Systematic Experimental Designs
For Mixed-species Plantings

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ABSTRACT

Systematic experimental designs provide splendid demonstration areas for scientists and land managers to observe the effects of a gradient of species composition. Systematic designs are based on large plots where species composition varies gradually. Systematic designs save considerable space and require many fewer seedlings than conventional mixture designs. One basic design incorporates a large triangular plot; in concept this plot is identical to the well-known soil textural triangle. The intent of the designs is to produce a response surface over species composition, rather than test for significant differences between 2 specific species compositions. Another design superimposes a species composition gradient on a Nelder's design, which systematically varies planting density. It is possible to study mixtures in multiple strata, such as overstory trees and herbaceous understory. The systematic mixture designs are most effective when considering 2 to 4 species.

KEY WORDS: competition experiment, diversity, interference, mixture experiments, multiple species, Nelder's design, response surface design

Planting of mixtures of species is often preferred to monocultures due to a desire for greater biodiversity or an appearance more similar to natural plant communities. Potentially, mixtures could be more productive than monocultures if the different species utilize different resources or if 1 species improves the productivity of the other, typically by fixing nitrogen (Assman 1970; Kelty 1992). However, productivity of different mixtures will vary, regardless whether the "products" are crops, timber, biomass, wildlife habitat, or aesthetics.

When mixtures are planted, it is desirable to determine how species composition determines growth and survival of individual plants, as well as overall productivity per unit area. Below, I describe some novel systematic designs after providing the context of traditional competition and mixture experiments.

Brief Review of Competition Experiments

Harper (1977), Radosevich (1987), Cousens (1991), and Goelz (1995b) review the design of competition experiments. Some of their main points are synthesized below. The standard design for two-species mixtures is the de Wit replacement series (de Wit 1960; Harper 1977). The replacement series involves treatment combinations where overall density per area is held constant, but the proportion of 1 species to another changes, usually in a symmetrical pattern; typically monospecific stands are included as the extremes of the treatments. The effect of overall density can be addressed with multiple replacement series. Replacement series designs are called "substitutive" designs.

Additive designs involve keeping the density of 1 species constant while varying density of the other species; thus the overall density of plants per unit area varies with the treatment. Harper (1977) believes that substitutive designs are easier to interpret than additive designs. However, Snaydon (1991) implores that substitutive designs are statistically invalid and biologically confusing. However, Sackville Hamilton (1994) refutes Snaydon's (1991) claims, and suggests that additive and substitutive designs address different issues. Additive designs quantify inter-taxa competition, regardless of of intra-taxum competition, and substitutive designs address questions concerning the similarity of competing taxa, and contrasts inter-taxa and intra-taxum competition. Thus, it seems that the choice of additive or substitutive designs depends on how one chooses to measure competition effects. Benefits of both types of designs may be obtained by establishing multiple replacement series with differing overall planting densities (Spitters and others 1989).

For 3 or more species, a design analogous to a diallel analysis in genetics may be employed. In this design, all possible two-species and single species stands would be planted (Norrington-Davies 1967, 1968; Norrington-Davies and Hutto 1972). If an extremely large number of species are employed, diallel designs without all possible combinations can be used. Usually monospecific stands and 50:50 mixtures of the 2 species are planted, however, Nance (1984) used 25:75 and 75:25 mixtures in a study involving different genotypes of the same species. These diallel designs could be viewed as a group of replacement series employing a limited choice of proportions. The diallel-like studies have the greatest promise in screening species that have (in genetics terms) high general combining ability (species that tend to do well regardless of which species it is mixed with) and high specific combining ability (species that combine well with a specific species).

