Eucalyptus Breeding for Clonal Forestry

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Abstract As global demand for wood increases, planted forests will also become increasingly important. Accepting and promoting them as the only way to address the wood scarcity problem and also to help suppress the demand for illegally logged timber from natural forests is a major issue globally. Eucalypt clonal forestry is proving to be an iconic alternative in this context, due to their fast growth, wood quality appropriate to many different uses, huge existing variability, and suitability to vegetative propagation. However, efficient breeding and deployment strategies are essential. The present chapter aims to present, based on the authors' practical experience, an overview on the most successful approaches that may be used during the different phases of eucalypt breeding programs for clonal forestry. Relevant topics covered are: identifying breeding objectives and related traits for the main eucalypt businesses worldwide; the major planted species and their value for different objectives; breeding strategies (recurrent selection methods, breeding cycle, etc.); recombination issues, such as effective population size, mating designs and controlled pollination methods; evaluation and selection procedures as applied to progeny and clonal trials; and deployment aspects, such as number of commercial clones, large scale vegetative propagation methods, and risk management.

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1 Introduction

The world population has recently reached seven billion people, and every day, everyone makes use of forest products at homes, offices and schools, but often don't realize how important they are. Forest products are present in buildings structural and decorative materials, furniture, printing and writing paper, toilet paper, steel products, pharmaceutical products, cosmetics and many other necessities of life.

Global demand for wood will increase, driven mainly by the following trends (FAO 2010, 2011):

- The world population is increasing, forecasts indicating that there will be nine billion people in 2050.
- Wood availability is decreasing. The global forest area and wood availability per capita has been reduced over time.
- Economic growth, especially in the emerging economies.
- Globalization of the forest products market. China, India and Brazil are now driving future forest products demand and investments.
- Climate change, with increasing temperatures and water scarcity trends.
- Environmental and energy policies, valuing the potential of wood as a renewable source, with positive environmental benefits.
- Demand for high quality value added and certified wood products.

In this scenario planted forests will become increasingly important. Accepting and promoting them as the only way to address the wood scarcity problem and to avoid illegal logging in natural forests is a major issue (Fenning and Gershenzon 2002). Expanding the range of commercial forests will be as important as increasing productivity in current plantation areas, in order to minimize competition for land, whether for agricultural, conservation or carbon capture needs, as well as for increasing the availability of wood while reducing its cost.

Eucalypt forests are an iconic alternative in this context, due to its astonishing fast growth (rotation ages ranging from 6 to 15 years), wood quality appropriate to many different uses, huge existing variability, and suitability to vegetative propagation. The global area of eucalypt plantations is already around 20 million hectares across all the inhabited continents, with large areas located in Brazil and India (more than 4 million hectares each) (FAO 2010, 2011).

According to the Brazilian Association of Planted Forests Producers (ABRAF 2011), in 2010 the harvest from Brazilian eucalypt forests was 112,955,222 m^3 of wood, with 48.5 % being used for pulp and paper production, 13.6 % for charcoal production mainly for the steel industry, 3.9 % for chipboard panels production, 3.1 % for added value solid wood products production (lumber for furniture, flooring, etc.) and 29.3 % for industrial firewood production. The estimated gross value of this forest production was ca. US$25,000,000,000.

In Brazil, nearly 50 % of current eucalypt forests are clonal. The development of clonal forestry for eucalypt in this country, including genetic and silvicultural improvements, is emblematic (Fig. 1) because it moved mean productivity from

Fig. 1 Eucalypt clonal forests, Veracel Company, Brazil

25–30 m^3/ha/year to 35–45 m^3/ha/year in the last 30 years. Productivity can exceed 60 m^3/ha/year in some areas where proper conditions (soil, climate, genetic material and silvicultural practices) are combined. However, clonal eucalypt forests are also successful in many other countries, such as China, South Africa, Portugal, Spain, Chile, Argentina and Uruguay, and are mostly used for pulp and paper production.

Given further developments, eucalypt clonal forestry could go a long way to fulfill present and future global wood needs. In this context, specific breeding and deployment strategies will be required to optimize gains in productivity and wood quality. The present chapter aims to present the authors' practical experience concerning the most relevant aspects for the development and implementation of such strategies.

2 Breeding Objectives

Breeding objectives must be aligned with the long term business strategy. This assumption, which is obvious at first sight, sometimes creates a huge challenge for breeders, simply because they must ensure all internal and external clients will

be satisfied when a specific clone is planted, harvested and converted into a final product. This is especially true for wood quality traits as they strongly impact the final product performance. The larger the number of traits involved, the smaller will be gain from selection in each one of them. That means the breeders must choose to work on relatively few individual wood quality traits, which explain the variability in the long term objectives for the most important product attributes.

The most relevant breeding objectives and related traits usually considered in eucalypt breeding programs worldwide, for the main eucalypt businesses (pulp and paper, charcoal for steel industry, biomass and solid wood production) are presented in Table 1. From this table some important observations can be made:

– Increased productivity is needed for any business. Forest productivity is the ultimate expression of adaptation. In highly productive areas (defined mostly by the absence of significant water stress), where there is broad adaptation of the breeding population, individual tree wood volume will play a major role as compared to survival. In poor or restrictive areas, especially those affected by drought or frost conditions, survival ability will be of increased importance.
– Increased tolerance to pests and diseases is a key determinant of forest productivity and forest production stability. For each point in time there will be specific agents to be monitored that might damage clonal forest plantations.
– Increased rooting ability is crucial for eucalypt clonal forestry, as this trait is the most important factor impacting clonal plant production costs. However, the application of this selection criterion must be done with parsimony, as the acceptable rooting ability of a given clone must take into account the cost/benefit relationship defined also by the gains obtained from its use (e.g. pulp productivity). Sprouting ability is also important, both in the nursery and the field, especially where coppice (second or third rotation) is a common practice.
– Wood basic density is relevant for almost all end uses, as it impacts both productivity and product quality. Special attention must be given to this trait, in any situation.

In this context, understanding the genetic control and correlations between traits is very important as this will impact different choices and activities related to the breeding program. Some important lessons obtained so far are as follows (Araujo et al. 2012; Assis 2000; Assis and Resende 2011; Borralho et al. 1993, 2008, Costa e Silva et al. 2006, 2009; Downes et al 1997; Drew et al. 2009, 2011; Greaves and Borralho 1996; Potts 2004; Raymond and Apiolaza 2004; White et al. 2007):

– Heritabilities for wood quality traits are higher than for growth traits (usually above 0.4 and below 0.25, respectively).
– Genetic control of growth and wood quality traits is predominantly additive (determined by the alleles mean effects) but evidence exist that for growth traits non-additive effects (effects from interactions between alleles or dominance effects, and between gene loci or epistasis) are important too.

Table 1 Most relevant breeding objectives and related traits in eucalypt breeding programs for pulp and paper, charcoal for steel industry, biomass and solid wood production

Business	General objectives	Specific objectives	Related or complementary traits (selection criteria)
Pulp and paper	Increased pulp productivity (adt/ha/year)[a]	Increased forest productivity (m^3/ha/year)	Increased tree volume (m^3/tree)
			Increased survival (%)
			Increased tolerance to biotic (pests/diseases) and abiotic (drought/frost) stress
			Increased rooting ability (%)
		Reduced specific consumption (m^3/adt)[a]	Increased basic density (kg/m^3)
			Increased pulp yied (%)
			Reduced lignin content (%)
			Increased S:G lignin type ratio[a]
	Increased product quality (major examples)	Printing and writing paper: increased bulk, stiffness and opacity	Increased basic density (kg/m^3)
			Increased number of fibers (million/g in pulp)
			Increased runkel ratio[b]
		Tissue paper: increased softness, bulk and tensile strength	Basic density upper limited to 600 kg/m^3
			Increased number of fibers (million/g in pulp)
Charcoal	Increased charcoal productivity (t/ha/year)	Increased forest productivity (m^3/ha/year)	Increased tree volume (m^3/tree)
			Increased survival (%)
			Increased tolerance to biotic (pests/diseases) and abiotic (drought/frost) stress
			Increased rooting ability (%)
		Reduced specific consumption on carbonization (m^3/t)	Increased basic density (kg/m^3)
			Increase gravimetric yield (%)
	Increased product quality (major examples)	Increased mechanical resistance and reduced specific consumption on pig iron making (m^3/t)	Increased basic density (kg/m^3)
			Increased lignin content (%)
			Increased particle size

(continued)

Table 1 (continued)

Business	General objectives	Specific objectives	Related or complementary traits (selection criteria)
Biomass	Increased biomass productivity (t/ha/year)	Increased forest productivity (m^3/ha/year)	Increased tree volume (m^3/tree)
			Increased survival (%)
			Increased tolerance to biotic (pests/diseases) and abiotic (drought/frost) stress
			Increased rooting ability (%)
		Reduced specific consumption (m^3/t)	Increased basic density (kg/m^3)
			Increased drying capacity
	Increased product quality (major example)	Increased calorific power (Kcal/kg)	Increased basic density (kg/m^3)
			Increased lignin content (%)
Solid wood	Increased lumber productivity (m^3/ha/year)	Increased forest productivity (m^3/ha/year)	Increased tree volume (m^3/tree)
			Increased survival (%)
			Increased tolerance to biotic (pests/diseases) and abiotic (drought/frost) stress
			Increased rooting ability (%)
		Increased industrial recovery (%)	Reduced taper
			Increased straightness
			Reduced spiral grain
			Reduced end splitting
	Increased product quality (major examples)	Increased clear and clean wood	Increased natural debranching
			Light-colored wood
		Increased dimensional stability	Reduced anisotropic factor[c]
		Increased mechanical resistance	Increased basic density (kg/m^3)

