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Experiment designs to evaluate inter- and intra-specific interactions in mixed plantings of forest trees

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Abstract

A review of three mixed-species trials reveals the utility of competition indices for evaluating inter- and intra-specific interactions between trees, the desirability of experiments that span a range of tree spacing and composition to inform calibration of these competition indices, the need for extremes of species composition and stand density to calibrate response surfaces, and the far-reaching impact of edge-effects. Experiment layouts commonly used for mixed-species trials in forestry (such as replacement series) rarely provide a strong basis to calibrate competition indices and response surfaces. Alternative designs involving systematic changes in species composition may offer a better basis for calibrating response surfaces, especially when used in conjunction with designs that vary tree spacing systematically. Systematic spacing designs (such as Pudden clinal plots, Marynen plaids and Nelder fans) are well established, but designs that vary composition systematically are less common. The Goelz triangle and an alternative are contrasted to illustrate how both may offer inspiration when designing trials for mixed-species research in forestry.

Key words: replacement series, response surface, polyculture, mixed-species, experiment design, systematic design

Introduction

Replacement series continue to be widely used in forestry to evaluate mixed-species plantings, despite a long-running debate about their utility (e.g., Connolly 1986; Snaydon 1991; Jolliffe 2000; Park *et al.* 2003). Problems reported with glasshouse studies and annual field crops are compounded in forestry field trials because of the long-term nature of these experiments. This paper examines some issues pertinent to long-term mixed-species trials in forestry, and suggests an alternative design for evaluating competition and growth in mixed plantings.

Increasing interest in mixed-species plantations has not been well informed by rigorous research on growth and yield in such mixtures. The desire to plant mixtures rather than monocultures may stem from considerations of biodiversity, of pests and diseases, or of marketing opportunities, but it is nonetheless important for forest managers to understand the consequences of mixed plantings in terms of timber production. Such an understanding requires field trials that allow the expression of mature growth habits, which inevitably must be conducted over several years. The long time frame compounds the difficulties associated with conducting and analysing such trials, including the serial correlation of observations, and the risk that some sample units may be damaged or destroyed. This context requires robust designs for experiments to evaluate mixed species plantings.

The Problem

Of four traditional experimental designs (pair-wise, replacement or substitution series, additive series, and response surfaces; Connolly *et al.* 2001a,b; Inouye 2001; Park *et al.* 2003) that are commonly used to examine species mixtures, replacement series have been used most frequently in forestry (based on abstracts in CAB International). Replacement series involve growing two species in varying proportions (often 100:0, 72:25, 50:50, 25:75 and 0:100%; Forrester *et al.* 2006), whilst maintaining a constant overall stand density (de Wit 1960; Jolliffe 2000). Despite its popularity (Gibson *et al.* 1999), the method has been criticised because estimated coefficients may depend on total stand density (Inouye and Schaffer 1981; Connolly 1986), because of the possibility of a size-

effect (Gibson *et al.* 1999; Connolly *et al.* 2001b; Kikvidze 2005), and because of the difficulty in discriminating between intra- and inter-specific competition (Firbank and Watkinson 1985; Snaydon 1991). In a forestry context, the demise of one or more trees in a replacement series can compromise the analysis: such a change in density may confound the interpretation of both per-tree and per-plot estimates, and in long-term studies, such mortality is not unexpected.

The inferences that may be drawn from a study are largely determined by the experimental design (Gibson *et al.* 1999; Freckleton and Watkinson 2000). Obviously, there can be no optimal design for competition experiments because the aims and objectives vary from study to study. However, a large number of studies have demonstrated the utility of regression techniques in appraising plant competition (Freckleton and Watkinson 2000, 2001). Designs that allow response surface analysis can avoid many problems inherent in other common methods (Gibson *et al.* 1999).

Designs for multiple species experiments are still largely untested, although several designs have been used. At the level of the individual plant, hexagonal fan designs (Nelder 1962, Antonovics and Fowler 1985, Kelty and Cameron 1995) permit study of the effects of varying the spatial pattern, and the densities and the relative proportions of interacting species, but have been criticised for lack of independence and lack of randomization (Inouye 2001). Studies in which only final yield is measured are severely limited as to the inferences which may be drawn, because effects due to interspecific interaction during the course of the experiment cannot then be separated from pre-existing differences, and interpretation may be biased towards species whose individuals were initially larger (Gibson *et al.* 1999). In addition, several re-measurement intervals are necessary to understand the changing dynamics of species interaction.

Specific Weakness of selected Mixed-species Trials

The limitations of some popular experiment designs are highlighted by examining a few published studies of mixed species plantings. An *Acacia-Eucalyptus* replacement series

trial at Atherton in north Queensland (Bristow *et al.* 2006), a Nelder wheel with two species in 18 spokes and 8 rings at Mt Mee in south-east Queensland (Lamb and Borschmann 1998), and a randomised block planting with 28 plots of 16 species at Mt Mee (Erskine *et al.* 2005; Lamb and Borschmann 1998) offer instructive insights. These trials have not been selected because they are inferior in design or conduct. On the contrary, these trials have provided useful insights for research and management of forests, have been reported in the formal literature, and have been included in conference and scientific tours. These trials have been selected because they are based on well-established designs, are relatively well known, and have offered useful insights, but still suffer significant weaknesses limiting the ability to make useful inferences for forest management.

Interpretation of results from the 10-year-old mixed planting of *Eucalyptus pellita* and *Acacia peregrina* at Atherton was confounded by natural mortality within the trial (Bristow *et al.* 2006). This mortality, exacerbated by tropical cyclones, altered the design from a traditional replacement series with equally-spaced levels (100:0, 75:25, 50:50, 25:75 and 0:100 Eucalypt:Acacia stems per plot) to 41:0, 41:8, 18:26, 11:19 and 0:48 stems per plot. This change in both the relative (i.e., the ratio of Eucalypt:Acacia) and absolute stocking (i.e., stems/ha) of the plots, made interpretation of the trial ambiguous, as the treatment with the largest trees was not the treatment with the largest yield. Bristow *et al.* (2006) showed that a competition index (based on Hegyi 1974) allowed useful insights into competition and facilitation in this experiment, by computing a competition index that accounted for local density, thus accommodating spaces left by dead trees and through edge-effects. Figure 1 illustrates inter- and intra-specific 'competition' experienced by the eucalypts in the Atherton trial. Clearly, the inter-specific interaction that a eucalypt experiences when an acacia is nearby, is very different to the intra-specific competition with other eucalypt trees, something not revealed in the traditional replacement series analyses based on relative yields (Bristow *et al.* 2006). *Observation 1*: Indices of local competition (such as the Hegyi index) can offer useful insights when unforseen events have confounded the original experiment design.

