# STATISTICAL DEFINITIONS, DESIGNS, RESPONSE EQUATIONS AND ANALYSES FOR EXPERIMENTS ON FIXED-RATIO MIXTURES IN AGRICULTURE

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## SUMMARY

Treatment designs (the selection of treatments for an experiment) are formed by selecting treatments from or combinations of entities from (i) controls or standards, (ii) factors with discrete levels, (iii) factors with continuous levels, and (iv) fixed-ratio mixtures. Fixed-ratio mixture designs are discussed with respect to applications in agriculture, reasons for utilization, statistical design, and statistical response model equations. A bibliography is also presented.

## INTRODUCTION

Research in and applications of statistical theory have been predominately for univariate responses and for the single type of treatment effect known as a direct effect. This is the effect of a treatment in the period when it is applied. In the real world of research investigations, responses are often multivariate in nature, and several types of treatment effects and side effects may be encountered. In tropical-zone agriculture, and to some extent in temperate-zone agriculture, the growing of mixtures of cultivars in specified proportions or of a specified succession of crops on a given unit of land have been common practices for centuries. The reasons are many and varied, some of which are (i) for increased yields by maximum utilization of environmental resources, (ii) for disease control, (iii) for

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insect control, (iv) for erosion control, (v) for stabilizing annual yields, (vi) for spreading labor and saleable products over a calendar year, and (vii) for decreasing use of commercial fertilizer. Appropriate and/or correct statistical analyses for investigations on mixtures of cultivars have lagged far behind the needs. In fact, it is postulated that the most important statistical problem associated with tropical agriculture investigations at the present time is the statistical design, appropriate response model equations, statistical analyses (both nonsequential and sequential), and statistical inferences for such investigations. Some of the problems and possible solutions are considered in the present paper.

We first present some definitions related to Statistics and statistical design to orient the reader with the context of this paper. Then some possible response model equations, minimal treatment designs, and statistical analyses are discussed. Finally, we present a number of references from agricultural research journals, related to experiments with fixed-ratios of cultivars in a mixture. The references from these journals are for the last twelve-year period.

## STATISTICAL DESIGN

The term "experimental design" is used in many ways, forms, and senses in statistical literature and by statisticians. It is, therefore, useful to define the context within which one is writing or speaking. Prior to this, however, it is useful to put forth a definition of Statistics, the subject. A frequently used definition is that Statistics is concerned with the characterization, development, and application of techniques for

(i) the statistical design of an investigation whether it be an experiment, a survey, an observational, or a model building study,

(ii) the summarization of the facts from the investigation, and

(iii) the inferences that can be drawn from the facts of the investigation about the parameters in the population (i.e., generalizing from the specific sample to the underlying population).

Statistical design of an investigation encompasses many items, and it is expedient in the interest of nonambiguity to clearly define their use relevant to the present context. Some of these items are:

1. Variables and Populations: A complete description of the variables of interest including the population characteristics for each variable separately as well as jointly. The nature of the variation and the goals of the investigation should be as precisely and completely specified as possible. The sampling unit, the experimental unit, and the observational unit should be precisely and clearly defined.

2. Treatment Design: The treatment design constitutes the selection of treatments (entities of interest) to be used in an experiment. It must be such that the objectives of the investigation can be achieved. Adequate points of reference (controls) must be included.

3. Experiment Design: The experiment design is the arrangement of the treatments in the experiment. It should be one that yields the desired contrasts among treatment parameters and the contrasts should have high precision and low bias.

## TYPES OF TREATMENT DESIGN

Conceptual and inferential errors may arise because of vague and imprecise definitions and formulations. This is the present situation in current statistical literature with respect to treatment design in that many writers do not distinguish between the various types of treatment design; they use the term "experimental design" as a catch-all for treatment design, experiment design, selection of sample size, and perhaps other items. Many textbooks with the term "design of experiments" in the title usually have little or nothing on the planning of experiments, but spend a considerable amount of space on statistical analyses for experiment and treatment designs as defined in the previous section. Writers frequently do not distinguish between factors which have discrete (qualitative) levels and those which have continuous (quantitative) levels. The statistical response model equations and analyses often differ for the two cases, although analyses for discrete data may be approximated with continuous models and vice versa.