[a]Specific consumption is the amount of wood (m^3) required for the production of one air dry ton of pulp (adt). S:G lignin type ratio means syringyl/guaiacyl ratio. High S:G ratios are advantageous for pulping process (cooking and bleaching) because S lignin type is more easily removed from wood

[b]Runkel index = (2 × cell wall thickness)/lumen diameter. High runkel indexes are advantageous for pulp and paper quality because it means the fibers are more consistent

[c]Anisotropic factor: relation between tangential contraction and radial contraction. Low anisotropic factors are advantageous for solid wood quality because they are associated with a low probability of splitting, cracking or warping

– Environmental correlations may occur between growth and wood quality traits, e.g. the same clone planted in both a more favorable adaptive region and in a less favorable one, will grow faster and present lower wood density and vice-versa, respectively.
– Genetic correlation exist between wood traits and between wood and final products traits.

– Within the same broad adaptation region, usually there is no significant genotype x environment interaction, either for volume or wood quality. This kind of interaction, however, is to be expected to some extent between regions with great differences in adaptive potential.
– Age effects occur both for growth and wood quality. Knowing general growing curves, at least for growth and basic density (basic density also increases with age), will help decisions in different phases of the breeding program.

3 Choosing Species

Eucalypt belong to the division *Angiospermae*, class *Dicotyledon*, order *Myrtales*, family *Myrtaceae*, genus *Eucalyptus* and *Corymbia*. There are around 900 species belonging to these genera (mostly *Eucalyptus*), originating from Australia, Papua New Guinea and parts of Indonesia. Thus, they naturally occur across a wide range of latitudes, meaning huge differentiation among species along with evolutionary processes and adaptation to very contrasting environmental conditions. Eldridge et al. (1993) and Boland et al. (2006) present detailed descriptions of eucalypt species and respective centers of origin. Recent reviews discuss several aspects of the biology and genetics of eucalypt (Grattapaglia et al. 2012; Myburg et al. 2007).

Despite the large number of existing species, less than 20 are commercially planted worldwide. *E. grandis*, *E. urophylla*, *E. globulus*, *E. camaldulensis*, *E. saligna*, *E. nitens*, *E. tereticornis* and *E. dunnii* are among the most important ones. Fonseca et al. (2010) present a more complete list of the main species planted around the world, as follows:

(a) Genus *Eucalyptus*

  – Subgenus *Symphyomyrthus*

    • Section *Transversaria*: *E. grandis*, *E. urophylla*, *E. saligna* and *E. pellita*.
    • Section *Maidenaria*: *E. globulus*, *E. nitens*, *E. dunnii*, *E. benthamii*, *E. viminalis* and *E. smithii*.
    • Section *Exsertaria*: *E. camaldulensis*, *E. tereticornis* and *E. brassiana*.

  – Subgenus *Idiogenes*: *E. cloeziana*.
  – Subgenus *Monocalyptus*: *E. pilularis*.

(b) Genus *Corymbia* (originally included in genus *Eucalyptus* as subgenus *Corymbia*): *C. citriodora*, *C. torelliana* and *C. maculata*.

All planted species have 2n = 22 chromosomes, predominantly allogamous (selfing may occur at rates between 10 % and 35 %), with hermaphrodite and protandric flowers. The most important natural pollination vectors are insects. Hybridization between species belonging to different subgenera is rare, but it does occur between species within the same subgenus and especially within the same section (Griffin et al. 1988; Assis 2000).

Table 2 Pulp productivity differences between *E.* "*urograndis*" and *E. globulus* (mean range values)

Species	Volume (m^3/ha/year)	Specific consumption (m^3/adt)	Pulp Productivity (adt/ha/year)
E. globulus	10–30	2.8–3.4	3–10
E. "*urograndis*"	30–50	3.4–4.2	7–14

The world-class benchmark for clonal forest productivity is the tropical inter-specific hybrid type between *E. grandis* and *E. urophylla* planted in Brazil and some other countries, usually known as *E.* "*urograndis*". This hybrid type was developed during the 1980s in Brazil and became a standard because it combines fast growth, increased tolerance to pests and diseases, excellent rooting ability, as well as wood quality suitable to different uses. *E.* "*urograndis*" clones are now widely planted in Brazil for pulp and paper, charcoal and solid wood production. This kind of material easily exceeds 40 m^3/ha/year in traditional planting areas.

On the other hand, *E. globulus* clonal forests grown in temperate climates, as in Portugal, Spain and Chile, are benchmarks regarding wood quality for pulp and paper production. This species presents reduced specific consumption, derived from its high basic density and high pulp yield, low lignin content and better lignin quality. Moreover, it presents excellent fiber morphology (increased fibers/g and runkel index). The combination of these characteristics makes its paper the most appreciated in different segments of the world market.

The main differences between *E.* "*urograndis*" and *E. globulus* regarding pulp productivity are presented in Table 2. It is easily seen that the *E. globulus* advantage in specific consumption is not sufficient to offset *E.* "*urograndis*" increased volume, resulting in huge differences in pulp productivity ranges. Moreover, rotation age for *E.* "*urograndis*" is 6–7 years whereas it is around 12 for *E. globulus*. These differences have important impacts in business competitiveness as affected by wood costs.

Ranges within the above "species" are due to both genetic and environmental variability observed in respective traditional plantation areas. Thus, one logical question arises from this analysis: is it possible to combine *E. globulus* wood quality with tropical species growth rates into an inter-specific hybrid clone? Because of significant adaptive distances between temperate and tropical species, this possibility has rarely been realized and many abnormalities or poor performances have been observed in hybrid progenies (Potts and Dungey 2004). However, there is some evidence of success, especially in temperate/tropical transition areas (e.g. southern Brazil), where some outstanding individuals have been found, regardless of their family behavior. Once vegetative propagation is established, value from these "transgressing recombinants" can be captured in clonal commercial plantations (Assis 2000; Bison et al. 2007).

The current success of inter-specific hybrid clones, especially *E.* "*urograndis*" in Brazil, regardless of the genetic control behind it (complementarity of additive effects, heterosis or epistasis), in combination with the huge unexplored genetic variability available between and within different species in genus *Eucalyptus* and genus *Corymbia*, and the need for increasing production goals to fulfill world

demands for wood, suggest that a multispecies hybrid development approach will be very attractive to breeding programs around the world. This approach, however, is unlikely to compete with programs dedicated to pure species in regions where the adaptation of these pure species is unquestionable and clear benefits exist from using them, e.g. the excellent wood quality obtained from highly productive *E. globulus* clonal plantations located in the maritime regions of northern Portugal and southern Chile.

A basic description of the main planted species potentialities, in accordance with the main objectives pointed out in Sect. 2, is presented in Table 3, subject to specific environmental effects. It is easily seen that countless combinations of species in different proportions might be used in a multispecies program, and could bring novel variation and complementary attributes to a synthetic population and to the specific clones within it. However a relatively limited number of core species must be carefully chosen, otherwise, the breeding program may become unmanageable.

4 Breeding Strategy

The vegetative propagation of outstanding individuals is a millenary technique extensively used in agriculture. Many important crops including potato, sugar-cane, banana, grape, etc., are produced by vegetative propagation. Its application to forests is not recent either (Zobel and Talbert 2003), but initially it was used only to preserve genotypes or to establish seed orchards, through grafting. The first attempts to root eucalypt cuttings were accomplished by French and Australians in northern Africa during the 1950s. Yet, the first commercial eucalypt clonal plantations were only established in Brazil during the 1980s, as previously mentioned.

The basic assumption underlying the use of clonal material is the possibility of fully transmitting to "offspring", by vegetative propagation, an outstanding individual genotype. This way, selection gains are maximized, once all kinds of genetic effects, additive (allelic mean effects) and non-additive (effects from interactions between alleles and between gene loci) are capitalized. Thus, any eucalypt commercial clone may be defined as a group of genetically identical plants, produced by vegetative propagation from the same common ancestor, which was selected for its all round outstanding performance.

However, the gains from clonal selection are a dead-end for an existing population at any given moment. Breeding programs, based on recurrent selection (recombination, evaluation and selection in successive generations), are required to allow the generation and deployment of new commercial clones. But breeding for clonal selection requires some extra effort, because individual performance evaluation in progeny trials is not robust enough to predict performance of the same individual as a clone over a range of different environmental and silvicultural conditions (Reis et al. 2011; White et al. 2007).