Brief Review of Statistical Mixture Experiments

Cornell (1990) provides an excellent review of mixture experiments that are common in research for developing products as diverse as fruit punch and concrete. The experimental designs largely follow from Scheffé's simplex lattice and simplex centroid designs (Scheffé 1958, 1963). The simplex is simply the projection of an n-dimensional space onto an n-1 dimensional coordinate system; this can be done because the proportions of the mixture are constrained to sum to 1.0. Thus, feasible combinations of 3 components can be projected onto a two-dimensional triangular field (Figure 1); the familiar soil textural triangle is a simplex. The simplex of a mixture of 4 components is a three-dimensional solid equilateral tetrahedron. The "lattice" part of the simplex lattice design reflects that treatment combinations are spaced regularly on the simplex (see Figure 2 for a simplex lattice design of degree three for 3 components). The degree of the simplex lattice is defined by the degree of the polynomial that may be used to fit the response surface over the simplex. The simplex-centroid design includes only even (equal proportion) mixtures. Thus, for a three-species design, the 3 monospecific design points are used (1:0:0; 0:1:0; 0:0:1), the 3 even two-species mixtures (1:1:0; 1:0:1; 0:1:1) are used, and the last point would be the even three-species mixture (1:1:1). Both the simplex-lattice and simplex-centroid may be generalized to any number of species. Numerous designs can be derived from these basic mixture designs (Cornell 1990).

Motivation for Something Different

The main problem in applying Scheffé's designs, and other alternatives, to restoration or reforestation plantings is that the designs all require large numbers of design points. For example, a three-species simplex-lattice design of degree three (a very modest design) requires 10 design points. If different planting densities or soil types are also considered in the experimental design, the number of experimental units could be multiplied severalfold. Then, the study would be replicated sufficiently to detect differences among treatments. If a study is to have adequate buffer areas around plots (Curtis 1983), large areas (and many seedlings) must be devoted to each plot. Even larger plot sizes are indicated if competition among individuals is to be investigated with competition indices involving distance to, and size of, competitors. All of these characteristics were true for a study I wished to install, and conventional designs were beyond my resources (I calculated that a minimal study would involve over 56.7 ha [140 acres] and nearly 100,000 seedlings—all of which would be measured). This provided motivation for the novel systematic design described below.

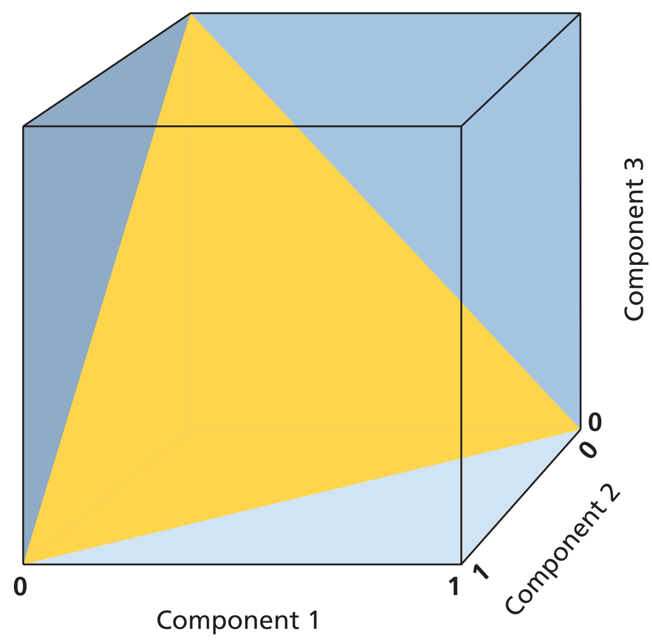

Figure 1 • The triangular simplex of a three-component system. The feasible region is defined by the equation $X + Y + Z = 1.0$.

METHODS

A Systematic Mixed-Species Plot

I chose to create a systematic design to study mixed-species plantations. Systematic designs are useful for fitting response functions, particularly at the early stages of a research program, although they are not well-suited to test for differences between 2 specific levels of a factor (Mead 1988). Nelder (1962) designs are well-known systematic designs in which plant density varies slowly across a large rectangular, circular, or fan shaped plot. In addition to providing data to assess the effects of the systematically-applied factor, systematic designs provide compact demonstration areas whereby land managers or other scientists can perceive effects of species composition.