[Figure 1 near here]

Hegyi's (1974) index is one of several competition indices that can be used to characterise local competition (Vanclay 1994; Mailly *et al.* 2003; Rivas *et al.* 2005), and is computed as the sum of the distance-adjusted relative size ($[h_i/h_j]/distance_{ij}$) of potential competitors *i* within a defined search radius of the subject tree *j*. Hegyi (1974) used a 10-foot (3 m) search radius, but most subsequent researchers have used a larger search radius (Bristow *et al.* 2006). Several authors (e.g., Hegyi 1974, Mailly *et al.* 2003) have used a search radius in the range 3-5 metres, a distance not well sampled within the 2 x 5 metre spacing adopted in the original Atherton design (Figure 2). In contrast, the Nelder design used by Lamb and Borschmann (1998) provided a better basis to calibrate the search radius for such a competition index. Figure 2 illustrates the frequency of inter-tree distances for the 144 trees (the number of trees in the Nelder wheel) arranged in different configurations. Clearly, it is possible to manipulate an illustration like Figure 2 through choice of the origin and width of classes. Nonetheless, it is apparent rectangular spacings offer a more restricted range of inter-tree distances, and that the nearest neighbour is more-or-less constant, in contrast to the wider range of spacings offered by Nelder wheels and other designs in which spacing varies systematically.
Observation 2: Designs which systematically vary spacing (such as Nelder wheels) inform the calibration of competition indices better than rectangular plantings on a regular grid.

[Figure 2 near here]

In Figure 1, the gradient of the inter-specific relationship is a surprise: a bigger Hegyi index means more neighbours, and bigger neighbours, and presumably less growth by the subject tree. The downward-sloping intra-specific trend is the norm, but the inter-specific relationship suggests that eucalypts 'like' to have a neighbouring acacia, more so than an empty space. This implies a facilitation role consistent with many studies of eucalypts with nitrogen-fixing plants (Forrester *et al.* 2006), but logic suggests that this relationship should be concave, facilitating growth when the competition index is small, but hindering

growth when the index is large, and competition and crowding may overwhelm any facilitation. While there is a hint of curvature in the inter-specific trend in Figure 1, there are no data above the index value of 2 to calibrate this trend. The Atherton design created indices greater than 3 for inter-specific competition in eucalypts, and approaching 7 for inter-competition experienced by acacias, but failed to create a high level of inter-specific competition in the variable of greatest interest: the indicator of facilitation by an acacia. This is partly due to the relatively wide spacing adopted (2 × 5 m), the slow growth rate of the acacias (relative to the eucalypts), and the higher mortality amongst the acacias (Bristow *et al.* 2006), but is substantially due to the decision to adopt 25E:75A as the most extreme treatment. In this treatment, some eucalypts are surrounded by a single row of eight acacias, but the design did not provide for any eucalypt trees surrounded by a double row of 24 acacias. Such a 4E:96A treatment would have offered useful insights into the nature of the apparent facilitation of eucalypt growth by acacias in the Atherton trial. Such extreme values can be particularly helpful in calibrating a response surface, and are much more useful than additional points near the mean (Vanclay 1991; Beetson *et al.* 1992).

Observation 3: Experiment designs that sample extremes of species composition and of spacing assist the calibration response surfaces.

It is desirable and customary to use guard (or buffer) rows to reduce edge-effects in experiments (Langton 1991). In the Atherton trial, the outermost row in each plot was designated a guard row, providing two guard rows between treatments. In the Mt Mee mixed-species trial (Erskine *et al.* 2005), there were no guard rows between plots, and a single guard row around the perimeter of the experiment, beyond which was pasture. It is instructive to consider the efficacy of the guard row, by examining the size of trees in the Mt Mee trial at a recent measure (2003, aged 13 years). Figure 3 illustrates how the size of trees of selected species depends on species and distance from the edge of the experiment. The edge effect does not appear strong in hoop pine (*Araucaria cunninghamii*), but appears to extend 3-4 rows in some other species. A least-squares regression fitting a simple additive model with species and truncated distance to the nearest edge explained 79% of the variation in tree size:

$$DBH = \beta_i - 2.2\ Dist$$

where β_i is a species-specific parameter, and *Dist* is the distance to the nearest edge in multiples of the standard spacing (3 m), or 3 (rows, hence 9 m), whichever is lesser. The parameter estimate for *Dist* is significant (s.e.= 0.3585, P<0.0001), and the model is adequate (regression P<0.0001) and sufficient (lack of fit P>0.8). Clearly, in this case, a single guard row is insufficient to protect the net plots from edge effects at the perimeter of the experiment. Discarding a further two rows around the perimeter of the Mt Mee trial involves the loss of 176 trees, 40% of the total number of trees in the experiment, a considerable sacrifice. In the Atherton trial, gross plots were 7 rows × 12 columns, and net plots were 5 rows × 10 columns. Providing for a minimum of 9 metres between treatments would reduce the net plots to 5 rows × 5 columns (because of the rectangular 2 × 5 m spacing), leading to a loss of half the trees. Neglecting an edge effect may inflate variances, conceal trends, or introduce bias into estimates and conclusions. Dispensing with the guard row and relying on estimates of local competition to accommodate edge effects, may be preferable to relying on a guard row which is inadequate.

Observation 4: Trees are large and their effects are far-reaching. Analyses should test for the extent of edge effects, and experiment designs (and analyses) should include sufficient guard rows, or adopt an experimental design (e.g., Nelder wheel) or analytical technique (e.g., competition indices) that dispenses with the need for guard rows.