Thus, it is usually necessary to go through two evaluation and selection phases in each breeding cycle (initial evaluation/selection in progeny trials and final evaluation/selection in clonal trials), as illustrated in Fig. 2. This has a huge impact on the time

Table 3 Main *Eucalyptus* and *Corymbia* species potentialities, in accordance to Table 1. Species marked +, ++ or +++ are respectively good, very good and excellent

	Individual volume	Drought tolerance[a]	Frost tolerance[a]	Disease tolerance[a]	Rooting ability	Basic density	Pulp yield[b]	Lignin content[b, c]	Fiber morphology[b]
Tropical species									
E. grandis	+++				++	+	++	++	++
E. urophylla	++	++		++	+++	++	++	++	+
E. saligna	++		+		++	+	++	++	+
E. pellita	+	++		+++	+++	+++			
E. robusta	+	++	+	+++	+++	+++			
E.camaldulensis		+++	+	++	+++	+++			
E. tereticornis		+++	+	++	++	+++			
E. brassiana		+++			+	+++			
E. cloeziana	+		+	+		+++	++	++	
C. citriodora	+	++		+		+++	++	++	
C. torelliana	+	+++	+++	++	++	+++	+	+	
Temperate species									
E. globulus	++	++	++		+	++	+++	+++	+++
E. nitens	+++		+++			+	+	++	+++
E. dunnii	++		++	+	+	++	++	++	++
E. benthamii	+++		+++	+	+	++	++	++	+++
E. viminalis	+		+++	++	+	++	+	+	+++
E. smithii	+		++	++		+++	+++	+++	+++

[a]Some other eucalypt species to mention due to evidence regarding disease, drought or frost tolerance are *E. resinifera*, *E. longirostrata*, *E. dalrympleana*, *E. rudis*, *E. badjensis*, *E. dorrigoensis*, *E.pilularis*, *E. paniculata*, *E. microcorys*, *E. cypellocarpa and C.maculata*

[b]Species marked according to suitability to pulp and paper production (e.g. low lignin content)

[c]Species presenting high lignin content are *E. camaldulensis*, *E. tereticornis*, *E. pellita*, *E. resinifera* and *E. paniculata* (*E. paniculata* presents very high density as well)

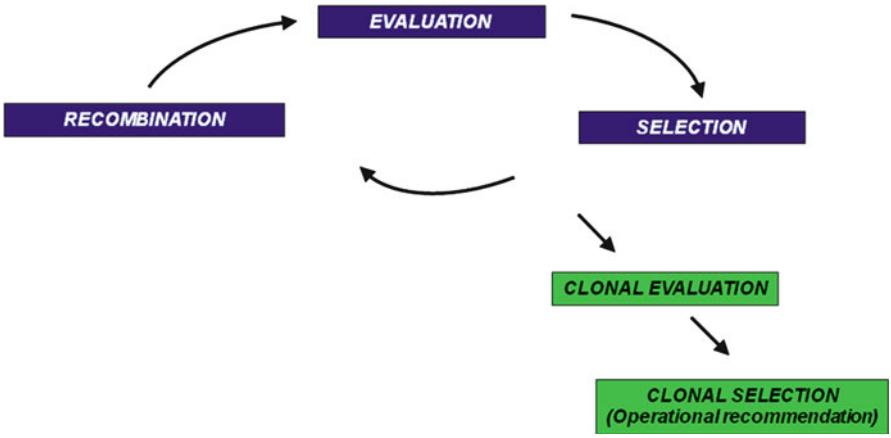

Fig. 2 Basic eucalypt breeding cycle (12–16 years from recombination to final recommendation)

required for clonal deployment. Even the most efficient programs, which make use of advanced techniques, such as flowering induction (see Sect. 5) and early selection (see Sect. 7), require about 12–16 years from initial recombination to clonal recommendation and deployment.

Some alternative approaches may enable short-cuts within this breeding cycle, but so far all of them also have limitations or still need to be proven operationally. The most relevant to mention are as follows:

- Simultaneous testing of families and clones: the vegetative propagation of seedlings as soon as they are obtained might avoid clonal tests at a later stage and save significant amounts of time. However, it is extremely difficult to multiply a representative minimum number of individuals from each progeny (see Sect. 6), so that a sufficient number of plants is available for all required trials. If a limited number of individuals from each cross are randomly selected, the variability may not be fully represented and some outstanding individuals will be lost. Moreover, only good rooters will pass on to the field trials, giving to rooting ability an excessive weight in the global selection process. Alternatively, if an effort is made to multiply a representative number of individuals from each progeny, costs rapidly increase and operational efficiency becomes a significant obstacle.
- Genome-wide selection: this relatively recent method (Meuwissen et al. 2001) relies on a genome-wide variant of marker-assisted selection (MAS) for quantitative traits. It is currently a hot topic in plant breeding and is being tested as a way to incorporate desirable alleles at many loci of small effect. In this approach, genomic breeding values (GBV) of individuals can be predicted in an experimental or "training" population, based on prediction models constructed from regressing phenotypes on whole-genome marker genotypes for several hundred to a few thousand individuals. These prediction models can then be used to estimate the GBVs of yet to be phenotyped individuals at early ages based on

marker data only (Grattapaglia and Resende 2011). Some results available for eucalypt suggest that the method might lead to elimination of progeny trials through genome based preliminary screening of seedlings, followed by cloning of only the top performing individuals for establishment in clonal field trials (Resende et al. 2012). In spite of this expectation, selection based on the phenotype evaluation is still unavoidable (Resende et al. 2011) and many standing issues still remain before the method can be widely adopted: will calibrations work both for small, short-term breeding populations and larger, long-term breeding ones? Will it work for both pure species and hybrid multi-species (multiple alleles) approaches? Will it be able to capture both additive and non-additive effects, allowing effective selection for parents and for clones? Will it be able to identify favorable transgressing recombinants, which might not be present in the training population? However, if this technology is successfully proven, it will be of great help to breeders.

– Clonal variety: this approach can be effective when there is great confidence about the performance of outstanding progenies or individual trees being tested, as compared to current commercial clones (controls). If so, clonal trials can be eliminated through the creation of a clonal variety composed of tens of outstanding individuals, which would be promptly propagated and deployed as a clonal mix into pilot plantations. From phenotypic evaluation of these pilot plantations and DNA fingerprinting of the best performers (Grattapaglia et al. 2004), purification of the clonal variety can be driven in successive steps, until an optimized mix composition of a few top clones is obtained, which can be individualized or not.

Regardless of the use of any above mentioned short-cuts, recombination of individuals selected in progeny trials according to their additive values must move forward at the same time as individuals selected from the same progeny trials according to their total genetic values are cloned for establishment in clonal field trials. This will make the breeding cycle a bit faster. Furthermore, it is mandatory to carry out parallel breeding sub-cycles (with sub-populations), every 3-4 years, to ensure periodic clonal portfolio renewal.

As previously indicated, one of the main assumptions justifying the use of clones is the maximization of selection gains by capturing all kinds of genetic effects (additive and non-additive). Although additive effects are the most important in determining total genetic variability available for growth related traits, current evidence suggests that non-additive effects also play an important role, both in tropical and temperate species (Araújo et al. 2012; Bouvet et al. 2009; Li et al. 2007; Rezende and Resende 2000). Reported non-additive genetic variance estimates for growth reach up to 80 % of additive genetic variance estimates, hence nearly doubling the total genetic variance available. More consistent information on the magnitude of heterosis and epistasis in eucalypt species and inter-specific hybrids is required, but this expected substantial amount of non-additive variance gives support to the continuing use of clonal propagation as a preferable deployment strategy for eucalypt.

There are many different efficient recurrent selection methods described in the literature (Comstock 1996), but in theory, reciprocal recurrent selection (RRS) methods and respective variants should work better for inter-specific programs and also for pure species with contrasting populations, because they allow capture of non-additive effects to some extent from one cycle to another. However, RRS methods are more time and money consuming, because they require recombination of individuals from pure species (or pure contrasting populations) based on respective "hybrid" progenies performance. As saving time and money is vital for any breeding program, and this becomes even more important in forest breeding due to the long cycles involved, in general terms the most effective approach suggested for eucalypt breeding is using simple recurrent selection for both pure species or multispecies programs (synthetic pure populations or synthetic hybrid populations, respectively) (Kerr et al. 2004; Resende and Assis 2008). In synthetic hybrid multispecies programs, the different types of hybrids available will be crossed each other, but pure species trees may be added to the group to improve specific attributes of the desired mix. As a consequence, parallel recurrent selection of pure species involved in the multispecies programs is desirable, although as a secondary priority.

It is important to draw attention to the fact that neglecting the capture of non-additive effects by choosing the simple recurrent selection method, does not mean neglecting the capture of non-additive effects during the clonal selection phase, as will be pointed out in Sect. 7.

5 Recombination

Combining short and long term breeding strategies is strongly recommended, especially for programs that are just starting, in order to provide results as soon as possible and address business needs and pressures. Short term programs involve narrowly based populations composed by elite individuals promptly available. If no information exists on additive and non-additive merit of available elite trees, recombining the best existing commercial clones is an interesting approach.