This design is based on a large triangular plot in which species composition varies gradually. The con-

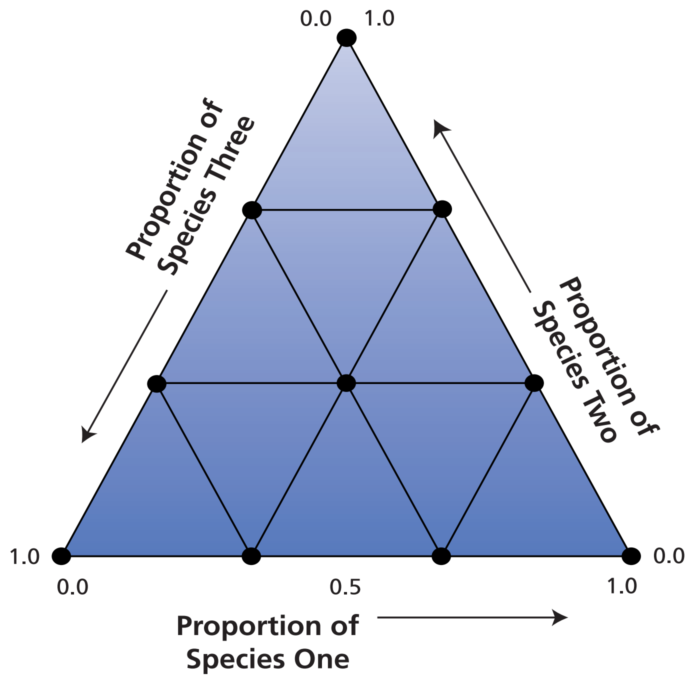

Figure 2 • A three-species simplex lattice design of degree three. The corners of the lattice represent monospecific stands. The other points along the faces represent two-species mixtures in the proportion of 1/3:2/3 . The point in the center represents an even three-species mixture. The lines on the interior of the triangle represent lines of 1/3 and 2/3 contribution of a given species (from Goelz 1995b).

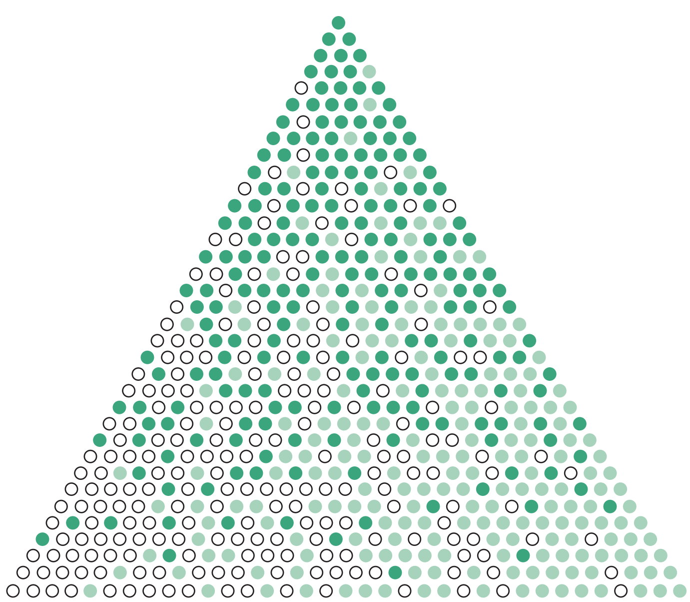

Figure 3 • The systematic mixed-species plot. There are 630 trees, 210 of each species represented by shading of the circle (from Goelz 1995b).

cept is equivalent to the commonly-used soil textural triangle, where the 3 "species" are clay, silt, and sand. Each vertex of the plot is dominated by a single species; the proportion of a given species decreases from 1 vertex to the opposite face. Each side of the triangle represents a two-species gradient. The middle of the triangle is a mixture of all 3 species (Figure 3).