Types of treatments that constitute a treatment design may be categorized as follows:

1. Controls, standards, checks, placebos, or other items required as experimental reference points.

2. Discrete levels of the variables or factors under study in an experiment. (These are sometimes denoted as qualitative factors. The commonly known factorial design falls in this category.)

3. Continuous levels of factors or variables under study in an experiment. (These are sometimes denoted as quantitative factors. The so-called "response surface" designs fall in this category.)

4. Mixtures of k of v factors with the proportion of each factor in the mixture being specified by the experimenter, i.e., there is only one level per factor. (The commonly known diallel crossing system in breeding, fixed ratios of densities of two or more crops in an intercropping system, fall in this category.)

5. Combinations of categories 1 to 4.

A treatment design consists of treatments from one or more of the above categories.

In category 1, each field of investigation, whether it be agronomy, medicine, education, physics, breeding, etc., should have treatises on points of reference which act as standards or checks with which to compare other treatments. However, this is not always the case, and most of this information is in the minds of senior investigators with each new entrant in the field being forced to learn by experience. Statistical literature virtually ignores this problem with the rationale being that this should be discussed by the subject matter specialist. The result is that category 1 is simply taken for granted in many fields and sometimes no, or inadequate, controls are included in the treatment design.

Categories 2 and 3, treatment designs, are well discussed in the literature. A subset of category 3 has been called "designs for mixture experiments", and a bibliography may be found in Cornell (1973, 1979). The factor space of these designs is continuous and this mixture problem is characterized by the fact that the relative proportions of the factors rather than the amount, influence the response. One is interested in obtaining estimated proportions giving maximum or minimum response as well as a characterization of the response function over the factor space. The total of the amounts of individual factors in a mixture of this type is held constant, with only proportions being varied.

The treatment designs under category 4 are also called "designs for mixtures", but their purpose is entirely different. The relative proportions of k of v factors <u>are</u> <u>specified</u> and inferences are made about these specified proportions. Thus, the population factor space is entirely different in nature for this type of design and the one for response surface designs. The fact that the treatment designs may be identical for this type of mixture design, for a response surface design, and for a fractional replicate of a factorial design has led to some confusion to which one of the authors had contributed (see, e.g., Federer (1967)).

There are many situations for category 4 designs for which the proportion cannot be varied. Many types of experiments such as nutritional, agronomic, etc., will involve fixed proportions of the factors involved. Statistical analyses and inferences possible with the mixture designs in category 3 are usually not appropriate for the mixture designs in category 4. Different concepts, population factor spaces, and hypotheses are required for all categories. The bulk of the work on statistical designs for category 4 designs has been for diallel cross experiments (see, e.g., Randall (1976) for a review and bibliography on the topic), matched pairs experiments (see, e.g., David (1963)), and tournaments. Mixtures of $k > 2$ have received little discussion (see, e.g., Federer (1979)) in statistical literature. In agriculture mixtures of pairs (either in the same experimental unit or in alternate rows) and pure stands (sole cropping) have been discussed by Sakai (1961), Williams (1962), Jensen and Federer (1964, 1965), McGilchrist (1965), McGilchrist and Trenbath (1971), and references listed under A14 in Federer and Balaam (1972).

## TYPES OF TREATMENT DESIGNS IN CATEGORY 4 FOR AGRICULTURAL EXPERIMENTS

We limit our discussion of <u>fixed-ratio mixture treatment designs</u> to agricultural research investigations which fall into category 4 of the previous section. Each writer has his own classification scheme, just as we have ours. Hence, the reader should not construe the scheme given to be the only one in the literature. The first distinction to be made is whether the experiment is a short term one (one year or less) or a long term (more than one year) investigation. Most statistical and agricultural research and effort has been concentrated on the former type, with long term response models, statistical analyses, and inferences receiving relatively little attention.

The second type of distinction made is whether one crop (single crop, monoculture, monocrop, sole crop, pure stand) or more than one crop, occupies the same experimental unit. Again, the majority of the statistical and agricultural literature is on single crop and single response investigations. Adaptations of response models and statistical procedures for sole cropped experiments were often used for multiple cropping investigations. Multiple cropping implies that more than one line, cultivar, crop, or species occupies the same experimental unit; it has also been denoted as mixed cropping, polyculture, intercropping, and perhaps other terms. We shall use the terms single cropping and multiple cropping. The latter involves treatment designs for mixtures of lines, cultivars, or species.