On the other hand, long term recombination must ensure maximum exploration of the population's improvement potential, which depends upon keeping an appropriate effective population size (number of unrelated individuals that effectively contribute with alleles to the breeding population along cycles). This will avoid favorable allele loss, in balance with the application of selection intensities high enough to provide satisfactory genetic gains. In general terms, regardless of the heritability and family structure concerned, keeping the effective population size between 30 and 50 along the breeding cycles will ensure the maintenance of adequate levels of genetic variability and consequent application of strong selection intensities (Namkoong et al. 1988; Resende and Barbosa 2005). But once a parent's gene pool is sufficiently sampled in progeny trials (see Sect. 6), it is not worth keeping it or the remaining seeds of its progenies for future breeding activities, as adopting this conservative practice reduces the breeding program overall efficiency and increases associated costs.

Recombination generates a new set of progenies. Mating designs used in this phase will determine family structure of progeny tests to be evaluated in the ongoing breeding cycle. Family structure is important because it will provide relevant information for decision support, namely the relatedness between individuals. This information is particularly important for estimating genetic parameters (e.g. additive and non-additive variances, heritabilities, etc.), for parent selection (keeping effective population size), and for potential clone selection (minimizing genetic vulnerability).

There are basically three types of family structures: half-sibs (HS), full-sibs (FS) and self-sibs (SS). The amount of additive and non-additive variance expressed between and within these types of families is obviously different. For example, comparisons between HS families provide a good estimate of the additive variance or general combining ability, and comparisons between FS families provide good estimates of the specific combining ability of parents, which has a non-additive variance expression component (Lynch and Walsh 1997; White et al. 2007).

The mating designs for developing recurrent selection programs and clonal selection should be analyzed in terms of four purposes: (i) efficiency in estimating the general combining ability of the parents; (ii) efficiency in identifying superior families; (iii) efficiency in selecting clones; (iv) the possibility of evaluating a large number of parents, which is desirable for enabling high selection intensity whilst maintaining adequate effective population size for future selection.

The main available designs are: polimix (half-sib families); single pair (full-sib families); disconnected factorial (half-sib families and full-sib families, simultaneously); partial diallel (half-sib families and full-sib families, simultaneously). The relative efficiency of the different designs for satisfying each above mentioned purpose was studied by Resende and Barbosa (2005), and the major conclusions were as follows:

- Polimix is the best design for estimation of general combining ability of parents (purpose i), as it maximizes efficiency in terms of heritability and accuracy. Disconnected factorial designs become almost as efficient as polimix when three or four crosses by parent are accomplishable.
- A single pair disconnected factorial or a partial diallel design with three or four crosses per parent is the best option for identifying superior families and selecting new clones (purposes ii and iii).
- The possibility of evaluating a large number of parents (purpose iv) should be considered by fixing the maximum number of crosses that can be afforded to be generated and evaluated in the field. For example, by limiting to 200 the total number of crosses or families that one can generate and evaluate in the field, the following number of parents can be used: 400 for single pair mating, 200 for polimix and 50 for disconnected factorial or partial diallel designs with four crosses by parent. Considering the recombination of the 30 best parents, it can be seen that selection intensities (in terms of the selected proportion) in the disconnected factorial and partial diallel designs are very high (60 %) providing low genetic gains.

It can be observed that none of the designs fulfill all the purposes in the most efficient way. For this reason, when good estimates of population genetic parameters are already available, i.e. in mature programs, it may be a good option to use polimix approaches, because of the significant gain in time and labor that is obtained. To complement this design, specific single pair crosses can be made emphasizing the best parents, i.e. the ones with highest breeding values, aiming at minimizing potential polimix disadvantages related to genetic sampling and selective accuracy of families and clones.

One major challenge faced by breeders during the recombination phase is getting the parents to flower as early and evenly as possible to enable the controlled pollinations. Significant advances have been achieved in recent decades, reducing the time required for flowering from the normal 4–8 years to 2–4 years (Fonseca et al. 2010; Griffin et al. 1993; Hasan and Reid 1995; Moncur and Hasan 1994). The methods combine grafting, water/nutrition management and hormonal treatments, both in the field and greenhouse (Fig. 3). But these methods are still very species and genotype dependent and further research into the physiology of flowering in eucalypt is required, as improvements in this area offer large opportunities for reducing breeding cycles.

Important advances have also been achieved in controlled pollination methods. Because eucalypt flowers are hermaphrodite (male and female structures are present in the same flower) and protrandric (the male structures mature prior to the female) complex manipulation (emasculation/isolation/pollination/isolation) is required for traditional controlled pollination. These operations are time consuming and negatively impact time, labor and seed productivity. However, in the late 1990s an important advance was made with the OSP (One Stop Pollination) technique, developed for *E. globulus* in Chile (Harbard et al. 2000). This method makes it possible to combine emasculation and pollination in a single operation, by cutting the female stylus tip just after emasculation and immediately applying pollen on top of it (Fig. 4). Some years later, further advances were achieved by Assis et al. (2005), who developed AIP (Artificially Induced Protoginy), with the purpose of avoiding emasculation for improved operational efficiency. The method consists of making the stylus receptive to external pollen prior to flower opening, by cutting the flower bud tip in the pre-anthesis phase (Fig. 4).

Eucalypt species present huge variation regarding flower size and this must be taken into account in inter-specific crosses. When crossing large-flowered species (e.g. *E. globulus*) with small-flowered ones (e.g. *E. nitens*), usually the former must be used as male parents because otherwise pollen tubes will not be robust enough to fertilize ovules.

At this point a brief discussion about the potential for genetic transformation is required. For breeders, genetic transformation is often regarded as no more than an alternative recombination system for "adding" a specific trait or trait expression controlled by one or few genes which cannot normally be found within the breeding population, into top performing clones. The reason behind this view is that the overall performance of a commercial clone in relation to adaptability (individual growth, survival) and product suitability (wood quality), is determined by thousands of

Fig. 3 Eucalypt recombination phase in greenhouse

Fig. 4 OSP (*left*) and AIP (*right*) controlled pollination techniques

genes and their interactions, which were naturally arranged in a very specific way to provide an overall favorable phenotype. Therefore, based on quantitative genetics principles and the most recent studies on QTL mapping and genomic selection efficiency in trees (Grattapaglia and Kirst 2008; Grattapaglia et al. 2009; Grattapaglia and Resende 2011; Resende et al. 2012), a single gene effect most certainly will not turn a bad performer into a good one. Detecting genes capable of providing a substantial

phenotypic benefit has often been frustrating, with many reported QTLs proving to have overestimated or inconsistent effects.

Moreover, as previously noted, recombination is only one in many phases of a breeding cycle. Using genetic transformation as an alternative recombination method for eucalypt is itself a challenging approach because significant technical bottlenecks related to genotype dependent regeneration/transformation protocols and expression stability persist, and the most amenable clones might not necessarily be the best performers available. Even if success is obtained in generating many transgenic lines of a specific clone, subsequent phases of field evaluation and selection are required. These field tests and eventual deployment of a transgenic clone will certainly take much more money and time than usual, due to regulatory, biosafety, intellectual property, certification and public acceptance issues involved.

However, genetic modification of trees is taking place in many countries. This effort will hopefully bring about knowledge and progress in sustainable forest production, especially for qualitative traits. But transgenic eucalypt clones are unlikely to be deployed commercially in much of the world for some more years, largely due to their cost and regulatory issues, greatly limiting the benefits that this technology is supposed to be capable of providing, as detailed in the related chapters of this book.

6 Evaluation

As previously mentioned, usually there are two evaluation steps in each breeding cycle, namely progeny trials and clonal trials (Fig. 2). In progeny trials seedlings obtained from the recombination phase are directly established in field trials. At this stage each tree represents a single genotype in the field. In clonal trials, the best trees identified in progeny trials are cloned and rooted cuttings are established in field trials over different locations. The objective of progeny and clonal trials is providing reliable data for the subsequent selection phases. In this context, it is important to take the following aspects into account:

– Locations: the number of locations where field trials must be established is as large as the number of different environmental conditions that have to be covered for the projected geographical extent of the breeding programs' ambitions. The larger this coverage is, the more contrasting will be the environmental parameters which in turn will cause complex type genotype × environment (G × E) interactions, with significant changes in progeny or clone ranking over locations. In the initial cycles, the identification of these contrasting areas depends upon soil (depth, stoniness, texture, fertility, etc.) and climate (rainfall, temperature, etc.) characterized as impacting forest performance. However, in more advanced cycles, the number of locations will be defined by a thorough analysis of G × E interactions over locations where the initial tests were established. At this stage, it is usually possible to group regions originally classified as contrasting based on soil and climate information, but which in fact, are

similar from a G × E perspective (Rezende and Resende 2001). Nevertheless, it is advisable for clonal trials and especially when only two or three contrasting grouped regions exist, to have an extra test in each macro-region, to ensure against the loss of genetic material and information if a natural or operational disaster occurs, such as fire, wind or mistaken harvest.

