Clonal screening plot type can impact growth predictions of clones deployed monoclonally

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Abstract

Productivity of ten clones (genotypes) was contrasted in clonal mixture plots and monoclonal block plots of radiata pine (*Pinus radiata* D. Don) to age 12 years at a site in Canterbury, New Zealand. The objective was to determine if plot type (monoclone vs clonal mixture) biased the growth and survival of any clones, and therefore impacted breeder's abilities to rate clones correctly for long-term growth.

The experimental design was a randomised complete block with three replications. Each plot contained 40 or more trees. The trial was installed at 1250 stems/ha and left un-thinned. Plots of deviations in diameter at breast height (DBH) and stand basal area were applied to critically evaluate each clone's growth sensitivity to plot type.

Overall DBH and survivals were similar in monoclonal and clonal mixture plots, but mode of deployment altered the DBH and basal area rankings of some clones. Thus, it appears that the standard single-tree-plot designs used by breeders, which are a form of intimate clonal mixture, could allow breeders to overlook clones that could grow more rapidly at older ages in monoclonal stands.

Introduction

Clonal forestry with radiata pine is feasible with the development of new techniques of clonal propagation, maintenance of juvenility and cryo-preservation (Carson *et al.*, 2004). However some issues need to be resolved to optimise its benefits. One is the challenge of finding clones that simultaneously exhibit rapid growth, resistance to potentially serious pests and diseases, and desirable wood qualities (Sorensson, in prep.). Another is the choice of mode of deployment to production forest (Ritchie, 1996; El-Kassaby and Moss, 2004; Sharma *et al.*, 2008).

There are two principal modes of clonal deployment: monoclones and intimate clonal mixtures. Opinions of some researchers differ markedly over their preferred mode of clonal deployment, in part due to a paucity of relevant research. A few studies have compared the productivity of monoclonal and clonal mixture plots, but many were on hardwoods and may not be relevant to radiata pine (Table 1).

The productivity of plantations to clearfell depends upon many factors. This could include environment, stresses (biotic, abiotic), silviculture, planting stock type and quality, genotype, inter-tree competition, as well as various interactions (Sharma *et al.* 2008).

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"Clonal Genetics", *i.e.* selection of superior genotypes and their mode of deployment to forest can strongly influence plantation development. In New Zealand, radiata pine clones are screened by breeders across multiple sites using single-tree-plot (STP) trials, which are effectively intimate clonal mixtures. Inter-clonal competition can bias the growth of clones, especially as trees grow older. Clonal trees that exhibit rapid early growth can dominate neighbours that grow more slowly (Sharma *et al.*, unpubl.).

Clones that begin growing relatively slowly in monoclonal plots will be slower, in a fixed silvicultural regime, to develop fierce inter-tree competition than clones that grow rapidly from an early age. In theory at least, clones that start out growing slowly could, over time, increase their rate of growth. Although there is little evidence as yet for this hypothesis of "sprinters and stayers", the notion is attractive because such trees would produce a desirably high proportion of mature wood.

Breeders recognise pros and cons of clonal mixture and monoclonal block-plots (BP) for use in clonal screening trials, but regard the land requirement of BPs as the most serious (Table 2). Typically STP trials test up to 10 trees (ramets) per clone at each site. In contrast, a common BP trial design involves three replications of 36-tree plots, or 108 trees per clone. One compromise is to greatly reduce the number of sites with BP trials, and thus the land requirement. Another is to test all clones in multisite STP trials, and select a handful of clones for testing (demonstrating) in BPs.

The objective of this study was to determine if plot type affected clonal growth, and thus whether it could

Table 1: Summarised findings of recent studies comparing the productivity of tree crops in monoclonal versus clonal-mixture plots.

Source	Tree species	Age (yr)	Relative productivity: Monoclones vs. clonal mixtures
Zhou *et al.* (1998)	Chinese-fir (*Cunninghamia lanceolata*)	9	27-30 % greater volume per hectare of monoclonal blocks of clones compared to single row plots over seedling check plots.
Markovic and Herpka (1986)	Poplars (*Populus sp.*)	4	Slightly higher volume, mean height and mean diameter growth in monoclonal plots.
Debell and Harrington (1997)	Populus	3	Similar productivity
Benbrahim *et al.* (2000)	Populus	8	Similar productivity
Dawson and McCraken (1995)	Willows (*Salix spp.*)	3	Greater biomass yield in clonal mixture plots compared to either the mean yield of component clones or individual yield of any component grown monoclonally
Sharma *et al.* (2008)	Radiata pine (*P. radiata*)	12	Similar productivity

significantly influence the ability of breeders to accurately rate the true growth potential of radiata pine clones.