There are several types of mixtures involved in short term multiple cropping situations; some of these are:

(i) Two or more crops are randomly mixed within the same experimental unit; the proportion of the mixture is specified by the investigator. Two types of response are possible, that is, only a total of the mixture for an experimental unit is available or the responses for each member of a mixture for an experimental unit, are available. Only total responses of a genetic cross, of a mixture of wheat lines, etc., are available for some types of investigations. In other types of investigations and when individual members' responses are distinguishable (e.g., different colored seeds, different plant types, different times of harvest, etc.), individual member yields are obtainable.

(ii) Individual plants are alternated according to some systemmatic plan. Plans of this type are often for the purpose of understanding the basic physiology of competition. They may also be utilized in insect and disease studies involving population levels of the insect or disease. Mead (1979) has designated the different aspects of competition experiments as given in Table 4.1. Here individual plant yields may be obtained, resulting in individual member responses for each experimental unit.

(iii) Rows of members of a mixture are alternated for crops requiring the same growing season. An example of this is the use of alternating sets of rows of soybeans and cowpeas to control insects affecting cowpeas in Nigeria. Another example is alternating rows of a legume (e.g., soybeans) which has been innoculated with nitrogen fixing bacteria and a non-legume (e.g., corn) in Iowa; the purpose is to find a natural source of nitrogen to replace the increasingly costly commercial fertilizers. Here responses are available for each member of the mixture in an experimental unit.

(iv) For crops with a different length of growing season, shorter term crops may be planted in between the rows of the longer season plants; they are harvested early and the longer season plants then occupy the experimental unit as a single crop during the last part of the growing season. An example would be melons or sweet corn with sugar cane. Another example would be melons and cassava where the melons mature in a few months but cassava requires a year-long growing season. Another example would be an orchard of fruit trees and a hay crop planted between the rows of trees. Still another example would be a stand of plantain trees underplanted with yams and/or tumeric.

(v) Vines or creepers are planted in a stand of trees or bushes when the vines or creepers require some sort of standard for climbing. An example would be betel or pepper vines on palm trees.

(vi) A sequence of crops on the same experimental unit in a specified period, say one year, is denoted as successive cropping. In many areas of the world it is possible to obtain more than one crop in a given year. For example, in Nigeria it is possible to obtain two crops of corn, soybean, or cowpeas but only one of cassava. In New York experiments are being conducted on sweet corn — soybean, sweet corn — sweet corn, sweet corn — melon, and sweet corn — soybean sequences of successive cropping in a single season. These are being coordinated with successive cropping investigations in Malaysia.

Other types may be possible. A similar outline could be constructed for long-term agricultural experiments. An outline of types of long-term experiments is presented in Table 4.2 which was adapted from Cochran (1939). It should be noted that investigations for short-term experiments are interested solely in the effect of the treatments in the periods when they are applied, that is, direct effects. When we come to long-term and/or repeated measures experiments, it is necessary to consider several other types of treatment effects such as the residual (effect of a treatment in periods beyond the period of treatment application), cumulative (the additional effect of application over no application), and permanent (direct plus residual). A description and discussion of these terms have been given by Yates (1949) (also in Federer (1955)). These additional effects in a linear model can considerably complicate the design, the analysis, and the inference structure. The majority of statistical work relates to investigations wherein only direct effects are present with relatively little literature on the more complicated treatment effects. Many repeated measures experiments are analyzed as if there were no effects other than direct. The existence of residual effects is mentioned only in a half dozen of the numerous textbooks on statistics.

In long-term agricultural experiments we can have a specified treatment applied to an experimental unit planted to a given crop as follows:

(i) applied in first year only,

(ii) applied at specified intervals, and

(iii) applied every year.