– Experimental procedures: accurate evaluation of the genetic merit of an individual tree, a family or a clone requires the application of appropriate experimental procedures which maximize environmental control and genetic material representativeness. In this regard, the most relevant recommendations to breeders are as follows:

  - Incomplete block designs (lattices) are recommended for eucalypt breeding field trials because large numbers of progenies or clones are usually tested (White et al. 2007).
  - Many plot sizes have been studied in forest species, but the current consensus is that the use of Single Tree Plots (STP) is the best approach, both for progeny and clonal trials, as it provides higher experimental precision (larger number of repetitions and smaller and more homogeneous blocks) (White et al. 2007). Nevertheless, STP requires additional care in tests planning, establishing, controlling and taking measurements.
  - Accuracy is also affected by the total number of individuals per family in progeny trials, and by the number of ramets per clone in clonal trials, at each location and among all locations. These numbers may vary depending on the heritability of the trait and the genetic structure of the material under evaluation (HS families, FS families, S1 families, clones). The studies carried out by Resende and Barbosa (2005) suggest that acceptable accuracy and variability levels can be achieved in progeny trials regardless the trait heritability and the family structure, by using a minimum of 100 individuals per progeny along all locations and a minimum of 30 individuals per progeny in each location. The genetic representativeness or effective size of a family is relevant to maximize selection accuracy of potential clones in progeny trials. The effective size (Ne) of a FS and a HS family is respectively given by $Ne = (2n)/(n+1)$ and $Ne = (4n)/n + 3$, where n is the number of individuals per family. Simulations showed that n = 100 provide 99 % and 97 % of representativeness of a FS family and a HS family respectively. So, including more individuals than this will barely add to the variability of the sample, as it is already sufficient to include the best individuals of FS and even HS families. In the clonal trials, acceptable accuracy levels are achieved by using between 20 and 30 ramets per clone per location.
  - Controls must be included in all field trials. One or two stable clones from the first recommendations shall be used as “permanent controls” in every progeny or clonal trial along cycles, allowing comparisons between materials established in different locations, ages or cycles and also the estimation of cumulative gains over time. In addition, current commercial clones should be used as controls in every planned progeny and clonal trial, to provide a reliable estimate of the candidates merit as compared to the best current planting material.

- Silvicultural practices as applied to field trials, including soil preparation, fertilization and weed control, must be similar to those used in large scale operational forestry, to ensure the suitability of the recommended clones to the standard conditions. Nevertheless, special attention should be given to environmental control, so that experimental precision is not compromised by environmental variation inside blocks or repetitions.

– Measurements: phenotyping progeny and clonal trials properly is a crucial step in any breeding program. Intensity, timing and type of measurements will largely depend upon the resources available. A sequential approach which privileges growth traits has been unavoidable especially due to the lack of non-destructive, efficient, cheap and large scale evaluation technologies for traits related to wood quality, rooting, and tolerance to pests, diseases and abiotic stress. Survival should be evaluated in the very early stages of field trials and for all individuals. Growth traits (diameter, height, health/physiological condition) should be evaluated at least from near to half the rotation age, allowing resources to be saved as well as early selection. Non-destructive large scale wood quality evaluation should, as far as possible, be applied at the same intensity as it is for growth traits, but this is difficult in practice because only a limited number of technologies are currently available for that purpose. Pilodyn has been used traditionally for basic density evaluation, but it presents low accuracy and its efficiency is limited to ranking purposes. Near-infrared spectroscopy (NIRS) from wood increment cores has been used successfully for wood chemical evaluation, including pulp yield and lignin content, but it requires significant investment in calibration models and its efficiency is also limited to ranking purposes (Apiolaza 2009; Downes et al 1997; Raymond and Schimleck 2002; Schimleck et al. 2006). More sophisticated methods such as *Silviscan*™, combine image and x-ray analysis, and provide accurate and detailed wood characterization (Buksnowitz et al. 2008; Raymond 2002; Wu et al. 2009; Wynne and Nelson 2006; Yang et al. 2006), but are very expensive for evaluating hundreds of trees every year, as required in regular breeding programs. Thus, a final complete and rigorous assessment of wood volume (including taper and straightness), wood quality, sprouting and rooting ability, nutritional efficiency, tolerance to pests and diseases, drought and frost, both in the field and laboratory, can only be accomplished for a limited number of pre-selected clones (20–50) in the final stages of clonal trials.

7 Selection

Breeding for clonal deployment involves three selection approaches (Fig. 2): selecting parents for recombination based on progeny trials evaluation; selecting candidate clones for clonal trials based on progeny trials evaluation; and selecting operational clones based on clonal trials evaluation. In every situation the most effective selection procedure is estimating the genetic value of individuals via BLUP (Best Linear

Unbiased Prediction). This statistical procedure maximizes selection accuracy by better separating environmental effects from genetic values (Henderson 1984). Furthermore, the method does not require balanced data, it accounts for trees genealogy (allowing prediction of additive and non-additive value), and combines all the information available including different locations, years, mating designs, field designs and breeding generations.

The whole process of genetic evaluation for selection purposes involves the use of the so called mixed model methodology in which the fitted model encompasses random effects such as genetic values and fixed effects such as locations and years. Treating genetic values as random effects leads to a better prediction of the genetic value of the candidates, which turn out to be both unbiased and with minimum prediction error variance (consequently more accurate). Besides correcting for environmental effects, the methodology takes into account the quantity and distribution of the information associated with each individual and also the heritability of the trait. As such, the procedure enables the comparison of individuals across time and space, enabling genetic gain to be maximized by the selection process.

This approach assumes knowledge of many genetic parameters as input data. As genetic parameters are not really known, they must be estimated by the most precise method available, which is the Restricted Maximum Likelihood (REML) procedure (Patterson and Thompson 1971). The REML method is superior to traditional Analysis of Variance (ANOVA) when the data is unbalanced and the designs are non-orthogonal. It takes into account the genetic relationship matrix to specify all the possible genetic relationships in the data set, and these relationships are then used to produce unbiased precise estimates of variance components. According to Searle et al. (1992) ANOVA estimates of variance components are unbiased only under the following conditions: data are balanced, meaning that there is 100 % survival and all families are planted in equal numbers of blocks at all test sites; parents are unselected, non-related and come from the same generation; and parents are inter-mated in a single, structured mating design (such as a factorial or diallel) to produce a single type of collateral relatives such as full-sib families.

By using REML estimates when fitting the mixed model, the so called empirical BLUP predictions are produced. Frequently, the REML variance components estimation and the BLUP prediction are performed simultaneously by the REML/BLUP procedure which corrects for environmental effects, estimates genetic parameters and produces the individual BLUP predictions at the same time. Different software, such as ASREML (Gilmour et al. 2002), can be used to perform this kind of analysis. Given the complexity involved, careful consistency analysis of data and results is recommended to software adoption and use at operational scales.

The BLUP approach is at least equal and often superior to other selection methods such as simple phenotypic selection (mass selection), between and within family selection and combined family – within family selection. The results diverge as the heritability of the trait under selection diminishes due to poor experimental precision, and the data becomes unbalanced. With BLUP there is a tendency to choose as winners the better tested candidates, while the opposite is true for classical methods. In other words BLUP penalizes the least tested genetic materials, which is a desirable

feature. For mass selection, each tree's phenotypic measurements are the only data used to predict the tree's genetic value for each trait, while BLUP entails all the information genetically linked to each selection candidate. However, uniform stands with low levels of environmental noise and high levels of trait expression give higher heritabilities, and, therefore, the genetic values are less regressed for these stands than others. In these situations, simpler selection methods, such as mass selection tends to be as efficient as BLUP selection (Reis et al. 2011; White et al. 2007).

Parental selection for recombination must be based on estimated additive genetic values while clonal selection must be based on estimated total genetic (or genotypic) values. The merits diverge and so do the rankings from these predictions, as the degree of allelic dominance and epistasis increases. Thus, for traits with some level of dominance such as growth and survival, top parents may not be top clones and vice-versa. Even if some high positive ranking correlations are observed for the whole population under evaluation, important changes may occur in the best elite material, which will impact the final program results. Moreover, the strength of the G×E interactions differs for the additive and total genetic values, the latter effect being the stronger (Araújo et al. 2012; Rezende and Resende 2001). This will be particularly relevant to the final selection of clones for operational deployment. Such features emphasize the need for thorough planning of the linear model to enable the BLUP prediction for the both additive and total genetic values.

While many traits are taken into account when selecting genetic material (Table 1), selection can be applied according to three different approaches: selection indices, tandem selection and independent culling levels (White et al. 2007). In practice combining selection indices and independent culling levels is often necessary for estimating the ultimate value of candidates (e.g. pulp productivity, as an index estimated from volume, basic density and pulp yield values, combined with minimum 70 % rooting). As previously mentioned, due to practical issues related to evaluation costs and time, a sequential approach is frequently used, meaning only a smaller group of pre-selected clones for volume and survival are evaluated/selected for wood quality traits, rooting ability, etc. Selection indices which consider the most relevant traits as affected by their economic importance, heritability and genetic correlations are useful when the relative economic importance of the measured traits can be accurately evaluated, and when these relative weights are expected to be stable across the time period that the selected genotypes are being deployed and bred. In general, they become more useful when there is no expected constraint to the long term wood supply, making the economic value of the wood more important than its existence in terms of productivity.

If breeding values are predicted for each trait separately (i.e. a separate BLUP analysis for each measured trait), then selection indices can be easily formed to combine data from all the traits under analysis into a single value for each candidate. The validity of this multiple-step approach rests on a property that the BLUP of any linear combination of traits is equal to that linear combination of the BLUP predicted values of the individual traits (White and Hodge 1989). So, for selection indices that are linear functions, e.g. pulp productivity, the indices values provide the best unbiased predictions of that linear combination of traits.