Materials and Methods

Site and experimental design

The experiment was established in September 1993 at Dalethorpe, 70 km west of Christchurch, Canterbury, New Zealand at an elevation of 520 m above mean sea level. The soil at the site was a well-developed silt-loam (NZ Soil Bureau, 1968). Mean annual precipitation averaged 1058 mm from 1993-2006 (NIWA, 2006).

The 10 clones were deployed in a randomized complete block design with three replications. Each replication had eleven treatments: 10 monoclones and one balanced 10-clone mixture. Each plot contained 40 trees (5x 8), except one clonal mixture plot with160 (5x32) trees. Trees were spaced 2 m within rows and 4 m between rows (1250 stems/ha). At age 7 years, all trees were pruned to a height of 2.5 m. The trial was left un-thinned during this study.

Planting material and establishment practices

All radiata pine clones used in this experiment were propagated by organogenesis (meristematic tissue culture). Thus, these clones were not regenerated fresh from cryostored embryos. Treestocks were hardened off in a nursery in the North Island (Fletcher Challenge Forests' Biotechnology Centre, TeTeko) with an undercutting and wrenching regime, and transplanted as bare-root plants.

The ten clones represented six different full sib families. Clones 3, 7 and 10 were propagated from different seeds of same cross. Clones 1 & 9 and clones 6 & 8 were also propagated from different seeds of two crosses. Clones 2, 4 and 5 were from three different crosses. All crosses were control-pollinations of well known NZ seed orchard parents.

Table 2: Key advantages and disadvantages of single-tree-plot (STP) and monoclonal block-plots (BP) for use in pine clonal screening trials.

Factors	STP	BP
Land area required	Less	More
Growth bias from inter-clonal competition	More	Less
Monoclonal stand productivity and growth pattern analysis	No	Yes

Trees were planted in pits 30 cm deep, in lines ripped to a depth of at least 30 cm. Each randomised complete block was planted by only one person. Plots were initially kept weed free using a mixture of hexazinone and turbuthylazene, and subsequently a mixture of turbuthylazine, clopyralid and haloxyfop herbicides for five years following planting.

Assessments and variables

Only the inner 18 trees in each 40-tree plot (and 90 inner trees in one big clonal mixture plot) were measured, to help remove bias from inter-plot competition. Diameter tapes were used to measure diameters at breast height over bark (DBH) to a precision of 0.1 cm. DBH was recorded annually from establishment year 1993 to 2005, except years 2001 and 2002. Assessments were made mid-winter (mid

August to mid September) when tree stem growth more or less stops in Canterbury. Mortality, wind-throw and stem damage were recorded at each assessment.

Mean DBH was separately calculated for every clone for monoclonal and clonal mixture plots. Stand basal areas per hectare for each plot were calculated at age 12 years from plot basal areas in monoclonal plots and from mean clonal basal area in clonal mixture plots.

Data analysis

Procedure GLM (General Linear Model) of SAS (SAS-Institute, 2000) was used to compare the productivity of clones in both modes of deployment, and interactions of clones and mode of deployment at age 12 years for DBH, stand basal areas and survivals.

The following model was used for analysis of variance:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\gamma)_{ik} + e_{ijk} \qquad 1$$

where Y_{ijk} is mean DBH or stand basal areas or survivals of i^{th} clone, j^{th} block and k^{th} mode of deployment, μ is overall mean, α_i is ith clone, β is j^{th} block, γ is k^{th} mode of deployment, $(\alpha\gamma)_{ik}$ is the interaction of i^{th} clone and k^{th} mode of deployment and e_{ijk} is error.

The Student-Newman-Keuls (SNK) multiple range test was used at P=0.05 to distinguish differences in mean DBH, stand basal areas and survivals of clones at age 12 years. The smallest critical range of the SNK test was used as measure of statistical power for each variable.

DBH and Stand Basal Area (G) were chosen to rate clones for three reasons: (a) high precision of underlying measurements, (b) G adjusts for differences in plot survivals, and (c) G is an area-based variable that is a standard input into crop growth models.

To critically explore the relative growth rates of clones and their sensitivity to plot type, two types of deviations were plotted:

- **DBH** Deviations of individual clone DBH means from overall DBH means were calculated for monoclonal plots and clonal mixture plots. These deviations for each clone were plotted against each other. Deviations more than 2 cm from the trend line were earmarked to identify clones whose growth rate changed markedly with deployment mode.
- **G** As for DBH above, deviations of individual clone stand basal area from overall stand basal area were calculated for monoclonal plots and clonal mixture plots, and the deviations plotted. Deviations above 4 m^2/ha from the trend line were earmarked to highlight clones whose growth rate changed markedly with deployment mode.