Instead of applying the same treatment to an experimental unit, one can have a succession of treatments. This is called a rotation. Repetitions of rotations through years are called cyclical rotations. Rotation experiments can be of many types and generally it is necessary to create a new design for a proposed new rotation experiment. The designs often become quite complicated and their construction and analysis is often time-consuming. The following represents a classification of rotation experiments:

(i) experiments comparing effects of treatments on the crops of a single rotation for one phase (the number of years it takes to complete the succession of crops applied to an experimental unit) or for several phases,

(ii) experiments in which different rotations, as well as the effects of individual rotations, are to be compared, and

(iii) experiments of type (i) or (ii) in which the treatments are modified or changed and treatments added or deleted.

It should be noted that rotation experiments are sequential in nature, but the primitive state of sequential analysis theory does not offer much help here. This should be an ideal place to apply sequential analysis, since there is usually an entire year between crop yields which would give sufficient time for an analysis.

There is a literature on the design and analysis of rotation experiments which includes Cochran (1939), Crowther and Cochran (1942), Yates (1949, 1952, 1954), Patterson (1964), and a number of other references listed under category E5 in Federer and Balaam (1972). As pointed out in Patterson (1964), crop rotation has been practiced in England for over 1500 years. Considerable increases in yield can be obtained using appropriate rotational practices. For example, many years ago in Western Iowa, continuous cropping of corn resulted in a 24 bushel per acre average. The introduction of a four course rotation of first year red clover, second year red clover, corn, and oats, increased the yield of corn fourfold. Other benefits of rotations are erosion, disease, and insect control. Just as crop rotation has a long history so does multiple cropping, especially in tropical agriculture. Mechanization tended to eliminate intercropping in the temperate zones, but here, as in the tropics, it is beginning to be realized that there could be a number of benefits from multiple cropping programs. Some of these are:

(i) Losses due to disease and insects can be reduced. The growing of mixtures of varieties of a cereal wherein the varieties are resistant to different diseases can stabilize yields.

(ii) A better utilization of soil nutrients and water can be achieved with a mixture when the members of a mixture utilize different nutrients or require the nutrients and water at different times or from different parts of the soil.

(iii) As energy becomes more costly, fertilizer costs will rise accordingly and the use of cultivars in a mixture which are mutually beneficial to each other will become more prevalent (e.g., a legume planted next to a nonlegume).

(iv) Certain crops require shade or a standard on which to grow. This necessitates planting taller cultivars with shorter ones. For example, perennial trees can be grown with creepers or vines.

(v) The growing of a profitable crop with a nonprofitable one such as corn and watermelons or cassava and melons. In the latter case the yield of cassava in one experiment in Nigeria was actually increased, but the yield of melons was reduced to one-third of the yield of melons grown alone. Thus, there was a net gain in the more profitable crop cassava, and a decrease in the less profitable crop. Often a second crop does not decrease the yield of the main crop and the yield from the second crop provides the additional profit, for example, corn and watermelons.

(vi) In parts of the tropics it is desired to spread the labor and saleable material over the entire year. This can only be done with multiple cropping systems. On some farms in Nigeria as many as 62 species on ten hectares have been recorded.

In light of the reawakening of agricultural researchers to the benefits derived from mixtures, it becomes necessary for the statistician to confront the statistical problems of design, analysis, and inference which accompany investigations on mixtures. The world of investigation in the real world is fraught with statistical problems whose solutions can provide great challenges to the statistician. In the next section we shall discuss a few of these.

Table 1. Different aspects of competition experiments

| | | Single Crop Species | Genotype Mixtures for Single Crop | Two or More Crop Species |
|---|---|---|---|---|
| Individual Plant Investigations | Spatial Arrangement Factors | Effects of different numbers of neighboring plants<br>Effects of local plant density | Pattern of competing plants | |
| | Other Factors | Plant interaction models | | The physiological basis of competition |
| Crop Mean Yields | Spatial Arrangement Factors | Response models for yield-density relationships<br>Designs for response model estimation | | Experimental designs for spatial arrangement treatments in intercropping<br>Methods for analyzing |
| | Other Factors | | Genotype competition models | Intercropping experiments<br>Genotype comparison experiments in intercropping |

Table 2. Types of long-term experiments

| Treatments | | | Information Supplied on | | Crops |
|---|---|---|---|---|---|
| Fixed | Applied on the same plots | Every year | Cumulative effects | x | Single crop: annual / perennial |
| | | First year only | Residual effects | | |
| | | At fixed intervals | Direct and residual effects | | Fixed rotation |
| Rotating | Applied on different plots in successive years | | Direct and residual effects | | Effects of different crops |