Indices combining more traits are capable of identifying candidates that are above average for that combination of traits, but not outstanding for any one. Therefore, when selecting parents for recombination it is important to include some trees that are outstanding for individual traits, even if their indices values are smaller than other candidates. This allows the capture of superior alleles in future breeding cycles, especially by crossing selections that are truly excellent for different sets of traits.

Some other issues to be considered while using BLUP are:

- Application requires compatible hardware processing capacity.
- It is important that an experienced person conducts the analysis, making sure data is clean and consistent prior to analysis.
- The quality of predictions can be improved by reducing the experimental error through environmental co-variables and spatial analysis. Incorporation of coordinates concerning the position of each experimental data point into mixed model analyses enables spatial analysis, and has the potential to reduce experimental error and increase heritability and gain from selection. However, spatial analysis is not currently widely used in forest genetic data analysis, as long as the trials are usually established according to adequate experimental designs. In such cases there is no advantage from spatial analysis as the models tend to be overfitted. Examples of complex models including spatial analysis in forest trees are reported by Resende and Thompson (2004).
- Taking into account any missing plots around each tree may also help reducing experimental error. Such numbers can be fitted as co-variables simultaneously to the REML estimation and BLUP prediction. Competition effects at the environmental and genetic levels can be also fitted to improve the accuracy of the REML/BLUP procedure (Resende et al. 2005).

After predicting the additive genetic values of candidate parents (recombination) some restrictions may still be necessary regarding the family contribution to the selected group of individuals. To do this it is necessary to determine the amount of relatedness to be permitted in the selected group. The concept of effective population size is important in this connection (see recommendations in Sects. 5 and 6). For selecting the candidate clones to be tested in clonal trials, the relatedness issue is less relevant but not negligible. Some care must be taken to avoid a high probability of deploying too many related materials as operational clones (see Sect. 8).

In any situation, an early selection approach, as applied both to progeny and clonal trials, is strongly recommended to reduce the generation time and maximize genetic gain per unit time (Borralho et al 1992; Osorio 1999; Rezende et al. 1994; Stackpole et al. 2010). Despite the lack of consistent scientific information on this matter, selection near to half the rotation age is an efficient and safe approach for the most important eucalypt commercial species and traits (White et al. 2007). Practical experience has shown that potential loss of some superior individuals may occur, but this negative impact is clearly offset by gains per unit time. However, it is always advisable to confirm the performance of selected clones at rotation age and also after harvest, in coppice trials.

8 Deployment

As previously mentioned, the use of clones is justified by the possibility of fully maintaining all kinds of genetic effects expressed in the founding ancestor, both additive and non-additive, in the plantation material. In eucalypt non-additive genetic effects for growth traits seems to be expressive, hence maximizing the total genetic variance available (Araújo et al. 2012; Bouvet et al. 2009; Rezende and Resende 2000; White et al. 2007). This expected importance of non-additive variance supports the use of clonal propagation as the preferred means of deploying improved eucalypt. In other words, gains associated with clonal forestry would be expected to be larger than if seedlings were used from an orchard, both based on the same original genotypes. Moreover, favorable "transgressive segregants" or "correlation breakers" trees, which might be neglected in a seedling population can be immortalized as clones.

So long as non-additive genetic effects are not being captured in the seeds from a seed orchard, but outstanding improved seed varieties are available, even better plantations can be established with the best clones selected from such varieties. Furthermore, there is evidence from a number of leading forestry companies that on average, commercial clonal plantations have historically provided at least 25 % higher realized gain in volume as compared to seedling plantations from the same breeding population, managed in the same locations and with the same silvicultural care. The use of clones also enables greater homogeneity of the wood products, which is no small advantage, and can capture the special attributes of a single tree, such as tolerance to diseases, drought or frost. Thus, in practical terms, clonal plantations present better productivity and uniformity, also resulting in improved efficiency in forest management.

Clonal forests may not represent the best option in every situation however. Improved seedlings may be a better option for plantations to be established in locations presenting specific edapho-climatic conditions, such as regular water deficits above the normal adaptation limits of species, very shallow or swampy soils, etc., for which suitable clones have not yet been sufficiently evaluated nor accurately selected. Therefore, when there is no clear evidence of adaptive superiority of available clones, it is usually a safer option to use batches of improved seed, provided that they were broadly developed for the target region. This is because the genetic variability and buffering ability of the seed population will help minimize any potential losses caused by biotic or abiotic stresses.

One frequent question breeders need to answer is "what is the ideal number of clones to be used in operational nurseries?" This number should not be as large as to reduce genetic gains or to cause operational constraints in the nursery. Nor yet should the number be as small as to increase the risk of genetic vulnerability or the risk of overall bad performance originated in mistaken selection.

Experience has shown that nurseries should operate with five to ten operational clones. These clones should be unrelated, although some slight relatedness between two or maximum three clones out of ten is often acceptable or unavoidable.

Stable clones are preferred because they allow gains in the operational efficiency of the production nursery due to the larger scale of their production and, if chosen judiciously, they can represent a sufficiently generic option for use in most situations. In short, they make the nursery managers life easier. Yet, given that G × E interaction can be expected to some extent (Sect. 7), clear good performers under specific environmental conditions should not be discarded, at the risk of losing potential gains in the shorter or longer term.

Another important issue is the timing of introducing any new clones into operational nursery production. Recommending new clones every year can be disruptive to the nursery planning and routine. It is important to allow time for learning about the propagation performance of new clones and to find solutions for overcoming any difficulties that might be encountered, such as water management, nutrition and disease control.

Breeders would rather recommend two to five top candidates for pre-commercial pilot plantations (at least 50 ha per year) every 3 or 4 years. This way periodic clonal portfolio renewal is built in accordance to the sub-cycles planned in the breeding strategy (Sect. 4). A good general approach, at any given moment, is to have nearly half the operational clones with a proven track record in commercial plantations accounting for 70–80 % of nursery production. The other half should consist of newly recommended clones, accounting for 20–30 % of nursery production.

Within the 3 or 4 years following the recommendation of a new set of clones, their operational performance shall be monitored in pilot plantations and in the nursery. Some of them will be confirmed as operational clones and will have their production expanded, replacing obsolete material. Some others will fall however, but when this happens other sets will be undergoing introduction to the nursery. Breeders must be deeply involved in this post recommendation monitoring phase, working very closely with operational foresters.

The procedures suggested above usually provide long term sustainable gains, rendering the large scale clonal production of tree material in nurseries and forests feasible, and also avoiding the risks of genetic vulnerability. Genetic vulnerability, especially to pests and diseases, is often assumed as a disadvantage of large scale clonal forests, but this potential problem is mostly overestimated. Factors that mitigate these risks are as follows:

– By recommending two to five unrelated top new clones every 3 or 4 years, a full replacement of the clonal nursery portfolio will happen every 10–15 years. This means that it is unlikely that any one specific clone will be in use in a specific area for more than two operational rotations.
– Simulations with the number of clones needed for managing risks in clonal forestry carried out by Bishir and Roberds (1999), suggested that the level of risk is unlikely to change significantly after the number of clones used in the whole plantation area exceeds 30–40. If about ten clones are constantly used in nurseries and these are completely replaced every 10–15 years, 30 or 40 different clones will have contributed to the total planted area after 30 or 40 years, which seems to be acceptable (one different clone per year on average).

Furthermore, this arrangement provides that any damage will not be significant if any one clone proves to be problematic, because its contribution to the planted area will never be predominant. Yet, problematic clones can be rapidly taken out of production as soon as difficulties are recognized and many alternatives will be immediately available.

– Local plantations are planned so as to use many different operational clones. A plantation will usually have many blocks, each one ranging from 5 to 50 ha, at a rate of only one clone per block, but having many blocks with different clones in the same area. This mosaic structure further reduces risks.
– Susceptibility to pests and diseases is determined more by species and provenances than by clones (e.g. Gonipterus beetle and *E. globulus*). Moreover, some pests/diseases are opportunistic and occur due to poor adaptation of planting material to environmental conditions or suboptimal silvicultural practices employed.
– Breeding programs are usually established with broadly based populations providing high levels of genetic diversity (including tolerance to biotic and abiotic stresses) and, as stated in Sect. 7, specific screening for pest and disease tolerance should occur in the final selection steps, prior to operational recommendation. In fact, it is important to understand that most often clonal deployment is the solution for overcoming problems with pests and diseases because it allows large scale production of tolerant individual trees. Overcoming the notorious problems caused by canker disease in Brazil during the 70s was a landmark example and irrefutable evidence to this end (Alfenas et al. 1983, 2009; van Heerden et al. 2005).
– In the last decades, severe genetic vulnerability problems related to the use of clones in forestry or agriculture were not widely reported. It should be remembered that cultivars belonging to autogamous (self-pollinating) species such as rice, soybeans, common beans and wheat, behave just like clones, since all the plants of a given cultivar are genetically identical. The same happens with simple hybrid maize cultivars which originate from pure homozygous lines. Yet, every year millions of hectares of a restricted number of cultivars of these crop species are planted around the world.