The proportion of a given species is determined by the location within the simplex. In a triangular spacing, there are 3 orientations of rows, rather than 2 orientations of rows, as in rectangularly-spaced plantations. In Figure 4, 3 gradients of species composition are identified by lines of equal proportion. Solid lines represent rows that have a specified proportion of species one. The proportion varies from zero on the left side of the triangle (the row that proceeds from the lower left of the triangle to the peak of the triangle), to 100% at the lower right vertex of the triangle (the 100% "row" is 1 planting spot). Similarly, the dashed lines represent proportions for species two, and the dotted lines represent proportions for species three. In a triangular plot with n planting spots on a side, there are n rows for each of 3 orientations. The number of planting spots per row varies from n to one. Rows associated with 1 species are indicated in Figure 5. The longest row lacks seedlings of this species (proportion is 0.0). The shortest row contains a solitary seedling of this species (thus this row, located at the top of the triangle, has a proportion of 1.0 for this species).

In Figure 4, an individual planted at location A would be species one 60% of the time, species two 20% of the time, and species three, 20% of the time. Similarly, location B would have probabilities of 30%, 40%, and 30% for species one, two, and three, respectively.

Each point on the triangular plot can be identified by a three-digit number representing the "row," i, j, k, corresponding to each species, 1, 2, 3. The row (i, j, or k) equals 1 for the longest row of each orientation and equals n for the shortest row. The probability of a given species at a given planting spot will be:

[1]
$$p_1 = \frac{i-1}{n-1} \qquad p_2 = \frac{j-1}{n-1} \qquad p_3 = \frac{k-1}{n-1}$$

Each side of the triangle has n planting spots. The total number of planting spots in the entire triangle will be n((n+1)/2). When n or (n+1)/2 is a multiple of three, the number of planting spots will be divisible by three, and this will allow equal representation of the three species.

These probabilities could be used to assign species to a planting spot. A uniform random number would be drawn. If the number is between 0.0 and p_1, then species one would be assigned. If the number is between p_1 and p_1+p_2, then species two would be assigned, and if the number is greater than p_1+p_2, then species three would be assigned. However, by assigning species completely randomly, the assignment of species might end up very different from the intended species proportion.

Assigning Species to a Spot

Three objectives could be desired for assigning species to planting spots: (1) symmetry; (2) equality; and (3) conformity to the intended pattern. Symmetry requires that for every planting spot assigned, the 2 corresponding planting spots be assigned corresponding species. For example, if the planting spot 6, 2, 2 (location “A” in Figure 4) was assigned species one, then planting spot 2, 6, 2 should be assigned species two, and planting spot 2, 2, 6 should be assigned species three. If planting spot 6, 2, 2 had been assigned species three, by chance, then planting spots 2, 6, 2, and 2, 2, 6 should be assigned species one and species two, respectively. Thus, to ensure symmetry, assigning species to 1 planting spot will also assign species to 2 corresponding planting spots. Thus, if symmetry is imposed, only one third of the planting spots will actually be randomly assigned, the other planting spots will be specified by the symmetry restriction. Equality merely requires that equal numbers of each species be assigned to each plot. This will be achieved if the number of planting spots per plot is a multiple of three and if symmetry is imposed.

Conformity to the intended pattern requires that the species proportion in any subsection of the triangular plot is close to the expectations. Several methods will help obtain this. An algorithm could be specified that assigns species to planting spots, given a symmetry constraint, and otherwise minimizes the deviation from expectations. However, when I attempted this, the algorithm produced unwanted behavior—such as “unlikely” species assignments were placed on the interior of the triangle and never on the margins. Given the objectives of such studies—to describe the effects of species composition rather than test for differences between any 2 specific species compositions—there is probably no need to specify “optimal” assignments of species. Still, it seems desirable that the assignment of species is not too much different from expectations. Below I have listed 1 algorithm that will tend to ensure assignment is close to expectations.

