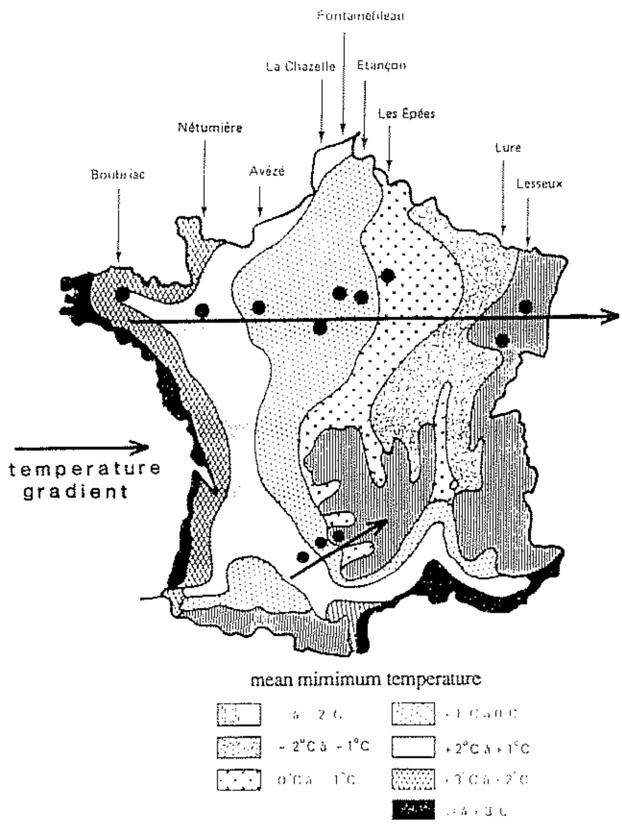

Figure 3 The location of the longitudinal (north) and altitudinal (south) transects along temperature gradients in France. The mean minimum screen temperature is also mapped (modified from Cauvin 1988).

Table 3. Minimum temperatures (°C) between extremities of the longitudinal and altitudinal transects from 1987 to 1990.

Year	longitudinal		altitudinal transect		
	west	east	600m	800m	1100m
1987	-10	-24	-14	-17	-20
1988	-1	-14	-	-10	-
1989	-4	-14	-5	-5	-8
1990	-6	-17	-10	-11	-17

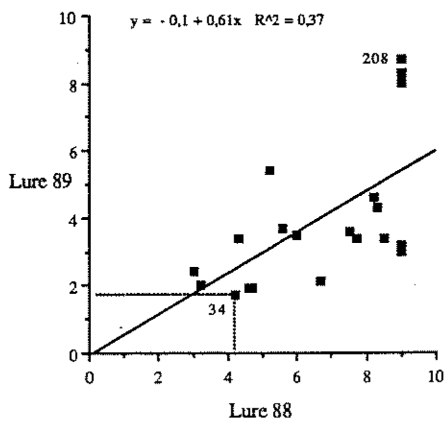

Figure 4. Relationship between frost damage recorded in the winters of 1988 (-14°C) and 1989 (- 14°C) at Lure (r = 0.61). (Scale: 1 = undamaged to 9 = dead)

Tests utilising artificial frosts: electro-conductivity method

The electro-conductivity method for testing frost resistance is now widely used for *Eucalyptus* (Raymond *et al.* 1986; Tibbits and Reid 1987a,b; Cauvin 1988). This method consists of measuring the leakage of cellular exudate from leaf disks subjected to frosts of varying temperature in a frost chamber. The leakage of cellular exudate from damaged cells is measured by the conductivity of the surrounding liquid and the conductivity is expressed relative to that measured after destruction of all tissue by freezing or heating (Cauvin 1988). The phenomenon of super-cooling is eliminated either by the addition of silver iodide or ice, or by using temperatures less than -8°C. Details of the artificial frost produced in the climatic chamber including the rate of temperature descent and stages of stabilisation are shown in Figure 5.