Large scale propagation methods have evolved impressively since commercial plantations were first established in the mid 1980s in Brazil. The first operational method was based on the use of macro-cuttings (two-leafed cuttings, 8–12 cm long) from sprouts collected from the stumps of early harvested commercial plantations or clonal banks. Because rooting ability declines with ageing, these commercial plantations were usually harvested prior to half the rotation age. These methods required large areas for producing the necessary numbers of macro-cuttings (usually 1 ha of clonal bank for 100 ha of planned forest plantations). Macro-cuttings also required hormonal treatment (IBA) for rooting induction.

In the late 1980s and early 1990s macro-cutting origin moved from clonal banks to clonal gardens, which were designed and managed with the specific objective of producing cuttings. In this system spacing is reduced and intensive management

Fig. 5 *E. globulus* clonal garden and macro-rooted-cutting, Viveiros Aliança, Portucel Group, Portugal

(topping, pruning, nutrition, etc.) is used to retard plant maturation allowing cuttings production from more juvenile material. Better rooting performance was achieved with this method, but the cutting still requires IBA treatment. Moreover, the area required for cutting production was reduced by 90 %. This approach is still used successfully in different countries, with different eucalypt species (Fig. 5).

Although clonal gardens brought important benefits, they have some well known limitations: silvicultural practices are not homogeneous and are strongly affected by climatic conditions; the success rate with macro-cuttings is very genotype dependent; and the architecture of their root system is often not so good.

During the 1990s in Brazil, Assis (2011) developed a new propagation method aimed at minimizing these problems. The method is based on the use of mini-cuttings (two or four leafed cuttings, 4–8 cm long) from sprouts collected in mini-stumps managed in mini-clonal gardens (Fig. 6). This system, although more labor intensive, is currently the mostly widely used in the world, especially for tropical species. Its main advantages are:

– Mini-clonal gardens are much smaller. They are established under homogeneous and protected conditions, allowing more precise control of water, temperature and nutrients, which positively affect the cuttings productivity as well as rooting ability and health.
– Mini-cuttings are much more juvenile, which promotes significant gains in rooting ability (often eliminating the need for IBA treatments), reduced nursery life-cycle and more balanced root system quality.

Choosing between macro and mini-cuttings is not always easy. The advantages of mini-cuttings might suggest this as the natural option. Usually it is, but macro-cuttings or mixed systems (macro + mini) can be justified in some specific situations, when species are poor rooters, or when there is a significant interaction between clones and cutting type, or when the demand for plants is limited. In summary, one size does not fit all and a common sense approach always helps.

Micro-propagation is another system with huge potential, due to the reinforced juvenility of the material produced via tissue culture. Yet, no large scale feasible method is currently available for eucalypt due to economic constraints, despite being used for the maintenance of germplasm and the rescue of rare or valuable genotypes for incorporation into the breeding programs. While the relevance of micro-propagation for large scale production of eucalypt plants is still unproved, it is advisable, whenever possible, to establish macro or mini-clonal gardens using micro-propagated plants. Practical evidence exists that rooted-cuttings produced from micro-propagated stumps produce plants with better juvenile attributes and performance, including rooting ability, precocity and plant quality. Being so, starting tissue cultures of new clones when they are just recommended for pilot operational plantations may be a good strategy because it allows the delivery of very juvenile material for macro or mini-clonal gardens establishment at a later stage, when some of them are definitely chosen for large scale production.

Regardless of the system in place, the physiology of clonal propagation is very complex. The operational efficiency of any eucalypt clonal nursery is highly sensitive to numerous variables, including physiological aspects (genotype rooting ability, cutting juvenility as affected by the maturation of the original stump, cutting size and nutritional status, C-effects such as topophysis, cyclophysis and periphysis, etc.) (White et al. 2007), substrate quality, container type and quality, water quality and management, fertilization, climatic control, sanitary control, operator effects, infra-structure, automation, etc. These variables will interact one with each other in multiple combinations throughout the plant production process (cutting production and collection, planting, rooting, elongation, acclimation, quality control, expedition), thus affecting the final nursery success rates. Much practical knowledge about

Fig. 6 *E.* "*urograndis*" mini-clonal garden and mini-rooted-cuttings, Brazil

these matters has been generated in the last decades and much more is expected in the forthcoming years. Nevertheless, such a complex theme cannot be more than superficially addressed in this article. Further information can be found in Alfenas et al. (2009).

Finally, it is very important to keep track of the genetic identity of operational clones. Contamination may occur at various stages of the process, from the development phase to gradual mixing in nursery operations, oversight of which requires regular sampling, followed by DNA fingerprinting using molecular markers where available (Grattapaglia and Kirst 2008). This kind of analysis can be easily and cheaply carried out these days, bringing important quality control to this long term, technically intensive and usually highly profitable integrated forestry investment chain.

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References

ABRAF (2011) Anuário estatístico da ABRAF 2011, ano base 2010. ABRAF, Brasília, Brazil, p 130

Alfenas AC, Jeng R, Hubbes M (1983) Virulence of *Cryphonectria cubensis* on *Eucalyptus* species differing in resistance. Eur J For Pathol 13:197–205

Alfenas AC, Zauza EAV, Mafia RG, Assis TF (2009) Clonagem e doenças do eucalipto, 2nd edn. UFV, Brazil, p 500

Apiolaza LA (2009) Very early selection for solid wood quality: screening for early winners. Ann For Sci 66:6

Araujo JA, Borralho NMG, Dehon G (2012) The importance and type of non-additive genetic effects for growth in *Eucalyptus globulus*. Tree Genet Genomes 8:327–337

Assis TF (2000) Production and use of *Eucalyptus* hybrids for industrial purposes. In: Dungey HS, Dieters MJ. Nikles DG (eds) Hybrid breeding and genetics of forest trees: proceedings of QFRI/CRCSPF symposium, Australia, pp 63–74

Assis TF (2011) Hybrids and mini-cutting: a powerful combination that has revolutionized the *Eucalyptus* clonal forestry. BMC Proc 5:I18

Assis TF, Resende MDV (2011) Genetic improvement of forest tree species. Crop Breed Appl Biotechnol 11:44–49

Assis TF, Warburton P, Harwood C (2005) Artificially induced protogyny: an advance in the controlled pollination of *Eucalyptus*. Aust For 68:27–33

Bishir J, Roberds JH (1999) On numbers of clones needed for managing risks in clonal forestry. For Genet 6(3):149–155

Bison O, Ramalho MAP, Rezende GDSP, Aguiar AM, Resende MDV (2007) Combining ability of elite clones of *Eucalyptus grandis* and *Eucalyptus urophylla* with *Eucalyptus globulus*. Genet Mol Biol 30:417–422

Boland DJ, Brooker MIH, Chippendale GM, Hall N, Hyland BPM, Johnston RD, Kleinig DA, McDonald MW, Turner JD (2006) Forest trees of Australia, 5th edn. CSIRO, Australia, 736 pp

Borralho NMG, Almeida MH, Potts BM (2008) O melhoramento do eucalipto em Portugal. In: Alves AM, Pereira JS, Silva JMN (eds) Impactes ambientais do eucaliptal em Portugal. ISAPress, Portugal, pp 61–110