Results

Survivals

Overall survivals at age 12 years were similar in both modes of deployment And only one clone (clone 9) had a significantly lower survival (65 %) in monoclonal plots.

Clonal survivals did not appear to differ significantly in clonal mixture plots using the SNK test (Table 3) but this may be misleading due to inadequate 'statistical power' for mixed clonal plots.

DBH

Overall DBH means were similar in both modes of deployment. However, the DBH of some clones did differ significantly with mode of deployment, as shown the significant interaction between clone x deployment mode (P=0.019). For instance, clone 7 had significantly lower DBH in clonal mixture plots compared to monoclonal plots (Table 3).

Stand basal area

Both modes of deployment generated similar overall stand basal areas (P=0.685). However, clones 5 and 9 significantly differed in stand basal area in monoclonal plots according to the minimum critical range of the SNK test. Clone 9 yielded the lowest stand basal area, and clone 5 the highest, at age 12 (Table 3).

Although there were considerable differences in stand basal areas among clones in clonal mixture plots, they were statistically non-significant. This is partly due to the low statistical power of clonal mixtures, but also reflects the greater within-clone variability of clones in clonal mixtures (Sharma *et al.* 2008).

Clonal productivity in monoclonal plots versus mixed clonal plots

As noted above, plots of deviations were used to identify any clones whose relative growth was sensitive to contrasting modes of deployment. These comparisons were affected by low survival rates of some clones, which in monoclonal plots would increase DBH of remaining trees while lowering basal area/ha. For this reason plots of both DBH (Figure 1) and basal area/ha were examined. These plots revealed that clone 5 grew rapidly, and nearly equally so, regardless of deployment mode (Figures 1 and 2). Of all the clones, it appeared to be the least sensitive to mode of deployment (plot type).

However, several of the ten clones showed some sensitivity in growth or growth pattern to deployment mode. Two distinct influences brought this about. Clone

Table 3: Mean clonal DBH, stand basal area and survival in monoclonal and clonal-mixture plots at age 12 years.

	DBH (cm)		Stand Basal Area (m2/ha)				Survivals (%)	
Clone	Monoclone	Mixture	Monoclone	Deviations from mean	Mixture	Deviations from mean	Monoclone	Mixture
1	26.0 cd	29.1 abc	67.0 ab	4.4	76.8 a	12.4	100 a	93 a
2	25.2 d	25.1 cde	56.8 ab	-5.8	61.4 a	-3	89 ab	97 a
3	24.7 d	25.6 bcde	60.3 ab	-2.3	63.0 a	-1.4	100 a	96 a
4	25.6 cd	28.3 abcd	56.6 ab	-6	53.1 a	-11.3	87 ab	67 a
5	27.4 bc	30.1 ab	70.7 a	8.1	90.7 a	26.3	94 a	100 a
6	25.5 d	24.8 cde	65.0 ab	2.4	51.4 a	-13	100 a	83 a
7	28.9 ab	23.5 e	67.3 ab	4.7	54.7 a	-9.7	82 ab	97 a
8	26.0 cd	26.4 bcde	66.3 ab	3.7	63.5 a	-0.9	98 a	92 a
9	29.4 a	31.5 a	54.3 b	-8.3	74.4 a	10	65 b	75 a
10	26.3 cd	24.1 de	61.3 ab	-1.3	54.9 a	-9.5	89 ab	94 a
Overall	26.5	26.8	62.6	0	64.4	0	90	89
SNK critical range	1.6-2.7	4.8-8.1	15.8-26.9		39.8-67.9		25-43	40-68

9 was sensitive to mode of deployment as a consequence of lower survival than average, and its location on Figure 1 differs from its location on Figure 2 because it suffered more competition in clonal mixture than in a monoclonal deployment. Other clones, however, had survival rates more similar to the average for clonal mixtures and demonstrated a tendency to grow more slowly in mixture than in monoclonal stands because they were slow starters and were more suppressed in mixture. Clone 7 is a good example of the latter type of clone. Clone 10 is a less extreme, but similar example.

Discussion

This study suggests that differences in survival between clones deployed monoclonally or in mixtures explains some of the measured growth sensitivity of ten radiata pine clones to mode of deployment (plot type) at age 12 years. Trees in clonal-mixture plots had more divergent growth patterns and morphologies, resulting in an earlier and sharper emergence of competition, both dominance and suppression (Sharma *et al.* 2007).