The first step is to break up the large triangular plot into triangular subplots of 15 to 55 planting spots (base of the triangle would be 5 to 10 spots wide). Calculate the expected number of each species for each subplot by summing the p_i for each species. Then use the following equation to calculate probabilities (this is equal to equation [1] for the first assignment of species to planting spot)

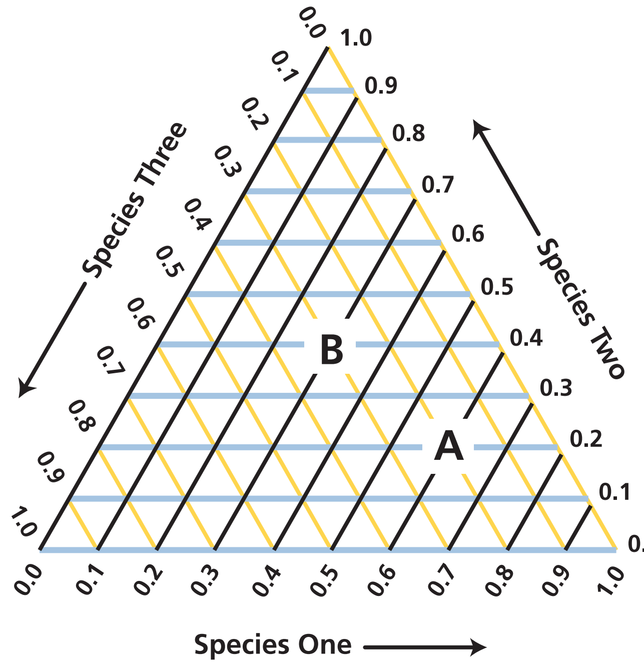

Figure 4 • A systematic mixed-species plot with 11 planting spots on a side. Black rows are labeled by proportion of species one, blue rows are labeled by proportion of species two, and yellow lines are labeled by proportion of species three. Planting spots are located at the intersection of rows.

[2]
$$P_i = \frac{p_i \dfrac{\eta_i - n_i}{\eta_i}}{\sum_{j=1}^{3} p_j \dfrac{\eta_j - n_j}{\eta_j}}$$

where P_i is the modified probability of species i, P_i is the unadjusted probability defined by equation [1], n_i is the number of planting spots already assigned to species i, and η_i is the expected number of seedlings of species i. This will ensure that species composition does not deviate by more than a fraction from expectations, at least at the scale of the subplots. Additionally, one could constrain the total for each row to differ by a fraction from expectations. The foregoing only represents one of many alternatives for approaching conformity to expectations.

If the planting is established as a demonstration area rather than a study, conformity could be achieved by arbitrarily swapping around species to break-up large blocks of 1 species, or to break up unlikely concentrations of a species where it should be rare. Alternatively, the distribution given in Figure 3 could be used in other studies.

For this, and all other designs described herein, border rows should surround the plot. Species assigned to a planting spot within a border row should reflect the adjacent row of the plot. In this

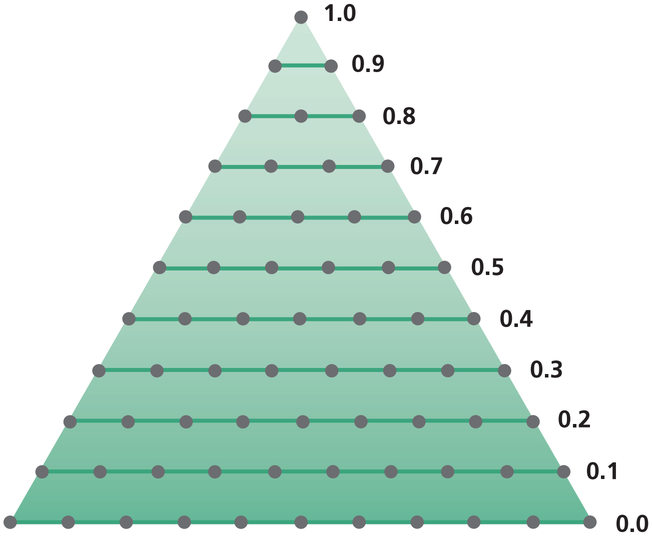

Figure 5 • One of the three series of rows is extracted from Figure 4. The rows vary from 11 planting spots to 1 planting spot in length.

design, a planting spot in a border row has 2 adjacent planting spots within the "measurement" plot, and the assigned species can be a 50:50 proportion of the species of those 2 spots. Of course, if the 2 spots were the same species, then species in the border row would be determined without any random draw. The concepts of symmetry and conformity may be applied to the border rows as well. The presence of border rows will also increase the number of individuals whose nearest neighbors are all conspecifics.