Borralho NMG, Cotterill PP, Kanowski PJ (1992) Genetic control of growth of *Eucalyptus globulus* in Portugal. II. Efficiencies of early selection. Silvae Genet 41(2):70–77
Borralho NMG, Cotterill PP, Kanowski PJ (1993) Breeding objectives for pulp production of *Eucalyptus globulus* under different industrial cost structures. Can J For Res 23:648–656
Bouvet JM, Saya A, Vigneron PH (2009) Trends in additive, dominance and environmental effects with age for growth traits in *Eucalyptus* hybrid populations. Euphytica 165:35–54
Buksnowitz C, Muller U, Evans R, Teischinger A, Grabner M (2008) The potential of SilviScan's X-ray diffractometry method for the rapid assessment of spiral grain in softwood, evaluated by goniometric measurements. Wood Sci Technol 42:95–102
Comstock RE (1996) Quantitative genetics with special reference to plant and animal breeding. Iowa State University Press, USA, p 421
Costa e Silva J, Borralho N, Araújo J, Vaillancourt R, Potts B (2009) Genetic parameters for growth, wood density and pulp yield in *Eucalyptus globulus*. Tree Genet Genomes 5:291–305
Costa e Silva J, Potts B, Dutkowski GW (2006) Genotype by environment interaction for growth of *Eucalyptus globulus* in Australia. Tree Genet Genomes 2:61–75
Downes GM, Hudson IL, Raymond CA, Dean GH, Michell AJ, Schimleck LR, Evans R, Muneri A (1997) Sampling plantation eucalypts for wood and fibre properties. CSIRO, Australia, 144 pp
Drew DM, Downes GM, Evans R (2011) Short-term growth responses and associated wood density fluctuations in variously irrigated *Eucalyptus globulus*. Trees 25:153–161
Drew DM, Downes GM, O'Grady AP, Read J, Worledge D (2009) High resolution temporal variation in wood properties in irrigated and non-irrigated *Eucalyptus globulus*. Ann For Sci 66:406
Eldridge K, Davidson J, Hardwood C, van Wyk G (1993) Eucalypt domestication and breeding. Clarendon, UK, p 288
FAO (2010) Global forest resources assessment, 2010 – main report. FAO, Rome, Italy, p 378
FAO (2011) State of the world's forests, 2011. FAO, Rome, Italy, p 179
Fenning TM, Gershenzon J (2002) Where will the wood come from? Plantation forestry and a role for biotechnology. Trends Biotechnol 20(7):291–296
Fonseca SM, Resende MDV, Alfenas AC, Guimarães LMS, Assis TF, Grattapaglia D (2010) Manual prático de melhoramento genetico de eucalipto. UFV, Brazil, 192 pp
Gilmour AR, Cullis BR, Welham SJ, Thompson R (2002) ASReml reference manual. Release 1.0. 2 ed. Harpenden: Biomathematics and Statistics Department – Rothamsted Research, UK, p 187
Grattapaglia D, Kirst M (2008) *Eucalyptus* applied genomics: from gene sequences to breeding tools. New Phytol 179:911–929
Grattapaglia D, Plomion C, Kirst M, Sederoff RR (2009) Genomics of growth traits in forest trees. Curr Opin Plant Biol 12:148–156
Grattapaglia D, Resende MDR (2011) Genomic selection in forest tree breeding. Tree Genet Genomes 7:241–255
Grattapaglia D, Ribeiro VJ, Rezende GDSP (2004) Retrospective selection of elite parent trees using paternity testing with microsatellite markers: an alternative short term breeding tactic for *Eucalyptus*. Theor Appl Genet 109(1):192–199
Grattapaglia D, Vaillancourt R, Shepherd M, Thumma B, Foley W, Külheim C, Potts B, Myburg A (2012) Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. Tree Genet Genomes 1–46. doi:10.1007
Greaves BL, Borralho NMG (1996) The influence of basic density and pulp yield on the cost of eucalypt Kraft pulping: a theoretical model for tree breeding. Appita J 49:90–95
Griffin AR, Burgess IP, Wolf L (1988) Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'Herit – a review. Aust J Bot 36:41–66
Griffin AR, Whiteman P, Rudge T, Burgess IP, Moncur M (1993) Effect of paclobutrazol on flower-Bud production and vegetative growth in 2 species of *Eucalyptus*. Can J For Res 23:640–647

Harbard JL, Griffin R, Espejo JE, Centurion C, Russel J (2000) "One stop pollination": a new technology developed by Shell Forestry technology unit. In: Dungey HS, Dieters MJ. Nikles DG (eds) Hybrid Breeding and Genetics of Forest Trees: Proceedings of QFRI/CRCSPF Symposium, Department of Primary Industries, Brisbane, Australia, pp 430–434

Hasan O, Reid JB (1995) Reduction of generation time in *Eucalyptus globulus*. Plant Growth Regul 17:53–60

Henderson CR (1984) Aplications of linear models in animal breeding. University of Guelph, Canada, p 462

Kerr RJ, Dieters MJ, Tier B (2004) Simulation of the comparative gains from four hybrid tree breeding strategies. Can J For Res 34(1):209–220

Li Y, Dutkowski GW, Apiolaza LA, Pilbeam D, Costa e Silva J, Potts BM (2007) The genetic architecture of a *Eucalyptus globulus* full-sib breeding population in Australia. For Genet 12(3):167–179

Lynch M, Walsh B (1997) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, MA, USA, p 980

Meuwissen TH, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Moncur MW, Hasan O (1994) Floral induction in *Eucalyptus nitens*. Tree Physiol 14:1303–1312

Myburg AA, Potts BM, Marques CM, Kirst M, Gion JM, Grattapaglia D, Grima-Pettenati J (2007) *Eucalyptus*. In: Genome mapping and molecular breeding in plants. Springer, USA, pp 115–160

Namkoong G, Kang HC, Brouard JS (1988) Tree breeding: principles and strategies. Springer, USA, p 180

Osorio LF (1999) Estimation of genetic parameters, optimal test designs and prediction of the genetic merit of clonal and seedling material of *Eucalyptus grandis*. School of Forest Resources and Conservation, University of Florida, Gainesville

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545–554

Potts BM (2004) Genetic improvement of eucalypts. In: Burley J, Evans J, Youngquist JA (eds) Encyclopedia of forest science. Elsevier Science, UK, pp 1480–1490

Potts BM, Dungey HS (2004) Interspecific hybridization of *Eucalyptus*: key issues for breeders and geneticists. New For 27(2):115–138

Raymond CA (2002) Genetics of *Eucalyptus* wood properties. Ann For Sci 59:525–553

Raymond CA, Apiolaza LA (2004) Incorporating wood quality and deployment traits in *Eucalyptus globulus* and *Eucalyptus nitens*. In: Walter C, Carson M (eds) Plantation forest biotechnology for the 21st century. Research Signpost, Kerala, India, pp 87–99

Raymond CA, Schimleck LR (2002) Development of near infrared reflectance analysis calibrations for estimating genetic parameters for cellulose content in *Eucalyptus globulus*. Can J For Res 32:170–176

Reis CAF, Gonçalves FMA, Rosse LN, Costa RRGF, Ramalho MAP (2011) Correspondence between performance of *Eucalyptus* spp. Trees selected from family and clonal tests. Genet Mol Res 10(2):1172–1179

Resende KFM, Santos FMC, Dias MAD, Ramalho MAP (2011) Implication of the changing concept of genes on plant breeder's work. Crop Breed Appl Biotechnol 11(4):345–351

Resende MDV, Assis TF (2008) Seleção recorrente recíproca entre populações sintéticas multi-espécies (SRR-PSME) de eucalipto. Pesqui Florest Bras 57:57–60

Resende MDV, Barbosa MHP (2005) Melhoramento genético de plantas de propagação assexuada. Embrapa Florestas, Brazil, p 130

Resende MDV, Resende MFR, Sansaloni CP, Petroli CD, Missiaggia AA, Aguiar AM, Abad JM, Takahashi EK, Rosado AM, Faria DA, Pappas GJ, Kilian A, Grattapaglia D (2012) Genomic selection for growth and wood quality in *Eucalyptus*: capturing the missing heritability and accelerating breeding for complex traits in forest trees. New Phytol 194:116–128

Resende MDV, Striger JK, Cullis BC, Thompson R (2005) Joint modeling of competition and spatial variability in forest field trials. Braz J Math Stat 23(2):7–22

Resende MDV, Thompson R (2004) Factor analytic multiplicative mixed models in the analysis of multiple experiments. Braz J Math Stat 22(2):31–52

Rezende GDSP, de Bertolucci F LG, Ramalho MAP (1994) Eficiência da seleção precoce na recomendação de clones de eucalipto avaliados no Norte do espírito Santo e sul da Bahia. Cerne 1(1):45–50

Rezende GDSP, Resende MDV (2000) Dominance effects in *Eucalyptus grandis*, *Eucalyptus urophylla* and hybrids. In: Dungey HS, Dieters MJ, Nikles DG (eds) Hybrid Breeding and Genetics of Forest Trees: Proceedings of QFRI/CRCSPF Symposium, Department of Primary Industries, Brisbane, Australia, pp 93–100

Rezende GDSP, Resende MDV (2001) Genotypic evaluation and genotype x environment interaction in *Eucalyptus* clones selection at Aracruz Celulose S.A., Brazil. In: Developing the Eucalypt of the Future: Proceedings of Iufro International Symposium, Instituto Forestal, Valdivia, Chile, pp 69–81.

Schimleck LR, Rezende GDSP, Demuner BJ, Downes GM (2006) Estimation of whole-tree wood quality traits using near infrared spectra from increment cores. Appita J 59(3):231–236

Searle SR, Casella G, McCulloch CE (1992) Variance components. Wiley, USA, p 528

Stackpole DJ, Vaillancourt RE, Aguigar M, Potts BM (2010) Age trends in genetic parameters for growth and wood density in *Eucalyptus globulus*. Tree Genet Genomes 6:179–193

van Heerden SW, Amerson HV, Preisig O, Wingfield BD, Wingfield MJ (2005) Relative pathogenicity of *cryphonectria cubensis* on *Eucalyptus* clones differing in their resistance to C-cubensis. Plant Dis 89:659–662

White TL, Adams WT, Neale DB (2007) Forest genetics. CABI, USA, p 682

White TL, Hodge G (1989) Predicting breeding values with application in forest tree improvement. Kluwer, UK, p 367

Wu Y, Wang SQ, Zhou DG, Xing C, Zhang Y (2009) Use of nanoindentation and silviscan to determine the mechanical properties of 10 hardwood species. Wood Fiber Sci 41:64–73

Wynne RH, Nelson RF (2006) SilviScan special issue – lidar applications in forest assessment and inventory - foreword. Photogramm Eng Remote Sens 72:1337–1338

Yang JL, Bailleres H, Evans R, Downes G (2006) Evaluating growth strain of *Eucalyptus globulus* labill. From SilviScan measurements. Holzforschung 60:574–579

Zobel B, Talbert J (2003) Applied forest tree improvement, 3rd edn. Blackburn Press, Caldwell, NJ, USA, p 505