## SOME STATISTICAL PROBLEMS RELATED TO RESPONSE MODEL EQUATIONS, STATISTICAL ANALYSES, AND INFERENCES

Statistical literature is overwhelmingly concerned with "the" linear model, univariate response, homoscedasticity, hypothesis testing, direct effect of treatments, single samples from identically and independently distributed populations, and attempts to make Statistics as easy as possible for the student. The real world is entirely different. One has to first describe variables and the population being sampled. Then, one must describe the sampling procedure used to obtain the experimental units and the nature of observations after the investigation has been conducted. The response model equation or a suitable approximation thereof must be determined, and not simply defined as it is in statistical literature. The entire subject of "model building" or "model selection" has been ignored in statistical text-books. Recent developments in data analysis can be utilized effectively here. Now, when one knows (not assumes) the sampling structure for a prescribed variable and population, the statistical design, the response model equation, and the distribution of random effects in the model, one may be in a position to perform a statistical analysis and aid in making interpretations of the data.

When one considers a statistical analysis for mixtures, many problems arise. The first question is how to make use of the responses obtained. To illustrate, suppose that we have an experiment involving one crop of cassava per year (treatment one), two crops of corn per year (treatment two), two crops of soy-beans per year (treatment three), and two crops of cowpeas per year (treatment four). The total yields of these four treatments are very different, have different error variances, different insect and disease problems, different nutritional values, and different economic values per kilogram. The point is, we wish to compare these four treatments instead of considering each crop individually as is usually done. What measure should be used? Some univariate contenders that come to mind are:

(i) total calories,

(ii) total yield in kilograms,

(iii) total economic value or profit,

(iv) land equivalent ratio (Willey and Osiru (1972)) or relative yield total (DeWit and Van den Bergh (1965)), (defined to be the relative land area for single crops to produce the amount obtained with a mixture), and

(v) yield per calendar month of a year.

Or, should one consider each treatment yield as a variate and use

(i) multivariate procedures,

(ii) linear programming procedures,

(iii) stochastic programming procedures, or

(iv) some other approach?

The exact approach to be used for a particular study requires thought, investigation, and applications to actual experimental data. Perhaps several approaches will be necessary.

One particular univariate approach for mixtures has been put forth by Federer (1979), who has given a linear model for a mixture of k of v cultivars with general mixing, and $n^{th}$-specific mixing effects, $n = 2,\cdots,k$ both for total yields of a mixture and for individual component yields of a mixture. The response model for experimental unit totals for $k = 3$ is given below for a fixed-ratio mixture treatment design and a randomized complete block experiment design:

$$Y_{ghij} = \mu + \rho_g + [\tau_h+\delta_h+\tau_i+\delta_i+\tau_j+\delta_j]/3 + 2[\gamma_{hi}+\gamma_{hj}+\gamma_{ij}]/3 + \pi_{hij} + \epsilon_{ghij} , \quad (1)$$

where $\mu$ is an effect common to every observation, $\rho_g$ is the $g^{th}$ block effect, $\epsilon_{ghij}$ have mean zero and common variance $\sigma^2_\epsilon$, $\tau_h(h,i,j=1,2,\cdots,v)$ is the $h^{th}$ cultivar effect when grown in pure stand, $\delta_h$ is the general mixing effect for cultivar h when grown in a mixture, $\gamma_{hi}$ is the bi-specific (interaction) effect of the pair of cultivars h and i when grown in a mixture, and $\pi_{hij}$ is the tri-specific mixing effect of cultivars h, i, and j when grown in a mixture.