It is likely that survivals of clones that exhibited higher mortality in monoclonal plots were also lower in mixed clone plots. Unfortunately our experimental design had more trees per clone in monoclonal plots than clonal mixture plots, and this reduced power made it hard to estimate some responses accurately.

Some clones were slow growers but survived well in monoclonal plots, and their survival boosted the overall productivity of these clones in monoclonal plots at least to age 12 years. In clonal mixtures, their productivities dropped from the 'double whammy' of relatively slow initial growth and suppression by their more aggressive clonal neighbours. Both clones 7 and 10 suffered more transplant stress than average (Sharma *et al.* 2007), and given nursery treatments that resulted in more stress-resistant trees their

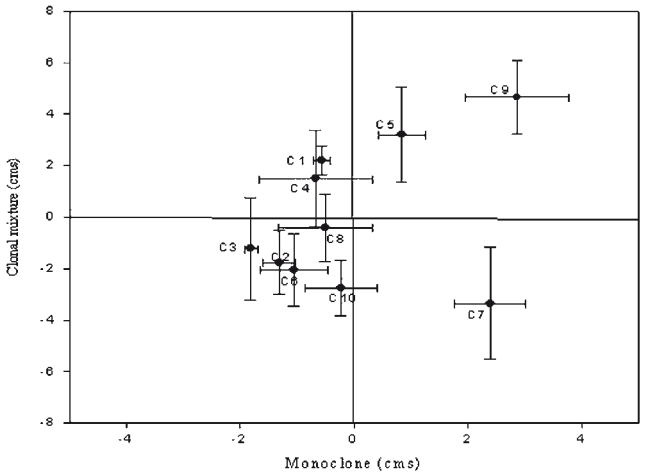

Figure 1: Deviations of mean DBH of clones from overall mode of deployment in monoclonal versus clonal-mixture plots. Lines show the extent of standard errors.

initial growth may not have been so slow relative to other clones.

This study suggests that screening clones in STP trials, which are a form of clonal mixture, will subtly mis-classify some clones that *would ultimately grow more rapidly* if deployed monoclonally. This would be particularly so if a clone began growing slowly and its growth sped up (relative to normal growth patterns) over time. Libby (1987) was perhaps the first to recognise that choosing clones only from clonal-mixture trials could lead to a biased view of the growth potential of clones that would be deployed monoclonally. However, these clones were all propagated in the same way. Had their nursery conditioning treatments been tailored to ensure that each clone was less likely to suffer from transplant stress then their growth patterns may have been more similar.

Block-plot (BP) trials require large land areas, enough so that sacrifices are required in the number of test sites and/or the number of clonal candidates that can be screened. The value of BP trials is arguably greatest for precisely determining the growth and growth pattern of high-ranked (pre-screened) candidate clones that would be deployed as monoclones. BP trials also have important uses in demonstration and education.

In our study, selection for DBH from clonal mixtures would have identified clone 9 as the fastest grower of the 10 test clones at age 12 years, whereas the fastest-growing clone, Clone 5, required *testing in monoclonal plots* to be correctly identified as the fastest grower under monoclonal deployment. Measuring growth as stand basal area at age 12 would have correctly identifed clone 5 as the fastest grower, but clone 9 produced a higher stand basal area until age 5 years (Sharma unpublished data).

The implications of these results for foresters are (1) that selections of rapidly growing clones from clonal

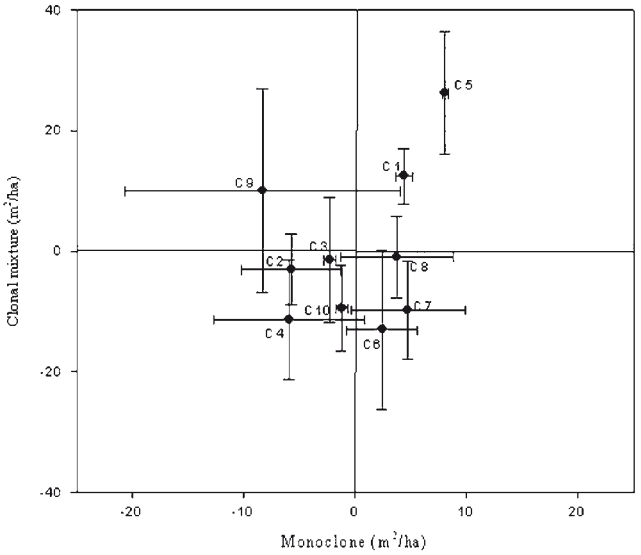

Figure 2: Deviations of mean stand basal area of clones from overall mode of deployment in monoclonal versus clonal-mixture plots. Lines show the extent of standard errors.

mixtures may miss some clones that would perform well in monoclonal plots; and (2) that deployment of clones in mixture may require careful selection of clones with similar growth patterns, otherwise slower starters may not contribute much to the final crop.