Experiments for Subsection of the Simplex

Situations exist where interest is restricted to a portion of the simplex. For example, 1 species may be most desirable for most products produced by the community, but diversity might also be desired. For example, if species one is the most-desired species, only the portion of Figure 4 that is to right of the $p_1 = 0.5$ line might be used. Any other subsection of the simplex could be used, and the resulting systematic plot could be an equilateral triangle, a hexagon, or parallelogram.

Applying the Design to Broadcast-seeded Species

The design could also easily be applied to broadcast-seeded species. In this case, rather than discrete planting spots, the entire triangular area would be seeded. Since the spacing in Figure 4 is triangular, the area around each planting spot is hexagonal (bisecting a line to each of the nearest 6 planting spots will locate the midpoints of each hexagonal side). These hexagonal areas could be seeded by actual mixtures of 3 species. For example, location A in Figure 4 could be seeded with a 6:2:2 mixture of the 3 species. If numerous individuals are planted in each hexagon, sampling could be restricted to an interior circle within the hexagon.

Expanding the Design for 4 Species

It is simple to apply this design to combinations of 4 species. In this case, the 4 different three-species combinations would be constructed. A supplemental area would be planted with equal proportions of all 4 species.

More than 4 Species

It would be possible to create all possible three-species combinations of a multitude of species. However, it would probably be prudent to carry out an initial screening study composed of a diallel-type design. The diallel study would identify promising species with regard to their general or specific combining ability and further study would be based on those selected species.

Mixtures in 2 Strata

An investigator may choose to study mixtures of more than 1 community strata (trees and herbaceous understory, for example). When 3 species of each strata are considered, 2 triangular plots could be superimposed on the same area. They could have different planting densities (there could be 50 rows of the trees along a side of the triangle, but 1000 rows of herbaceous planting spots). For each combination of the tree species (species A, B, and C), there would be 3 combinations of the herbaceous species (species X, Y, and Z), (XYZ triangle would be rotated while keeping the ABC triangle stationary, thus producing 3 different combinations, one where the "X" dominated corner coincided with, in turn, each of the A, B, and C-dominated corners). Alternatively, a single mixture of the understory species could be planted, and the growth and survival across the gradient of tree species composition could be observed.

Systematic Density and Species Composition for 2 Species

Competition among individuals will be affected by the distance to neighbors, in addition to the species composition. Thus, it is desirable to vary both planting density and species composition. Planting density could be varied by establishing the mixed-species plots at different spacings. Alternatively, a design could be used that systematically varies both spacing and species composition. This can easily be accomplished by superimposing a species composition gradient tangential to a Nelder's (1962) design. Thus, the Nelder's design will systematically vary stand density radially, and the species composition will vary systematically along the arc (Figure 6). Figure 6 only includes 2 species, but several species could be incorporated into the design by using a relay of species as rotation progressed along the arc. This might be more easily enacted if Figure 6 represented a larger sector (a Nelder's design can comprise a complete circle or circular ring). If interest was only in species composition of a two-species mixture, a simple systematic gradient of species compostion could be applied to a square-spaced plantation.

Results from Systematic Species-mixture Studies

There are 3 ways to derive inference from the mixed-species studies described herein. First, and by no means trivially, is as a physical demonstration area for land mangers and scientists to visualize a gradient of species composition. Second, if inference is only directed at identifying overall success or failure of a mixture, is to provide summary statistics for each species, averaged across entire systematic plots. Third, more formal modeling is indicated if inference is directed at identifying trends in some variable in response to trends of species composition. The modeling may be relatively simple, such as using polynomials of species proportion as independent variables, or it may be more complex, such as spatially explicit models derived to estimate biologically-relevant parameters or test biological theories concerning plant competition. Results could be graphically represented as contour plots based upon raw data or upon modeled values (Figure 7).