When individual yields of a mixture are available, a response model equation would be obtained for each member of the mixture. For k = 3, Federer (1979) has given the following equation:

$$Y_{gh(ij)} = (\mu+\rho_g+\tau_h+\delta_h+\gamma_{hi}+\gamma_{hj}+\pi_{hij})/3 + \epsilon_{gh(ij)} , \quad (2)$$

where the subscript h, i, or j not in parentheses indicates the yield for that cultivar in the presence of the cultivars in the parentheses. Thus, $Y_{gh(ij)}$ is the yield for cultivar h grown in the mixture hij. The effects $\mu$, $\rho_g$, and $\pi_{hij}$ as defined for equation (1) are split equally between cultivars h, i, and j. Likewise, a bi-specific mixing effect, say $\gamma_{hi}$, is split equally between the pair involved. This may not be a tenable model for some fixed-ratio mixture designs. In fact, most of the bi-specific mixing effects could be allocated to one of the cultivars involved. The same situation could prevail for the tri-specific mixing effect $\pi_{hij}$. One would need to alter the response model equation (2) as follows:

$$Y_{gh(ij)} = (\mu+\rho_g+\tau_h+\delta_h)/3 + \gamma_{h(i)} + \gamma_{h(j)} + \pi_{h(ij)} + \epsilon_{gh(ij)} , \quad (3)$$

where $\gamma_{h(i)}$ is a bi-specific mixing effect of cultivars h and i grown in a mixture that is attributable to cultivar h and $\pi_{h(ij)}$ is a tri-specific mixing effect of cultivar h when grown in a mixture involving the three cultivars h, i, and j. From results on wheat variaties (see Jensen and Federer (1964)) this would appear to be a more suitable response model equation than equation (2). It would appear that a variety of statistical response models may be required.

Hall (1976) has provided a generalization of response equation (1). He considers that the general and $n^{th}$-specific mixing effect depend upon the the number in the mixture. The general mixing effect $\delta_h$ say, depends upon whether $k = 2,3,\cdots$. Likewise, a bi-specific effect, $\gamma_{hi}$ say, depends upon the number of cultivars, k, in a mixture; a bi-specific mixing effect in mixtures of size two is different from the same effect in mixtures of size three.

Minimal treatment designs to obtain unique solutions (denoted as $\hat{\tau}_h$, $\hat{\gamma}_{hi}$, $\hat{\pi}_{hij}$, etc.) for effects under the restraints $\Sigma_1^v\hat{\tau}_h = 0$, $\sum_{\substack{1\\h\neq i}}^{v}\hat{\gamma}_{hi} = 0$,

$$\sum_{\substack{h,i,\text{ or }j\\h\neq i\neq j}}^{v}\hat{\pi}_{hij} = 0, \text{ etc., or } \Sigma_1^v\hat{\tau}_h = 0, \sum_{\substack{i=1\\\neq h}}^{v}\hat{\gamma}_{h(i)} = 0, \sum_{\substack{i\text{ or }j\\\neq h}}^{v}\hat{\pi}_{h(ij)} = 0, \text{ etc.}$$

has received some discussion by Hall (1976), but further work is required in this area. The restraint $\Sigma_1^v\hat{\delta}_h = 0$ is <u>not</u> imposed on the solutions because $\delta_h$ could all be positive (or negative) in a given experiment, e.g., in single cross hybrids in corn the $\delta_h$ are all positive. The analysis will follow a general linear models analysis with the above restraints.

For $v = 4t+3$, $t = 1,2,\cdots$, a symmetrical balanced incomplete block design is available for which v = b, the number of blocks. These designs form the minimal designs for obtaining unique solutions for $\tau_h + \delta_h$ when there are no $n^{th}$-specific mixing effects. In general, any balanced incomplete block design will do this. For obtaining solutions for both the $\tau_h$ and $\delta_h$, it will be necessary to include experimental units of pure stands of each of the v cultivars. To obtain solutions for the $\tau_h$, $\delta_h$, and $\gamma_{hi}$ only, it will be necessary to have v pure stand treatments and all possible pairs, v(v-1)/2, of the v treatments, i.e., v(v+1)/2 treatments in the mixture design. To obtain solutions for the $\tau_h$, $\delta_h$, $\gamma_{hi}$, and $\pi_{hij}$ effects, it will be necessary to have v + v(v-1)(v-2)/6 treatments in the mixture design. Thus, as the size of the mixture increases and as the number of effects is increased, the number of treatments in the fixed-ratio mixture design increases in order to obtain unique solutions for the parameters in the response model equation. To illustrate, all possible triplets of v = 7 cultivars are given in Table 5.1. Either blocks 1 to 7 or blocks 8 to 14 form a minimal design to obtain solutions for $\tau_h + \delta_h$ only. Blocks 15 to 35 are necessary to obtain solutions for $\tau_h + \delta_h$ and the $\gamma_{hi}$. All 35 blocks are necessary to obtain solutions for the $\tau_h + \delta_h$, $\gamma_{hi}$, and $\pi_{hij}$.