[Figure 3 near here]

Randomizing is a wise precaution in experimental design (Greenberg 1951), but it is not always desirable to randomise species locations within a trial, because the interactions between species form a key part of the treatment (cf. dose), and randomizing species locations will not always strengthen conclusions that may be drawn. The Mt Mee trial involved 28 adjoining plots, each with 16 species, randomly located within each plot. Since each planting position has 8 adjacent positions (counting rows, columns and diagonals), and each species occurred only once in each plot, the expectation is that each species would be adjacent to each other species 13-14 times (accounting for edge effects). It is also expected that on average, each species would be adjacent to a tree of the same

species in an adjoining plot four times within the experiment. However, randomization does not guarantee the average outcome: for instance in the Mt Mee trial, two species (3: *Argyrodendron trifoliolatum* and 8: *Dysoxylum muelleri*) were never adjacent to another individual of the same species, while another (12: *Gmelina leichhardtii*) was adjacent to itself 14 times (Table 1). Excluding adjacencies in the guard row, adjacencies between *Acacia melanoxylon* (species 1) and *Araucaria cunninghamii* (2) were under-represented (3 occurrences) and adjacencies between *A. melanoxylon* and *Khaya nyasica* (14) were over-represented (22 occurrences), both very different from the expected average of 13-14. Similar discrepancies occur in the Grant *et al.* (2006) trial reported in this special issue; for instance, *Gmelina leichhardtii* is adjacent to itself only once, while *Flindersia australis* is adjacent to itself five times (Figure 1 in Grant *et al.* 2006). In both these cases, randomization has not helped to draw inferences about species interactions and their impact on resource utilization and growth rates, and hampers the ability to examine inter- and intra-specific interactions between trees.

Observation 5: Randomization, especially of the placement of species within plots, is not always helpful when the object of the experiment is to appraise species interactions.

[Table 1 near here]

Autocorrelation may also pose some problems with some designs. For instance in the Nelder wheel (Lamb and Borschmann 1998), the correlations between numeric density, local basal area and tree size are sufficiently high (>0.8, Table 2) that they hamper efficient estimation of the diameter increment response surface (when a single measure interval is used). The problem obscures the characteristic sigmoidal growth pattern of many trees and makes it difficult to establish whether an observed growth trend is in response to tree size or to local competition. This is the “picket-fence” problem (Lang and Kalivas 1993), in which a response *vector* is visible, but a response *surface* cannot be calibrated reliably (like a table with only two legs at opposite corners, a slight perturbation can make it can tilt unpredictably). The problem is relieved to some extent by employing a series of regular remeasurements over an extended time interval, but the best way to overcome the problem is to disrupt the correlations by thinning, or to

complement the Nelder wheel with some conventional plots in which these variables have less autocorrelation.

Observation 6: Some systematic designs do not provide orthogonal predictor variables (such as stand basal area and tree diameter) required for efficient calibration of response surfaces.

[Table 2 near here]

Towards a Solution

Response surface analysis avoids many of the problems with the usual interpretation of replacement series experiments, especially when the response surface is formulated for individual trees and explicitly accounts for the partial density (partitioned by species) of competitors with the subject tree. The term response surface design has been used in a wide range of contexts, often to refer to an experiment series in which the density (number of individuals per unit area) of two species is varied independently (Inouye 2001), but here it refers to an analysis which estimates the growth rate of an individual in response to attributes of that individual and its potential competitors. Such a response surface is individual-based rather than plot-based, and relies on the relative size as well as numbers of competitors. Such a response surface can reflect the expected growth of each individual tree as a function of its initial size and the intra- and inter-specific competition experienced. Such an equation (Increment = f(dbh, competition$_i$), for all species i) looks deceptively simple, but is a powerful tool for silvicultural analysis, and can be used to evaluate optimal species composition, spacing, thinning and rotation length (Vanclay 2006).

Design criteria for a field experiment to calibrate such a response surface may include:

- Robust, able to yield useful results even with the death of individual trees;
- Wide range of intra- and inter-specific competition, including both high and low relative densities;

- Compact, to minimize within-site variation and reduce establishment and maintenance costs; and
- Practical, especially with regard to on-going maintenance.

These requirements are not onerous, and can be fulfilled using a series of conventional plots, or with a systematic design.

All of the observations offered above suggest that systematic designs warrant serious consideration. Some systematic designs such as Nelder fans pose some practical difficulties (e.g., with access for mechanised operations such as weed control) if they are to be included within an operational plantation estate. However, they also offer an advantage that is not often recognised: although often implemented as a wheel or fan, Nelder designs can be arranged in irregular areas unsuitable for more conventional designs (Freeman 1964). These practical difficulties are overcome to some extent with rectangular systematic designs such as Pudden clinal plots (Dawkins 1960; Vanclay *et al.* 1995; Webster and Osmaston 2004 p.35), plaid designs (Marynen 1963; Lin and Morse 1975), and with the triangular designs demonstrated by Goelz (2001a,b). Some commentators (e.g., Inouye 2001) advocate designs in which the density (plants per unit area) of both species are varied. While this is desirable, it need not be attained within a single plot. It is possible to achieve this with a series of plots, by employing a series of Goelz (2001a,b) triangles with different spacings, or by employing a series of clinal plots with differing species compositions. However, in planning such series, it is important to consider practical (e.g., in operational forestry, row spacing may be dictated by equipment rather than by silviculture) and temporal issues (i.e., local competition is influenced by plant size as well as by the number of plants, the former increases with time).

The great majority of mixed species trials in forestry are binary mixtures established as a replacement series or some other configuration (Forrester *et al.* 2006). Many of these trials often disappoint their originators by failing to demonstrate conclusively any benefit from mixed plantings. Some initiators of these trials subsequently lament that if they had their time again, they would choose to evaluate a different species combination. Such

trial-and-error progress is inefficient with annual crops and glasshouse trials, but is a serious setback for long-rotation crops such as forest trees. This leads to the suggestion that multi-species trials, rather than binary trials, may be more efficient for examining competition and facilitation amongst forest tree species.

Goelz (2001a) has established a series of triangular plots in which the relative density of each of three species varies systematically from each corner, and has reported the 7-year results (Goelz 2001b). His design was inspired by the simplex lattice (Cornell 1973, 1990), and represents an optimal design for three species, provided that species interact only with their immediate neighbours. The Goelz design provides for all possible combinations of three species in a 7-tree hexagon (a central subject tree surrounded by 6 neighbours), including 0:0:7, 0:1;6, 1:1:5 etc, in a compact design involving only 210 trees of each species. Goelz (2001a) discussed how the design can be expanded to accommodate more than three species, and randomization can be introduced into the design without compromising the range of species composition. His design is near perfect for small trees, but suffers a weakness because as trees grow, the zone of influence of each tree may extend beyond one spacing unit, and may affect the next concentric hexagon of trees. The two spacings used by Goelz (2001b), 1.8 and 2.7 metres, are sufficiently close that trees within these trials are likely to interact with the next hexagon (i.e., the trees 3.6 and 5.4 m away) before trees reach maturity. At that time, a 7-tree hexagon is inappropriately small, and a 19-tree hexagon forms a more appropriate unit for evaluating a tree's local environment. When scrutinized as a series 19-tree hexagons, the Goelz triangles no longer sample extremes (e.g., 0:0:19, 0:1:18, 1:0:18, 1:1:17 and other extremes are no longer represented). While the Goelz triangles still span a wide range of species composition, the loss of the extremes is regrettable (cf. Observation 3 above), and the missing monospecific case may preclude comparisons with other work (Forrester *et al.* 2006). This observation of the temporal change in the utility of the design highlights how the research questions about species mixtures in forestry differ from those more commonly considered (Cornell 1973, 1990), because the prescribed mix varies over time as trees grow and die.