SUMMARY

Systematic designs are useful for investigating competition among species, and the effects of species composition on yield. Relative to conventional designs for mixtures, they conserve space, seedlings, and effort while additionally providing splendid demonstration areas. Although best suited for combinations of 2 to 4 species, the designs may be applied to annuals, herbaceous perennials, and are ideally suited for trees or other large plants. Furthermore, multiple strata may be investigated coincidentally, such as both overstory trees and herbaceous understory.

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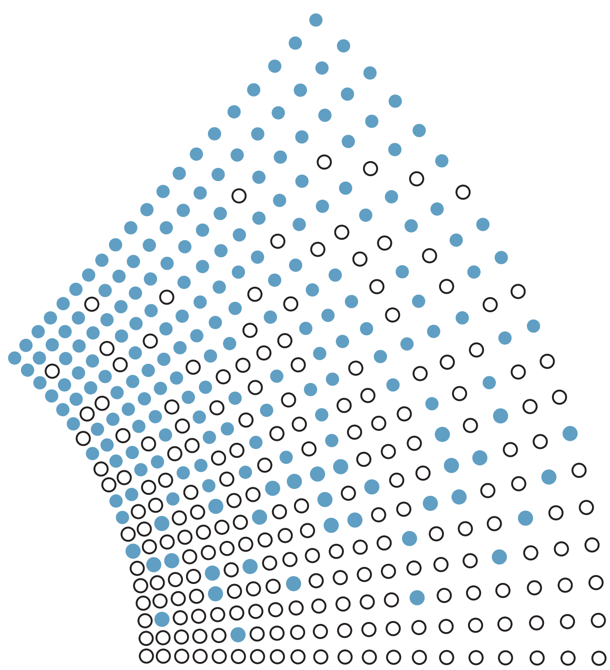

Figure 6 • A Nelder's (1962) design for studying the effects of stand density is superimposed with a gradient of species composition. Shading indicates species. This systematic design allows exploring the effects of density and species composition simultaneously (from Goelz 1995b).

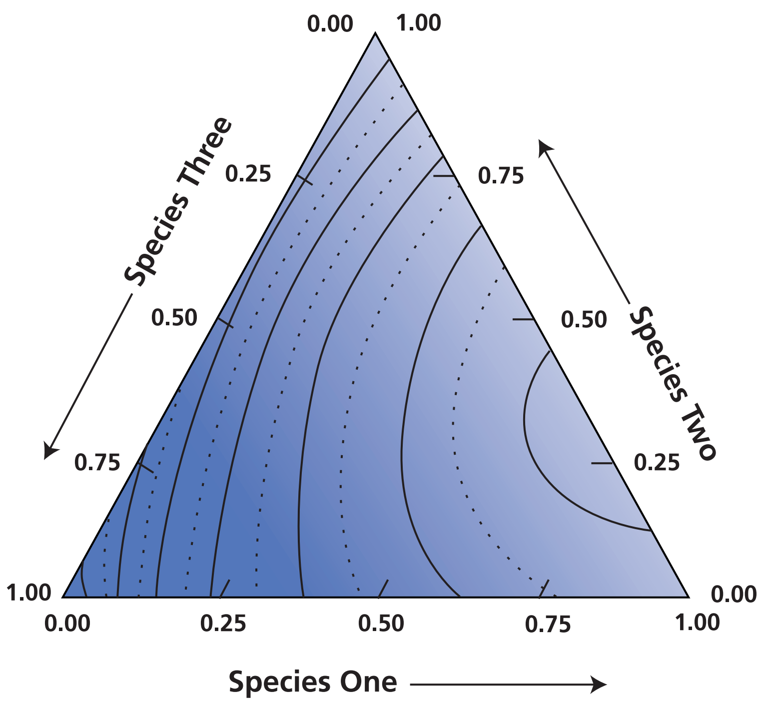

Figure 7 • *A example contour plot describing yield, as related to species composition. Yield is represented as a percentage of maximum yield.*

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