An Application of Spring Balance Designs to

Crop Estimation with Special Reference to Legumes

and Mixtures of Crops

by

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Abstract

Forage crop researchers often need to determine the proportion of legume, weed, and grass contents of hay. This is often done by sampling and hand-separation or by visual estimates of the relative proportions. From the proportions it is possible to estimate the weight of legume in each plot of hay. The sampling and hand-separation method is costly and time-consuming and both procedures are subject to biases. In this paper, a method employing spring balance weighing design theory is presented as an alternative to the presently used methods. It is free of the biases and eliminates the sampling and hand-separation or the visual-estimate procedures and at the same time decreases the variance of a difference of two legume strain means by a factor of $1/t$ where $v = 4t-1$ = the number of legume strains.

Since there is current interest in blends or mixtures of crops, a procedure is also presented to measure the general competitive effects of strains and to determine how much more (or less) effective a blend is than when the strain is grown alone.

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1. INTRODUCTION

A current procedure utilized in forage crop experimentation on legume content of hay yields, is to compare v varieties (strains) of a legume (alfalfa, clover, etc.) in an experiment which has been overseeded with a single grass strain. Thus, the weight of the hay from an experimental plot receiving the ith alfalfa strain is composed of the weight of the ith alfalfa strain, the weight of the grass, and weight of weeds. Each plot yield also contains an experimental error component and a blocking component.

The objective of many forage trials with mixed species is the determination of differences of total mixture (hay) weights. Determination of legume content of the hay provides additional information relating to the contribution of the legume strains to the total hay weight. Therefore, if an experimenter is interested in comparing different strains of alfalfa for legume content of hay, the present method used in practice is to draw a sample from each experimental plot, to segregate the sample into its component parts of alfalfa, grass, and weeds, to determine their respective proportions, and to use the proportions

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to estimate the weight of alfalfa in each of the experimental plots. An alternative procedure is to obtain visual estimates of the relative proportions of weeds, grass, and legumes.

The hand separation of plot samples is a tedious and costly operation, and the method of sampling is often not optimal. Both the sampling and the visual procedures may introduce biases which invalidate the computed proportions. Hence, procedures resulting in less labor and eliminating personal biases would be highly desirable. The experimenter is often interested in weight of hay (alfalfa plus grass plus weeds) and in the weight of alfalfa for each strain. Procedures are given below for obtaining these characteristics with some of the procedures resulting only in unbiased estimates of the differences in alfalfa yields between two strains. Also, the procedure described herein will discontinue the need for drawing samples and for making hand separations. Before presenting the proposed procedure, a discussion of some of the assumptions is in order. Later on in the paper, methods for meeting these assumptions are given.

2. ASSUMPTIONS FOR THE PROPOSED PROCEDURE

It is assumed that: (i) The experimenter is interested in the alfalfa weights (or, at least, differences in alfalfa weights) for his comparisons of strains. (ii) In experimental plots where 3 or more alfalfa strains along with grass are grown, the proportional weight of the weeds and of the grass remains constant from plot to plot within a block. (iii) Each of the experimental plots (units) receives an equal amount of seeds (plants or area) of the strains allotted to that plot. (iv) More than one alfalfa strain can be grown in each experimental plot and that the competitive effect between strains is negligible.

In the event that these assumptions do not hold, alternatives are discussed in section 7.

3. STANDARD TECHNIQUE VERSUS PROPOSED PROCEDURE

An example is used to illustrate the currently used or standard procedure and the proposed one. Suppose that an experimenter is interested in determining the alfalfa content of hay for $v = 7$ strains of alfalfa, which we designate as A,B,C,D,E,F, and G, that a randomized complete block design with r blocks is used, and that the following 8 treatments are used:

Treatment	
1	grass plus strain A
2	" " " B
3	" " " C
4	" " " D
5	" " " E
6	" " " F
7	" " " G
8	grass alone.

Now after harvesting and with the help of hand separations of samples, the alfalfa weights are determined for each of rv plots and the means are compared. The standard error of the difference between the means of any two alfalfa strains is $\sqrt{2EMS/r}$ where EMS is the estimated error mean square for an experiment designed as a randomized complete block design.