The Goelz design could be modified to retain these extremes for mature trees, by expanding the triangle and varying species composition more slowly across the triangle, or by distorting the data space by "stretching" the apexes and compressing the centroid of the triangle (e.g., with a sine transformation), so that species composition varies slowly near the apexes and more rapidly near the centre of the triangle. The appropriate approach depends on the context: if resources are sufficient and the shape of the response surface is unknown, then the former is preferable. However, if the nature of the response surface is known and resources are limited, then the latter alternative may be the preferred option.

A further limitation of the Goelz triangle is that it may not help to define the search radius of a competition index, because potential neighbours lie in equally-spaced concentric hexagons (cf. Observation 2). This may be addressed by employing a series of triangles with different spacings (Goelz 2001a), or by ensuring that the experiment employs two components: one which varies species composition systematically (e.g., a Goelz triangle), and another that varies spacing systematically (e.g., a clinal plot or Nelder fan as advocated by Goelz 2001a).

[Figure 4 near here]

Figure 4 illustrates an alternative design to evaluate competition and facilitation with four species, whilst overcoming some of the weaknesses discussed above. The design is presented as a square to illustrate symmetry, but a rectangular spacing could also be considered. The design has a number of noteworthy features. It has

- Constant density (stems per unit area), consistent with de Wit replacement series;
- Progressive change in relative density of each species from corner to corner;
- Partial symmetry allow contrasts to be made with alternate species combinations;
- Redundancy to be robust to case of natural mortality; and is
- Compact, requiring only 400 trees in 20 rows and columns.

The design retains some limitations. Unlike the Goelz design, this design is not truly optimal, and is not fully symmetrical. The Goelz design covers all possible combinations

of the three species in each triangle, and can be expanded to examine four (or more species) by adding more triangles, each dealing with triplets. The Figure 4 design does not include *all* combinations of any of the pairs, triplets or quartets involved, and includes only a small proportion of the possible combinations of species located at opposite corners of the design. Nonetheless, it may be of interest, because it is compact, relies on a square rows-and-columns layout, and deals with four species simultaneously.

The design is intended to inform a response surface based on an individual-tree competition index, but may be viewed in other ways to allow comparisons earlier work. It is possible to view the design as a de Wit replacement series comprising

- 36 subplots each with 9 trees (3x3) surrounded by a single external guard row, with the range of pairwise densities including 9:0, 8:1, 6:3, 3:6, 1:8 and 0:9;
- 25 subplots each with 16 trees, with pairwise densities including 16:0, 15:1, 8:8, 1:15 and 0:16; or as
- 16 subplots each with 25 trees, with pairwise densities including 25:0, 19:6, 6:19 and 0:25.

In each case, the data can be analysed in the traditional manner for de Wit replacement series, but with a greater range of relative densities than is usual in forestry (cf. Forrester *et al.* 2006). In addition to these pair-wise possibilities, the design deals with four species, and can be viewed as a four-species replacement series comprising

- 36 subplots each with 9 trees surrounded by a single external guard row, with the range of relative density including variations on 9:0:0:0, 8:1:0:0, 6:3:0:0, 6:2:1:0, and 3:2:2:2;
- 25 subplots each with 16 trees, with relative density including variations on 16:0:0:0, 15:1:0:0, 14:2:0:0, 8:8:0:0, 7:7:1:1 and 4:4:4:4; or as
- 16 subplots each with 25 trees, with variations on 25:0:0:0, 19:6:0:0, and 14:5:3:3.

Because of the systematic nature of the design, rows outside but adjacent to these subplots have a composition similar to the adjoining row within the subplot, thus reducing the need for guard rows between subplots, provided that the trees are sufficiently small that inter-tree effects extend for only one or two planting units. It is

likely that at maturity, this subplot view of the design will no longer be valid, and that a response surface approach will be the only reliable way to interpret long-term results.

It may be more effective to consider a response surface based on each individual tree and its competitors as gauged by a competition index such as Hegyi's (1974) index. In such an analysis, the design would help calibrate species-specific coefficients for the competition term (cf. Canham *et al.* 2004, Bristow *et al.* 2006). It would also provide data to help resolve questions such as:

1. Does species *A* grow better in a monoculture, or in a mixture of 1, 2 or 3 other species?
2. If there is a beneficial effect of another species, how far does it extend: 1, 2, 3, 4 or 5 planting spaces?
3. Is such a benefit directional, or is it conferred equally in all directions? (e.g., uphill vs downhill on sloping sites, or shaded vs sunny in temperate and higher altitudes).
4. Is such a benefit unequivocal, or does it apply only between certain species combinations, or at certain relative densities?

Of these four questions, the Goelz triangle is better able to address question 3, but may be handicapped in addressing question 1 because the monoculture is only represented while species interactions do not exceed the unit spacing. The design presented in Figure 4 emphasises extremes, so is better able to inform question 2. Neither design can fully inform question 4 without supplementary material (either a series of plots of the same design at different spacings, or a complementary design which varies spacing).

Systematic designs such as that in Figure 4 may be criticised for a lack of randomisation. While it is possible to randomize the placement of species in such a design (Goelz 2001a), it is not always desirable to do so (cf. Observation 5). In Figure 4, the spacing between individuals of the various species *is* the treatment, and randomising the placement of individuals may weaken rather than strengthen the ability of the design to estimate a response surface indicating inter- and intra-species competition. One would not argue that the dose of fertilizer to be applied in a nutrition trial should be randomized;

instead, a series of doses are prescribed to help establish the response surface. Thus it is with the placement of the various species in Figure 4. Where practicable, it may be desirable to randomize the location of the experiment in the field, to randomize its orientation, and to randomly assign species to the symbols in Figure 4, but this may not be desirable in all cases.