Conclusions

Monoclonal and clonal mixture modes of deployment were similar overall in average clonal productivity and survival at age 12 years in this study. Mode of deployment significantly altered the relative growth of some clones. This appears to be partly attributable to the effect of different plot types (monoclone vs. mixture) on survivals of clones with unusually high or low competitiveness.

Some clones appeared to grow more rapidly in monoclonal plots than they did in clonal mixture plots, and *vice versa*. One clone among the ten studied was highly productive in both modes of deployment. At present, one would have to test a candidate clone both in a clonal mixture and as a monoclone to fully rate its suitability for both modes of deployment.

References

Benbrahim, M.; Gavaland, A.; Gauvin, J. 2000: Growth and yield of mixed polyclonal stands of populus in short-rotation coppice. *Scandavian Journal of Forest Research* 15: 605-610.

Carson,M.J.; Carson, S.D.; Hayes, J.D.; Riini, C.T. 2004: Clonal forestry with radiata pine. *In*: Li, Bailian and Steven Mckeand (Eds.) proceedings of IUFRO Forest Genetics Meeting, at Charleston, South Carolina, USA from Nov. 1-5, 2004.

Dawson, W.M.; McCracken, A.R. 1995: The performance of polyclonal stands in short rotation coppice willow for energy production. *Biomass and Bioenergy* 8 (1): 1-5.

Debell, D. S.; Harrington, C.A. 1997: Productivity of Populus in monoclonal and polyclonal blocks at three spacings. *Canadian Journal of Forest Research* 27: 978-985.

El-Kassaby, Y.A.; Moss, I. 2004: Clonal Forestry: options, deliveries and benefits. *In*: Proceedings of 2004 IUFRO Forest Genetics Meeting held at Charleston, South Carolina, USA from Nov. 1-5. pp. 55-64.

Libby, W.J. 1987: Testing and deployment of genetically engineered trees. *In*: Cell and tissue culture in forestry. (Eds.) J. M. Bonga and D. J. Durzan Volume 1. Martinus Nijhoff Publishers. pp. 167-197.

Markovic, J.; Herpka, I. 1986: Plantations in short rotations. *In*: Guzina, V. (Ed.), Poplar and Willows in Yugoslavia. Poplar Research Institute, Novi Sad. Yougoslavia. pp.182-197.

NIWA 2006: National institute of water and atmospheric research, PO Box 8602, Christchurch,10 Kyle Street, Riccarton, Christchurch.

New Zealand Soil Bureau. 1968: Soils of New Zealand. Part 1. Jeab Luke. (Ed.), Govt. Printer, Wellington.

Ritchie, G.A. 1996: Operational use of vegetative propagation. Forestry: world overview of cloning and bulking. *In*: National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW-GTR-389. Portland, USDA, Forest Service, Pacific Northwest Research Station. Pp. 192-197. http://www.fcanet.org/proceedings/1996/ritchie.pdf.

SAS Institute, 2000: SAS User's Guide: Statistics, SAS Institute, Cary, North Carolina

Sharma, R.K.; Mason, E.G.; Sorensson, C.T. 2007: Impact of planting stock quality on initial growth and survival of radiata pine clones and modelling initial growth and survival. *New Zealand Journal of Forestry* 52 (1): 14-23.

Sharma, R.K.; Mason, E.G.; Sorensson, C.T. 2008:. Productivity of radiata pine (*Pinus radiata* D. Don.) clones in monoclonal and clonal mixture plots at age 12 years. *Forest Ecology and Management* 255 (1): 140-148.

Sorensson, C.T. (in prep.): Improving the relevancy of wood quality breeding in softwoods. *NZ Journal of Forestry Science*. (re-publication of Australasian Forestry Research Working Group 1 (Genetics) http://www.cdesign.com.au/afgc2007/)

Zhou, T.; Zhou, J.; Shelbourne, C.J.A. 1998: Clonal selection, propagation, and maintenance of juvenility of Chinese fir, and afforestration with monoclonal blocks. *New Zealand Journal of Forestry Science* 28 (3): 275-292.