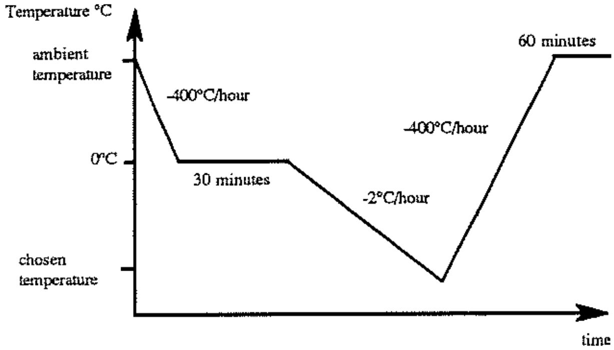

Figure 5. Temperature descent and stages of stabilisation used in the climatic chamber for artificial frosting.

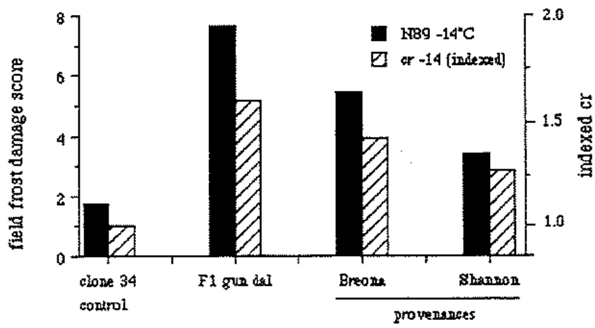

Figure 6. The frost resistance of clones according to genetic origin. The figure indicates the mean relative conductivity (at -14°C) indexed on the resistant control clone (No. 34 = 1) and the mean frost damage score at Lure (temp = -14°C; n 89; 1 = undamaged to 9 = dead) for clones of *E. gunnii* x *dalrympleana* F1 hybrids and *E. gunnii* clones from the Breona and Shannon Lagoon provenances..

The conductivity method is a good means of discriminating provenances or families when they differ markedly in frost resistance (e.g. Figure 6 - where the *E. gunnii* x *dalrympleana* hybrids are more frost sensitive than the *E. gunnii* clones). As with the results from field transects, there are changes in clonal rankings at the individual level which are essentially related to their physiological state (i.e. degree of hardening). However, only clones which consistently show good resistance in all of the tests undertaken are retained in the final selection. The age of material also appears to affect the results of the electro-conductivity tests. We have compared the relative conductivity of a sensitive (208) and a resistant (34) clone under varied physiological conditions (e.g. juvenile versus 4 years old, summer versus winter) and frost temperatures (Figures 7 and 8). The results clearly show that a clone is more resistant as an adult field-grown plant compared with the juvenile stage in the nursery. It is difficult to differentiate material during the growing season (e.g. summer) when it is not hardened (Figure 7a). However, when hardened in winter the clones are clearly differentiated and the rank remains the same for both adult and juvenile stages (Figure 7b).

As with the correlation between the frost damage scores from different sites, the relationship between the electro-conductivity results and those from the field transects has proven quite variable and can not be strong if the frost obtained in the field is not as discriminating as that obtained in the climatic chamber. Figure 8 shows the association between the frost damage of clones as assessed from a field trial in the north of France (Lure) compared to the relative electro-conductivity scores for the same clones measured during winter at another trial in the south of France (Pujaudran). While the resistant and sensitive control clones are clearly differentiated in both cases, the overall correlation between the field and electro-conductivity scores is only 0.46. However, this is only slightly less than between the correlation between two frost damage scores at the same site in 1988 and 1989 (Figure 4). In contrast, high correlations between the field frost damage and the results from the electro-conductivity method have been obtained when material growing at the same site has been assessed by both methods (r = 0.83; Figure 9).

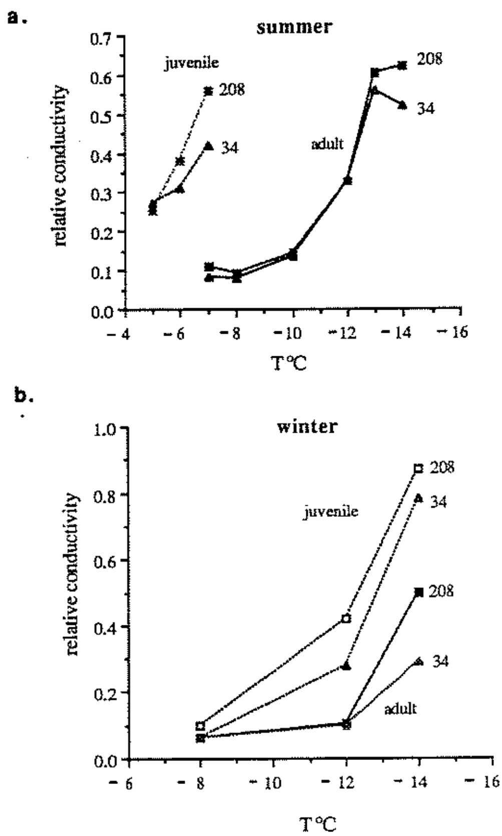

Figure 7. The relative conductivity at different frost temperatures of two clones (34 resistant; 208 sensitive) at the juvenile and adult stage measured during (a) the growing season and (b) winter during.