For the above situation the following procedure is proposed. Set up 8 composite treatments as follows:

Treatment

1	=	grass plus strains A, B, C, D, E, F, G
2	=	" " " A, B, D
3	=	" " " B, C, E
4	=	" " " C, D, F
5	=	" " " D, E, G
6	=	" " " E, F, A
7	=	" " " F, G, B
8	=	" " " G, A, C

and use a randomized complete blocks design with r blocks as before. The data will be analyzed in the same manner as the previous experiment to determine the error mean square, EMS, which should be an estimate of the same parameter in both cases. Let $T_1, T_2, \ldots, T_8$ be the total weights for the 8 treatments listed above. Now W_i, the mean weight of the ith alfalfa strain, may be estimated as follows:

$$\hat{Y}_A = \left(T_1 + T_2 - T_3 - T_4 - T_5 + T_6 - T_7 + T_8\right)/4r$$
$$\hat{Y}_B = \left(T_1 + T_2 + T_3 - T_4 - T_5 - T_6 + T_7 - T_8\right)/4r$$
$$\hat{Y}_C = \left(T_1 - T_2 + T_3 + T_4 - T_5 - T_6 - T_7 + T_8\right)/4r$$
$$\hat{Y}_D = \left(T_1 + T_2 - T_3 + T_4 + T_5 - T_6 - T_7 - T_8\right)/4r$$
$$\hat{Y}_E = \left(T_1 - T_2 + T_3 - T_4 + T_5 + T_6 - T_7 - T_8\right)/4r$$
$$\hat{Y}_F = \left(T_1 - T_2 - T_3 + T_4 - T_5 + T_6 + T_7 - T_8\right)/4r$$
$$\hat{Y}_G = \left(T_1 - T_2 - T_3 - T_4 + T_5 - T_6 + T_7 + T_8\right)/4r$$

A comparison of strains may be made with the estimated weights above and the standard error of a difference between any two $\hat{Y}_i$ is equal to $\sqrt{EMS/r}$. Thus our present procedure not only eliminates the tedious and costly hand separating method, but has also halved the variance of a difference between two means. The proposed procedure increases the precision of the experiment. Even if the grass plot is not included in the standard procedure, only r additional plots are required for the proposed procedure. Any bias in the sampling procedure has also been eliminated.

It should be noted that the weights of the strains given above are slightly underestimated as they are based on 1/7th of the plot size for treatment 1 and 1/3rd of the plot size for the other treatments. However, the difference in true weights of any pair of strains is an unbiased estimate of the difference between the corresponding W_i.

To obtain the estimated hay weights $\hat{H}_i$ for the ith strain, only the last seven treatments are used as follows:

$$\hat{H}_A = \left[T_2 + T_6 + T_8 - (T_3 + T_4 + T_5 + T_7)/2\right]/3r$$
$$\hat{H}_B = \left[T_2 + T_3 + T_7 - (T_4 + T_5 + T_6 + T_8)/2\right]/3r$$
$$\hat{H}_C = \left[T_3 + T_4 + T_8 - (T_2 + T_5 + T_6 + T_7)/2\right]/3r$$
$$\hat{H}_D = \left[T_2 + T_4 + T_5 - (T_3 + T_6 + T_7 + T_8)/2\right]/3r$$
$$\hat{H}_E = \left[T_3 + T_5 + T_6 - (T_2 + T_4 + T_7 + T_8)/2\right]/3r$$
$$\hat{H}_F = \left[T_4 + T_6 + T_7 - (T_2 + T_3 + T_5 + T_8)/2\right]/3r$$
$$\hat{H}_G = \left[T_5 + T_7 + T_8 - (T_2 + T_3 + T_4 + T_6)/2\right]/3r$$

The difference $\hat{H}_i - \hat{H}_{i'} = \hat{Y}_i - \hat{Y}_{i'}$, and is an estimate of the <u>difference</u> in alfalfa yields between strains i and i'.

4. A COMPETING ALTERNATIVE PROPOSED PLAN TO COMPARE THE 7 STRAINS OF SECTION 3

As an alternative to the proposed procedure in the preceding section, one may lay out the following 8 treatments in a randomized complete block design:

Treatment

1	=	grass alone
2	=	grass plus strains A, B, D, G
3	=	" " " B, C, E, A
4	=	" " " C, D, F, B
5	=	" " " D, E, G, C
6	=	" " " E, F, A, D
7	=	" " " F, G, B, E
8	=	" " " G, A, C, F