[Figure 5 near here]

The utility of this design can only be determined through field trials, but simulation studies (Figure 6) with plausible models of tree growth suggest that good estimates of the original parameters can be recovered through statistical analyses, provided that several measurements interval are available. Obviously, the details of such simulations depend heavily on the assumptions made (in particular, regarding the number, frequency and accuracy of measurements, and on the amount of stochastic variation introduced into the simulation), but the possibility of recovering good parameter estimates suggests that the design should offer good insights into competition and facilitation in mixed species plantings. Contrary to the usual experimental situation (where great care is taken to standardize everything), simulation studies suggest that some variation in initial size and in planting positions, and modest mortality is not detrimental to the ability to calibrate the response surface (provided that this variation and mortality is measured and recorded). Simulation studies also indicate that parameter estimates can be improved by supplementing the design in Figure 4 with a companion plot with spacing that varies systematically (e.g., a Pudden clinal plot as in Figure 5, Dawkins 1960, Vanclay *et al.* 1995).

[Figure 6 near here]

Figure 6 illustrates some results of simulation studies with the Goelz and proposed designs. The effects of species interactions and edge effects are apparent. However, the simulation is not entirely fair to either design. The simulation has omitted guard rows, although Goelz (2001a) specifically emphasised the need for adequate buffers, and

employed 4 or 5 guard rows in his trials (Goelz 2001b). Parameters for eucalypts and acacias are based on real data (Bristow *et al.* 2006), but the additional species are hypothetical, calibrated in one case by averaging the parameters of the eucalypt and acacia (because species exhibit a continuum of such responses, Sheil *et al*. 2006) , and in the other by adopting the acacia parameters but ignoring any interspecific interaction. Unexpectedly, the simulation reveals antagonism between the eucalypt and the 'averaged' hypothetical species, with high mortality where these species interacted. Another unexpected outcome of the simulation studies was that the facilitation experienced by eucalypts (cf. Figure 1 and Observation 3) did not rise above index=2 in the simulations, and when simulations were initiated at higher levels (by assuming dense planting), the index quickly subsided to levels comparable to those in Figure 1. This illustrates that special efforts may be required to sample extremes. Yet another surprise is that the eucalypt trees which benefit from the edge effect (because of the absence of guard rows) are not as large as the trees that benefit from the patches of high mortality, or from the facilitation provided by acacias (cf. Observation 4).

Figure 6 also illustrates how the Goelz design provides uniform representation for all species combination, whereas the proposed design emphasises the extremes. It is possible to interrogate the simulation results to contrast the data space created by these two alternatives, but because of the subjective nature of some of the inputs, publication of such a comparison is not warranted. It is however, useful to draw attention to the fact that it is useful to use simulation studies as part of the process of appraising a potential experiment design.

Which design should a researcher use? They should adopt neither, but should be inspired by both. Researchers should re-examine their specific needs, and test them against the concepts underpinning these and systematic spacing designs. If researchers need a plot that comprehensively explores all composition alternatives, and are confident that their research interests will conclude before inter-tree effects exceed the unit spacing, then the Goelz design (2001a,b) seems ideal. If larger inter-tree effects are anticipated, the Goelz design could be expanded or supplemented with adjacent monospecific plots, or the

Figure 4 design could be considered. In other situations where resources are limiting and the primary interest is to calibrate a response surface, then the design presented in Figure 4 may offer some useful ideas. In either case, it is probably desirable to employ a series of plots planted at different spacings, or to have a pair of plots, one in which composition varies (e.g., Goelz triangle or Figure 4), the other in which spacing varies (e.g., Nelder fan of Marynen plaid).

Finally, researchers are encouraged to replicate their chosen design in time as well as in space. It is much more informative to discover a consistent trend in comparable trials from more than one place, planted in more than one year, than it is to obtain a single datum, with the no certainty as to how that result may depend on the prevailing site or weather conditions.

Conclusion

There are many factors to take into account in designing a mixed-species experiment in forestry, and it appears that convention and practical considerations have often taken precedence over considerations such as robustness in the event of mortality, the utility of extreme conditions for calibrating response surfaces, and the extent of inter-tree interactions. As a result, many established trials are less informative than they could have been. The Goelz (2001a) triangle and the design presented in Figure 4 reflect different interpretations of these considerations, and may provide a useful basis for researchers to reappraise their specific needs and to formulate an experimental set-up well-suited to their specific needs.

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Table 1. Frequency of inter- and intra-species interactions in the Mt Mee trial. Expected values are 4 for diagonals and 13.5 for off-diagonal cells (excluding the guard trees, here the bottom row of the Table). Exceptional cells mentioned in the text are highlighted.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Species
4	3	9	14	13	14	12	14	16	12	11	17	17	22	20	11	1
	4	11	9	16	11	11	8	20	19	20	11	16	13	12	20	2
		0	16	11	13	11	15	17	20	13	16	18	10	6	15	3
			2	21	15	19	12	10	17	13	10	13	11	12	15	4
				2	15	16	16	14	17	18	10	19	12	7	13	5
					6	13	9	10	12	9	9	11	18	16	10	6
						4	18	13	6	16	14	7	14	15	18	7
							0	11	13	17	15	13	16	17	18	8
								4	11	12	14	11	15	13	13	9
									2	13	17	7	13	10	12	10
										2	12	15	6	17	18	11
											14	13	18	11	8	12
												6	17	12	12	13
													4	9	12	14
														4	14	15
															6	16
15	20	23	15	4	33	17	12	20	23	12	15	17	14	29	9	Guard

Table 2. Correlations in the 2003 remeasure of the Mt Mee Nelder wheel.