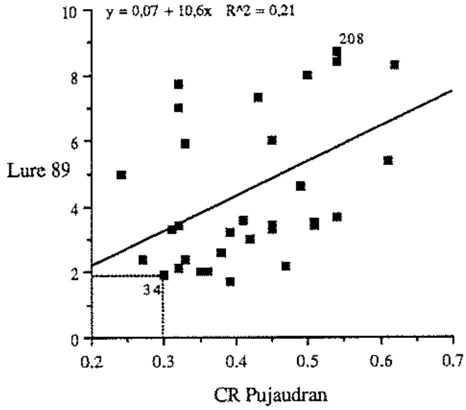

Figure 8. The relationship between the relative conductivity after an artificial frost of -12°C measured from a clonal trial at Pujaudran (1 year old, winter) and field frost damage scores of the same 29 clones in a trial at Lure (-14°C) after the 1989 winter (Scale: 1 = undamaged to 9 = dead). The two reference clones are indicated (34 = resistant; 208 = sensitive).

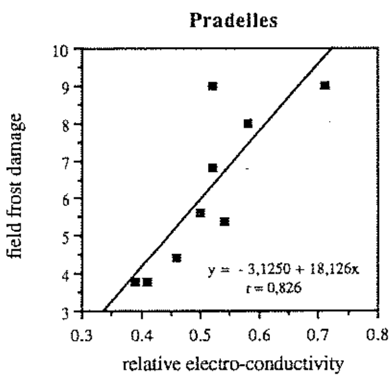

Figure 9. The relationship between the relative electro-conductivity (-12°C) and frost damage score (Scale: 1 = undamaged to 9 = dead) of clones measured at the same site (Pradelles).

Conclusions

The necessity to select a range of clones for industrial plantations which can tolerate very low winter temperatures and also tolerate precocious, "out-of-season", frosts where the temperature change is often extremely rapid, necessitated a radical modification of AFOCEL's improvement strategy with a change in the genetic base and research shifted toward artificial, early selection procedures. These changes included sampling the full natural range of *E. gunnii* and neighbouring species in Tasmania and identifying the most resistant provenances and families. This involved four successive collections of seed and pollen which were mainly concentrated on the Central Plateau.

The selection of elite clones for mass vegetative propagation was undertaken in two stages. Firstly, by selection of ortets having the best frost resistance from specialised field trials established along natural temperature gradients (transects) and, secondly, by confirming this aptitude from the response of clones in either the transects and/or by the electro-conductivity method using leaf discs.

Up to now, selection has primarily been on frost resistance followed by selection for growth traits amongst the most resistant clones. However, the screening of adult material by the electro-conductivity method will now allow the possibility of first selecting for growth traits amongst individuals within the most frost resistant families and then subsequent elimination of individuals with a poor response in the electro-conductivity tests. The latter method may be more practical because screening for frost resistance in field transects and even using the electroconductivity method is long and relatively expensive. It appears to be essential to test clones under differing types of frost in field transects or, in the case of the electro-conductivity method, under differing physiological states in order to identify clones with a stable response.

Since 1987 AFOCEL has selected 318 ortets at various temperatures between -12°C and -20°C, depending on the site. These selections encompasses six species -

E. gunnii -archeri, 217 individuals (including 203 from natural Tasmanian provenances, 7 from French provenances and 7 from controlled pollinations)
E. glaucescens, 12 individuals
E. dalrympleana , 24 individuals (Tasmanian provenances)
E. parvifolia , 49 individuals (Victorian provenances)
E. pauciflora , 15 individuals
E. subcrenulata , 1 individual (Tasmania)

These ortets are propagated by hardwood cuttings in sufficient numbers to enable the planting of transects designed specifically for evaluating frost resistance (minimum 60 cuttings per clone) with 40-50 clones tested per year. At the same time clones are included in multi-location trials in order to assess their productivity, form and stability.