The $\hat{Y}_i$, the estimated alfalfa yield, may be computed as:

$$\hat{Y}_A = \left(- T_1 + T_2 + T_3 - T_4 - T_5 + T_6 - T_7 + T_8\right)/4r$$

$$\hat{Y}_B = \left(- T_1 + T_2 + T_3 + T_4 - T_5 - T_6 + T_7 - T_8\right)/4r$$

$$\hat{Y}_C = \left(- T_1 - T_2 + T_3 + T_4 + T_5 - T_6 - T_7 + T_8\right)/4r$$

$$\hat{Y}_D = \left(- T_1 + T_2 - T_3 + T_4 + T_5 + T_6 - T_7 - T_8\right)/4r$$

$$\hat{Y}_E = \left(- T_1 - T_2 - T_3 - T_4 + T_5 + T_6 + T_7 - T_8\right)/4r$$

$$\hat{Y}_F = \left(- T_1 - T_2 - T_3 + T_4 - T_5 + T_6 + T_7 + T_8\right)/4r$$

$$\hat{Y}_G = \left(- T_1 + T_2 - T_3 - T_4 + T_5 - T_6 + T_7 + T_8\right)/4r.$$

However, the yield of grass in treatment 1 and of the weeds growing with grass alone cannot be expected to be the same as weeds and grass growing with an alfalfa strain. Hence, the estimated alfalfa yields cannot be validly estimated with this design, which it should be noted, has the same precision as the previous design, i.e., $\sqrt{EMS/r}$. But the estimated differences in alfalfa yields may be validly obtained by the method of this section.

The estimated hay yields from this design are:

$$\hat{H}_A = \left(T_2 + T_3 - T_4 - T_5 + T_6 - T_7 + T_8\right)/4r$$
$$\hat{H}_B = \left(T_2 + T_3 + T_4 - T_5 - T_6 + T_7 - T_8\right)/4r$$
$$\hat{H}_C = \left(- T_2 + T_3 + T_4 + T_5 - T_6 - T_7 + T_8\right)/4r$$
$$\hat{H}_D = \left(T_2 - T_3 + T_4 + T_5 + T_6 - T_7 - T_8\right)/4r$$
$$\hat{H}_E = \left(- T_2 + T_3 - T_4 + T_5 + T_6 + T_7 - T_8\right)/4r$$
$$\hat{H}_F = \left(- T_2 - T_3 + T_4 - T_5 + T_6 + T_7 + T_8\right)/4r$$
$$\hat{H}_G = \left(T_2 - T_3 - T_4 + T_5 - T_6 + T_7 + T_8\right)/4r$$

as with the proposed design in section 3 the difference $\hat{H}_i - \hat{H}_{i'} = \hat{Y}_i - \hat{Y}_{i'}$ and is an unbiased estimate of the difference in alfalfa yields between strain i and strain i'.

5. SPRING BALANCE WEIGHING DESIGNS FOR THE PROPOSED PROCEDURE

Weighing designs were studied by Hassler [1832], by Yates [1935], and by various other authors. A detailed list of references and material on this topic is presented in chapter 17 of a book by Raghavarao [1971] (see also, section XV.4 of Federer [1955]). These designs have so far been viewed as calibration designs but their use as "treatment designs" (see Federer [1959, 1960]) has not been discussed. The proposed plan presented in section 3 corresponds to an optimum spring balance design to weigh 7 objects in 8 weighings whether or not the spring balance has a bias.

6. GENERAL PROPOSED PROCEDURE

When the number of strains, v, to be tested is of the form $v = 4t-1$, then the treatment design may be formulated as follows:

Form a $(v+1) \times (v+1)$ square array of plus ones and minus ones such that the first row and the first column have plus ones everywhere and such that the sums of the cross products of the corresponding elements of any two rows is zero. An example for $v = 7$ and with the ones omitted is:

+	+	+	+	+	+	+	+
+	-	+	-	+	-	+	-
+	+	-	-	+	+	-	-
+	-	-	+	+	-	-	+
+	+	+	+	-	-	-	-
+	-	+	-	-	+	-	+
+	+	-	-	-	-	+	+
+	-	-	+	-	+	+	-

Such arrangements as the above are known as Hadamard matrices in mathematical and statistical literature and are presumed to exist for all $v + 1 = 4t$. When such an arrangement is written down, the rows are identified with the 4t treatments with the first column being identified with grass and the other 4t-1 columns being identified with the $v = 4t-1$ alfalfa strains. A plus (one) (or a minus (one)) in the (i,j)th-position is interpreted to mean that in the ith treatment the jth strain is included (or not included (minus one)) while the grass is included in every treatment.