Dbh	BA	Density (stems/ha)	
0.50	-0.56	-0.45	Increment (cm dbh/yr)
	-0.81	-0.81	Dbh (cm)
		0.92	BA (m^2/ha)

Figures

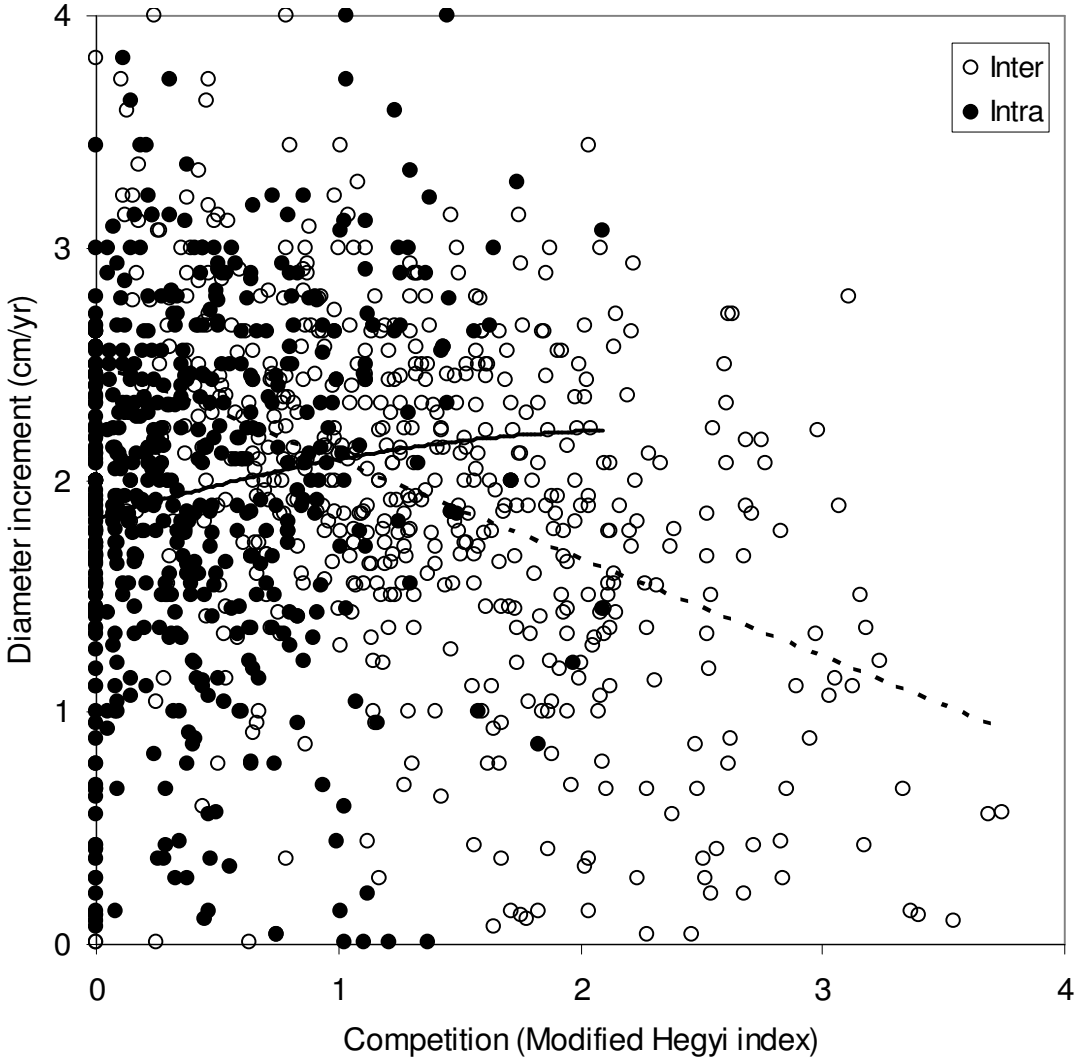

Figure 1. Inter- (○) and intra-specific (●) competition experienced by eucalypts in a 10-year-old mixed planting of *Eucalyptus pellita* and *Acacia peregrina*. The competition index is a modified Hegyi index (Vanclay *et al.* 2006).

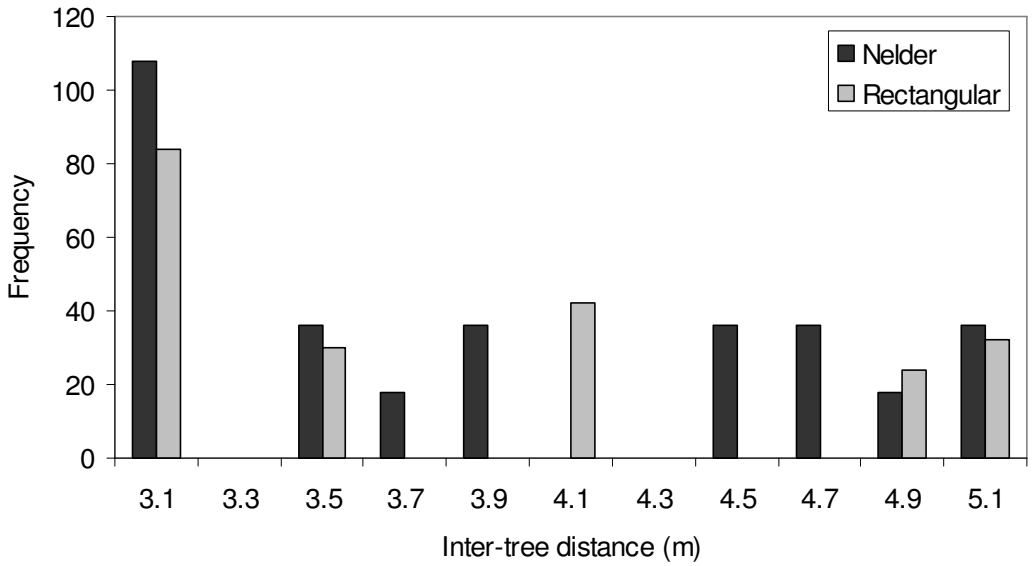

Figure 2. Frequency of inter-tree distances when 144 trees are arranged in different configurations.