References

Cauvin, B. (1982). Réjuvénilisation multiplication d'ortets séniles. *Annales de recherches silvicole 1981*. (AFOCEL, Paris.) pp.74-105.
Cauvin, B. (1984). - *Eucalyptus* hybridation controlée - premiers resultats. *Annales de recherches silvicole 1983*. (AFOCEL, Paris.) pp.86-111.
Cauvin, B. (1988). *Eucalyptus* : Les tests de résistance au froid. *Annales de recherches silvicole 1987*. (AFOCEL, Paris.) pp.161-195.
Cauvin, B. and Marien, J. N. (1979). La multiplication vegetative des *Eucalyptus* en France. *Annales de recherches silvicole 1978*. (AFOCEL, Paris.) pp.142-175.
Cauvin, B. , Potts, B. M. and Potts, W. C. (1987). *Eucalyptus*: Hybridation artificelle - Barriers et hérédité des characters. *In* 'Annales de recherche silvicole 1986'. pp. 255-303. (Association Forêt-Cellulose: Paris.)
Chippendale, G. M. (1988). *Eucalyptus, Angophora (Myrtaceae), Flora of Australia* (Australian Government Publishing Service, Canberra).

Davidson, N. J. and Reid, J .B. (1985). Frost as a factor influencing the growth and distribution of subalpine *Eucalyptus*. *Aust. J. Bot.* 33, 657-667.

Eldridge, K. G. and Griffin, A. R. (1983) Selfing effects in *Eucalyptus regnans*. *Silvae Genetica* 32, 216-221.

Griffin, A. R. and Cotterill, P. 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, 124-131.

Marien, J. N. (1980). La sélection juvénile des *Eucalyptus* pour leur resistance au froid. *Annales de recherches silvicole 1979*. (AFOCEL, Paris.) pp. 226-253.

Marien, J. N. (1985). A propos d'une récolte de graines d'*Eucalyptus* en Tasmanie. *In* 'Annales de recherche silvicole 1984'. pp. 395-435. (Association Forêt-Cellulose: Paris.)

Marien, J. N. and Cauvin, B. (1984). Les *Eucalyptus* en France : tarifs de cubage et production de biomasse. pp. 174-193 in 'Proceedings of the IUFRO International Conference on Frost Resistant *Eucalyptus*, Bordeaux, France, 1983'.

Marquestuat, J.,Thibout, H. and Cauvin B. (1978). Essais d'introduction d' *Eucalyptus* dans le midi de la France. *Annales de recherches silvicole 1977*. (AFOCEL, Paris.) pp.161-195.

Potts, B. M. (1985). Variation in the *Eucalyptus gunnii- archeri* complex. 111. Reciprocal Transplant Trials. *Aust. J. Bot.* 33, 687-704.

Potts, B. M. and Potts, W. C. (1986). Eucalypt breeding in France. *Aust. Forestry* 49, 210-218.

Potts, B. M. and Reid, J. B. (1985). Variation in the *Eucalyptus gunnii- archeri* complex. 1. Variation in the adult phenotype. *Aust. J. Bot.* 33, 337-59.

Pryor, L. D. and Johnson, L.A.S. (1971). 'A Classification of the Eucalypts.' (Aust. Natl. Univ. Press: Canberra.).

Raymond, C. A., Harwood, C. E., and Owen, J. V. (1986). A conductivity method for screening populations of eucalypts for frost damage and frost tolerance. *Aust. J. Bot.* 34, 377-93.

Tibbits, W. N. and Reid, J. B. (1987a). Frost resistance in *Eucalyptus nitens* (Deane & Maiden) Maiden: Genetic and seasonal aspects of variation. *Austr. For. Res.* 17, 29-47.

Tibbits, W. N. and Reid, J. B. (1987b). Frost resistance in *Eucalyptus nitens* (Deane & Maiden) Maiden: physiological aspects of hardiness. *Aust. J. Bot.* 35, 235-50.

van Wyk, G. (1980). Inbreeding effects in *E. grandis* in relation to degree of relatedness. *S. A. For. J.* 116, 60-63.