If one uses Hall's (1976) model for the above it will be necessary to have v treatments of pure stands, v(v-1)/2 treatments of mixtures of two cultivars, and v(v-1)(v-2)/6 treatments of mixtures of three cultivars. This gives a total of $v(v^2+5)/6$ treatments. Thus, it can be seen that the number of treatments for a fixed-ratio mixture design becomes large as v, the number of cultivars, increases. This means that serious thought and screening needs to be used prior to conducting these experiments. The supplemented block designs have been suggested by Federer (1979) as possibilities for screening new material to be used in a mixture with standard material.

We are currently pursuing a number of aspects concerned with the statistical design and analysis of fixed-ratio mixture designs. One aspect would be to consider the yields in (2) or (3) as multivariates and use a multivariate analysis. Another is to consider alternative models such as (3). Still another aspect is to investigate further minimal treatment designs for this type of experiment.

Table 3. Thirty-five possible combinations (blocks) of size $k = 3$ for $v = 7$ treatments

| block | | block | | block | | block | | block | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 2 3 | 8 | 3 5 6 | 15 | 1 2 3 | 22 | 1 4 7 | 29 | 2 5 7 |
| 2 | 2 3 5 | 9 | 4 6 7 | 16 | 1 2 5 | 23 | 1 6 7 | 30 | 3 4 5 |
| 3 | 3 4 6 | 10 | 5 7 1 | 17 | 1 2 7 | 24 | 2 3 4 | 31 | 3 4 7 |
| 4 | 4 5 7 | 11 | 6 1 2 | 18 | 1 3 5 | 25 | 2 3 6 | 32 | 3 5 7 |
| 5 | 5 6 1 | 12 | 7 2 3 | 19 | 1 3 6 | 26 | 2 4 6 | 33 | 3 6 7 |
| 6 | 6 7 2 | 13 | 1 3 4 | 20 | 1 4 5 | 27 | 2 4 7 | 34 | 4 5 6 |
| 7 | 7 1 3 | 14 | 2 4 5 | 21 | 1 4 6 | 28 | 2 5 6 | 35 | 5 6 7 |

It may not always be reasonable to assume that the effects due to growing two or more crops in a mixture are additive. However, within a certain range of plant densities, the assumption that yield per unit area is proportional to density is tenable and could be incorporated in the model.

Thus, a third type of model for analyzing and evaluating mixtures of two crops is suggested. The treatments would consist of n different densities of each of the mono-cultures and m mixtures of different densities of the bi-blends. A response model would be given by

$$Y_{ii\ell k} = \mu_i + p_{i\ell}\tau_i + \epsilon_{ii\ell k} \tag{4}$$

where $Y_{ii\ell k}$ = yield for $\ell^{th}$ density of $i^{th}$ crop, $p_{i\ell}$ = density relative to optimum density of $i^{th}$ mono-culture with respect to yield, $1 \le i \le n$, $0 < p_{i0} \le p_{i\ell} \le 1$ [linear relationship of yield and density is assumed in the range $(p_{i0} \le 1)$], $\mu_i, \tau_i$ = parameters associated with yield of $i^{th}$ crop, $\epsilon_{ii\ell k} \sim$ iid $N(0,\sigma_i^2)$, k denotes replicates $1 \le k \le r$.

$$Y_{ij\ell k} = \mu_i + q_{ij\ell}\tau_i + \gamma_{ij\ell}(q_{ij\ell}, q_{ji\ell}) + \epsilon_{ij\ell k} \tag{5}$$

where $Y_{ij\ell k}$ = yield for $\ell^{th}$ density of $i^{th}$ crop when grown in a mixture with $j^{th}$ crop, $q_{ij\ell}$ = density of $i^{th}$ crop relative to its optimum density in mono-culture with respect to yield, $1 \le \ell \le m$, $0 < p_{i0} \le q_{ij\ell} \le 1$, $\gamma_{ij\ell}(q_\ell)$ = effect due to mixture of $j^{th}$ and $i^{th}$ crops on $i^{th}$ crop when grown in the density ratio $q_{ij\ell}:q_{ji\ell}$, and $\epsilon_{ij\ell k} \sim N(0,\sigma_i^2)$ $\mathrm{Cov}(\epsilon_{ij\ell k}, \epsilon_{ji\ell k}) = \sigma_{ij}^2$ .