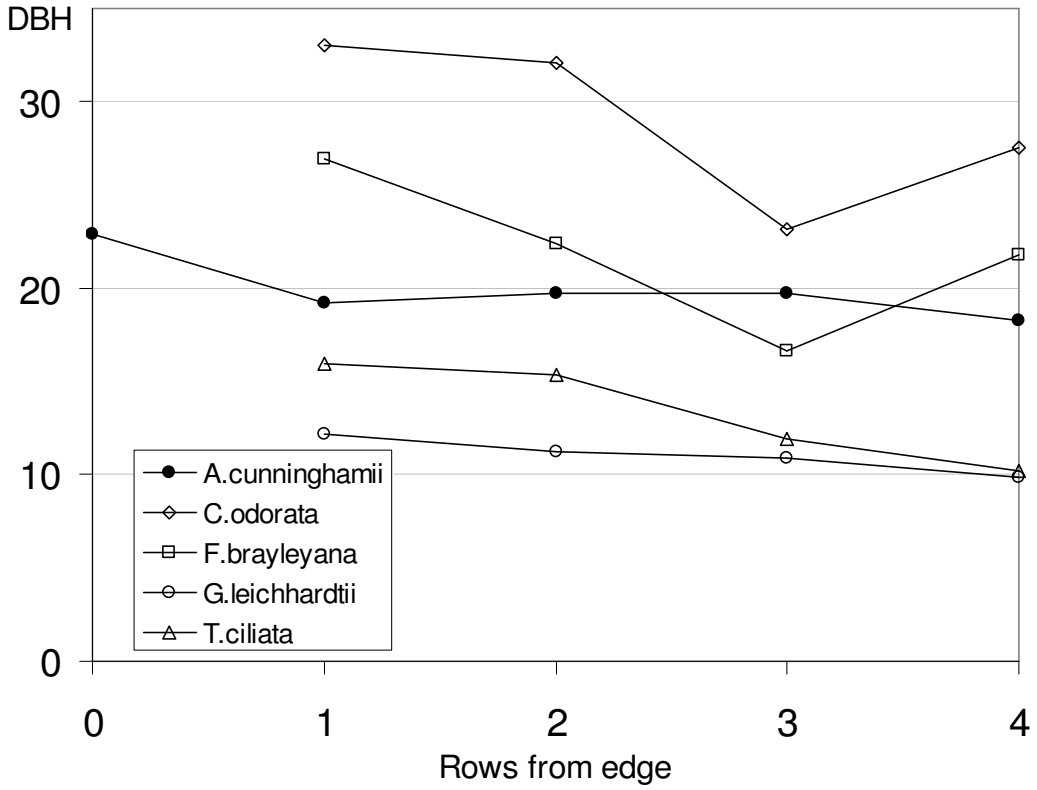

Figure 3. Apparent edge effects in the Mt Mee mixed species trial, illustrated by plotting the mean diameter of trees of selected species (*Araucaria cunninghammii*, *Cedrela odorata, Flindersia brayleyana, Gmelina leichhardtii* and *Toona ciliata*) at a given distance from the edge of the experiment. The trial includes a single guard row of hoop pine (*A. cunninghammii*), here 0 rows from the edge.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	o	o	o	o	o	o	o	o	o	+	o	+	+	+	+	+	+	+	+	+
2	o	o	o	o	o	o	o	o	+	o	+	o	+	+	+	+	+	+	+	+
3	o	o	o	o	o	o	+	o	o	+	o	+	+	o	+	+	+	+	+	+
4	o	o	o	o	o	o	o	o	+	o	+	o	+	+	+	+	+	+	+	+
5	o	o	o	o	o	o	o	o	o	+	o	+	+	+	+	+	+	+	+	+
6	o	o	o	o	o	o	o	o	+	o	+	o	+	+	+	+	+	+	+	+
7	o	o	x	o	o	o	□	o	o	□	x	+	+	x	+	+	+	□	+	+
8	o	o	o	o	o	o	o	□	+	o	+	o	x	+	+	+	+	+	+	+
9	o	x	o	x	o	x	o	x	o	+	o	+	□	+	□	+	□	+	□	+
10	x	o	x	o	x	o	□	o	x	□	x	□	+	x	+	□	+	□	+	□
11	o	x	o	x	o	x	+	x	o	+	o	+	□	o	□	+	□	+	□	+
12	x	o	x	o	x	o	x	o	x	□	x	□	+	□	+	□	+	□	+	□
13	x	x	x	x	x	x	x	+	□	x	□	x	o	□	□	□	□	□	□	□
14	x	x	o	x	x	x	+	x	x	+	o	□	□	o	□	□	□	+	□	□
15	x	x	x	x	x	x	x	x	□	x	□	x	□	□	□	□	□	□	□	□
16	x	x	x	x	x	x	x	x	x	□	x	□	□	□	□	□	□	□	□	□
17	x	x	x	x	x	x	x	x	□	x	□	x	□	□	□	□	□	□	□	□
18	x	x	x	x	x	x	□	x	x	□	x	□	□	x	□	□	□	□	□	□
19	x	x	x	x	x	x	x	x	□	x	□	x	□	□	□	□	□	□	□	□
20	x	x	x	x	x	x	x	x	x	□	x	□	□	□	□	□	□	□	□	□

Figure 4. Design for a mixed species trial, showing planting positions for four species (o, +, x and □) in a 20 x 20 grid, showing three different viewpoints: the 3x3 viewpoint with 36 plots (top left), the 4x4 viewpoint with 25 plots (top right), and the 5x5 viewpoint with 16 plots (bottom left).