Using the theory of linear models, we formulate the model as $\underline{Y} = \underline{X}\underline{\beta}$ where $\underline{Y}' = (Y_{1111},\cdots,Y_{111r}, Y_{1121},\cdots,Y_{11nr}, Y_{2211},\cdots,Y_{22nr}, Y_{1211},\cdots,Y_{12mr}, Y_{2111},\cdots,Y_{21mr})$, $\underline{\beta}' = (\mu_1, \mu_2, \tau_1, \tau_2, \gamma_{121}(q_1),\cdots,\gamma_{12m}(q_m), \gamma_{211}(q_1),\cdots \gamma_{21m}(q_m))$, and $\underline{X}$ a matrix of p's, q's, 0's, and 1's, with

$$V(Y) = V = \left[\begin{array}{cc|cc} \sigma_1^2 I & & & 0 \\ & \sigma_2^2 I & & \\ \hline & & \sigma_1^2 I & \sigma_{12} I \\ 0 & & \sigma_{12} I & \sigma_2^2 I \end{array}\right] .$$

The least squares estimates of $\underline{\beta}$ are given by $\hat{\underline{\beta}} = (X'V^{-1}X)^{-1}X'V^{-1}Y$ with $V(\hat{\beta}) = (X'V^{-1}X)^{-1}$, i.e.,

$$\hat{\tau}_i = \sum_{\ell=1}^{n} \frac{(p_{i\ell}-\bar{p}_{i.})(\bar{Y}_{ii\ell.}-\bar{\bar{Y}}_{ii..})}{\sum_{\ell=1}^{n}(p_{i\ell}-\bar{p}_{i.})^2}$$

$$\hat{\mu}_i = \bar{Y}_{ii..} - \bar{p}_{i.}\hat{\tau}_i$$

$$\gamma_{ij\ell} = \bar{Y}_{ij\ell.} - \hat{\mu}_i - q_{ij\ell}\hat{\tau}_i \quad .$$

A solution for $V(\hat{\beta})$ may be obtained by estimating $\sigma_1^2, \sigma_2^2$ and $\sigma_{12}$ as follows:

$$\hat{\sigma}_i^2 = \frac{\sum_{\ell=1}^{n}\sum_{k=1}^{r}(Y_{ii\ell k}-\bar{Y}_{ii\ell.})^2 + \sum_{\ell=1}^{m}\sum_{k=1}^{r}(Y_{ij\ell k}-\bar{Y}_{ij\ell.})^2}{(r-1)(m+n)}$$

$$\hat{\sigma}_{ij} = \frac{\sum_{\ell=1}^{m}\sum_{k=1}^{v}(Y_{ij\ell k}-\bar{Y}_{ij\ell.})(Y_{ji\ell k}-\bar{Y}_{ji\ell.})}{m(r-1)} \quad .$$

t and F statistics may then be used to test hypotheses about the parameters and their relationships to each other.

This idea could be extended to mixtures of three or more crops by defining the "mixture effect" in terms of the deviation from the assumed linear regression of yield vs. density in the mono-culture. E.g., for a mixture of three crops

$$Y_{ijt\ell k} = \mu_i + q_{ijt\ell}\tau_i + \pi_{ijt\ell}(q_\ell) + \epsilon_{ijt\ell k}$$

where $\pi_{ijt\ell}$ defines the effect on the $i^{th}$ crop of growing the crops in a mixture of $i^{th}$, $j^{th}$, and $t^{th}$ crops at a particular ratio of densities.

This model is clearly not as over-parameterized as the previous ones, and is easily extendable to mixtures of more than two crops. Further, it does not assume any of the restrictions placed on the previous models, which makes it possible to use it when only a small number of mixtures of a particular crop are of interest and not the whole spectrum of possibilities.

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