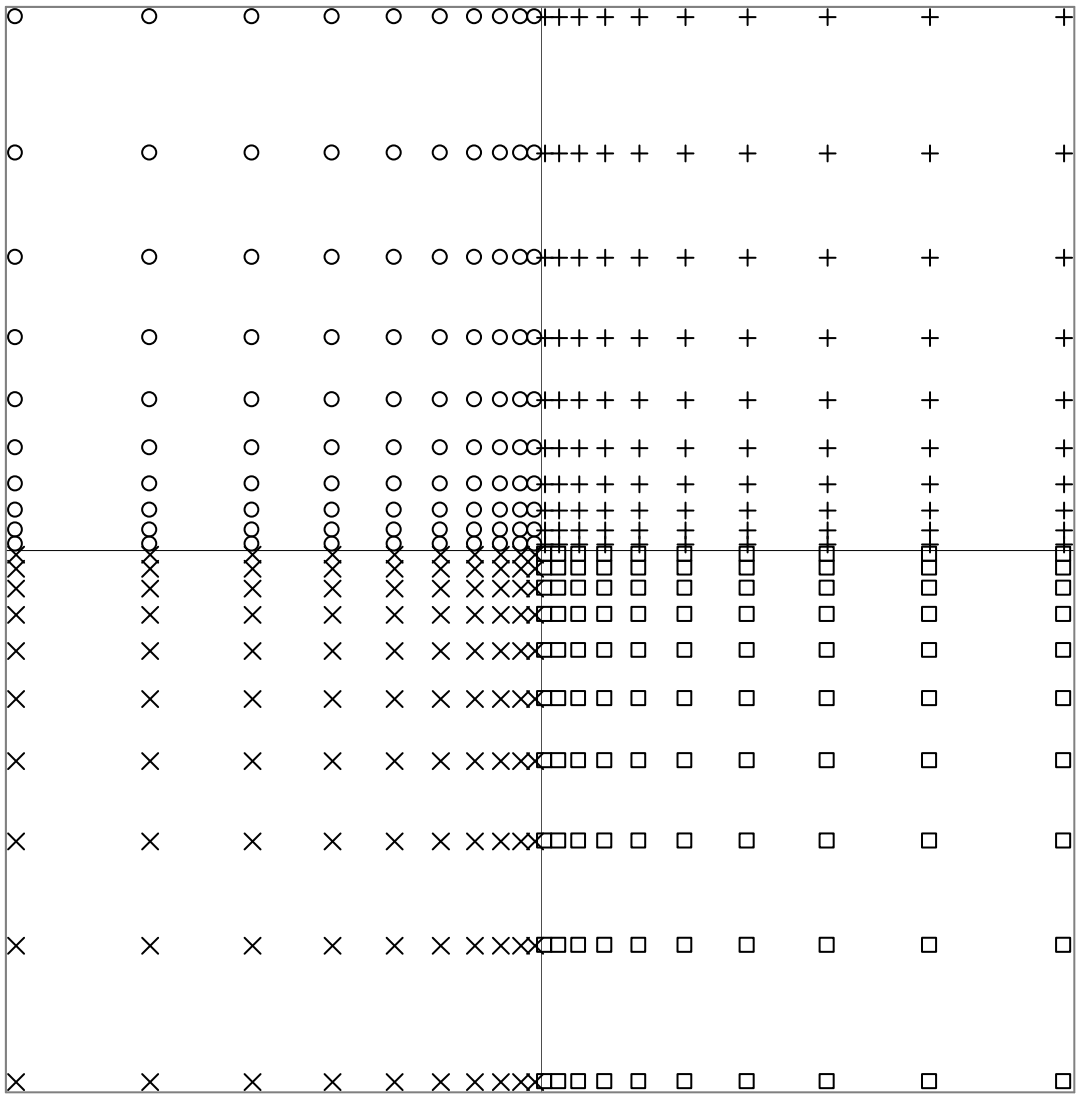

Figure 5. A possible design for a clinal planting to supplement the design in Figure 1, showing planting positions for 100 trees of each of four species (shown as o, x, + and □).

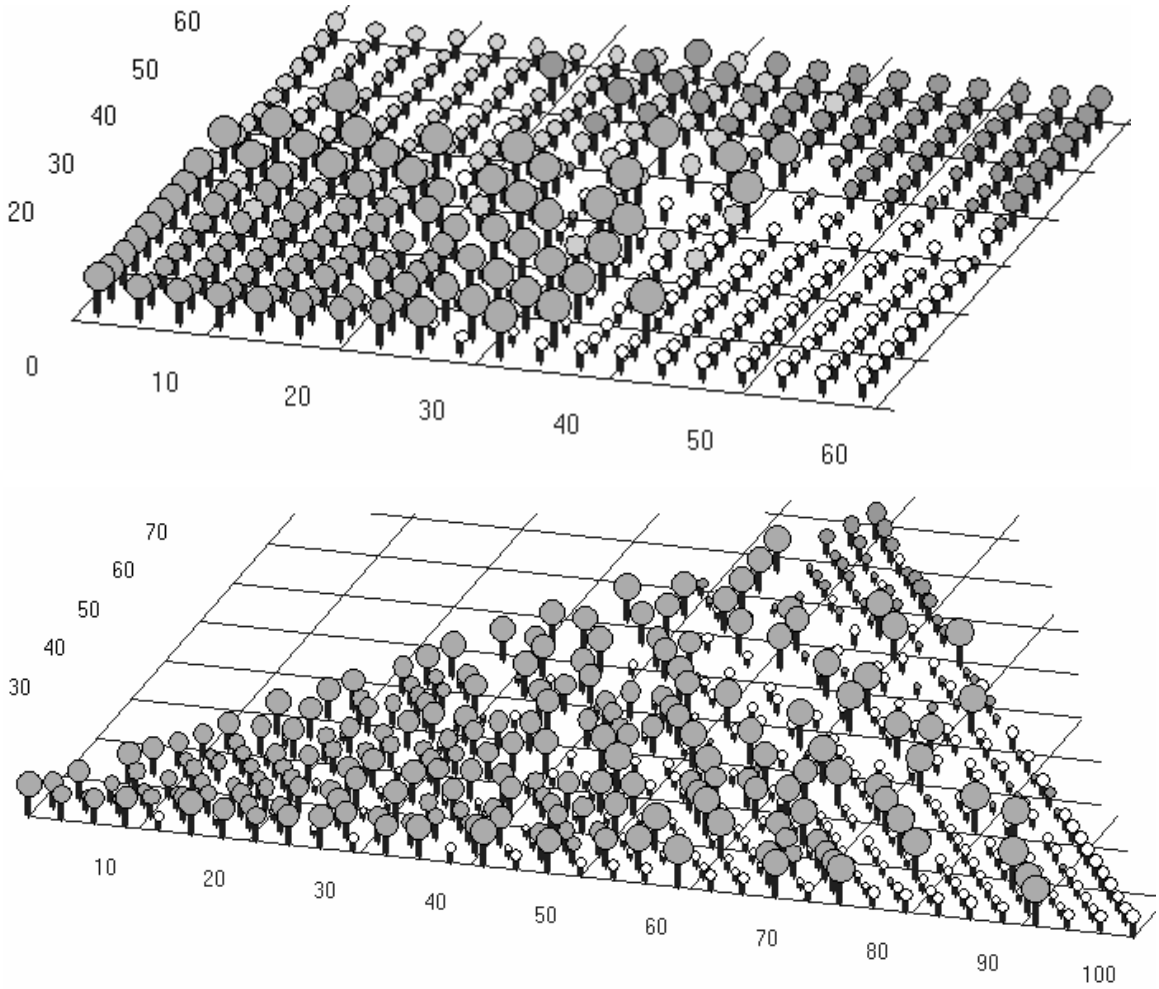

Figure 6. Simulation output using Simile (Muetzelfeldt and Massheder 2003, Vanclay 2006) illustrating how a mixed planting of acacia, eucalypt and one or two hypothetical species may develop in two different trial layouts. Acacia (white) is simulated in the right front apex, and eucalypt (grey) is simulated in the left front apex. One of the hypothetical species (dark grey, right rear apex) is based on the average of the eucalypt and acacia parameters, while the other hypothetical (light grey, left rear apex, square only) is based on the acacia parameter, but is not influenced by and does not influence other species. Guard rows have not been simulated.