After forming the $v + 1$ treatments, they may be laid out in a randomized complete block design if $v + 1$ and the heterogeneity within blocks are reasonably small; the data may be analyzed in the usual manner for estimating the error mean square. The estimate of the ith strain weight is obtained by dividing the difference of the total yield containing the ith strain from the total yield of all plots not containing this strain, by 2rt. The difference between the weights of any two strains has a standard error equal to $\sqrt{2EMS/rt}$. Had the current standard procedure been used, the standard error of a difference between two means would be $\sqrt{2EMS/r}$; thus, a reduction in the standard error of $\sqrt{(2EMS/r)/2EMS/rt} = t^{-\frac{1}{2}}$ has been achieved by the proposed procedure.

If $v + 1 = 4t$ is large, then an incomplete block design may be used and the estimated treatment means are obtained in the usual manner from this design. Then, the estimated treatments can be utilized in the manner described previously to obtain the estimated strain means.

When the number of strains v is not of the form 4t-1, then the treatments can be determined from the best spring balance design with bias. There are no tables readily available but for $v = 4t - 2$ or $v = 4t - 3$ such designs may be easily constructed from designs for $v = 4t - 1$ simply by deleting the last column(s) of a Hadamard matrix.

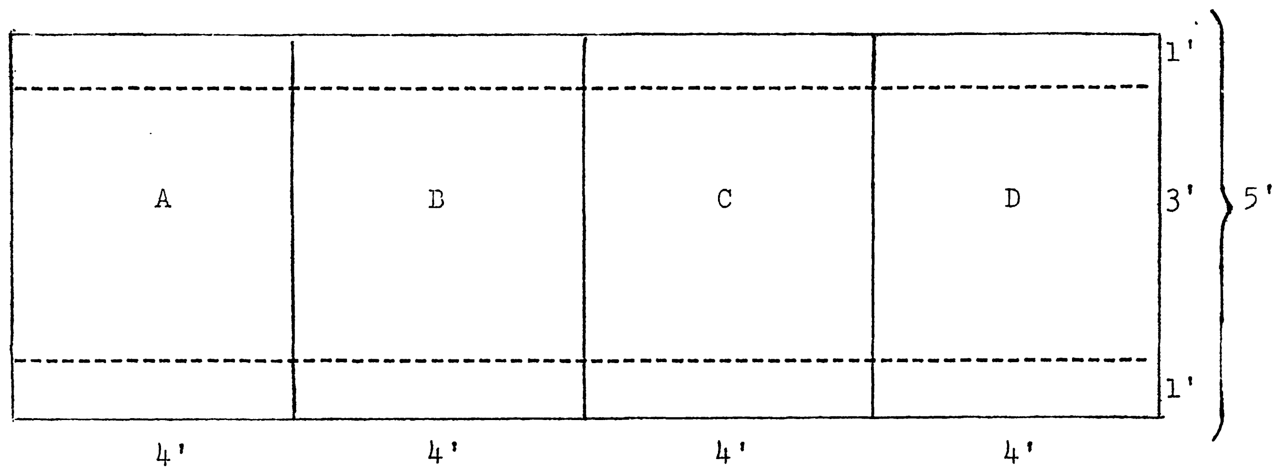
7. VALIDITY OF THE ASSUMPTIONS OF SECTION 2

Of the assumptions made in section 2, number (i) is more of a limitation rather than an assumption for the situations in which the newly proposed technique holds. From a practical viewpoint, when a plot contains many strains, it is only reasonable to allot equal area or material in the plot to each strain and thus (iii) is justified.

Though assumption (ii) would appear to be a major restriction on the proposed procedure, it appears to be a fairly valid one in light of the following argument. In an experimental plot consisting of the ith alfalfa strain, let the yield apart from experimental error and blocking effects be equal to $Y_i + W_i + G_i$ where Y_i is the weight of the alfalfa, W_i is the weight of weeds, and G_i is the weight of grass. Now, if k strains are grown in the plot, then the yield of the plot will be $\sum_{i=1}^{k} Y_i/k + \sum_{i=1}^{k} W_i/k + \sum_{i=1}^{k} G_i/k$. If k is sufficiently large (perhaps as small as 3,4, or 5), then the last two components will vary little and hence will essentially be a constant, K, and consequently independent (almost) of which k strains are included. Thus, the experimental plot yield for a set of k strains will be $\sum_{i=1}^{k} Y_i/k + K$. Since the $\hat{Y}_i$ (the estimated alfalfa weights) are computed as differences (see section 3), the constant K drops out.

Assumption (iv) could be a drawback of the proposed procedure in that competitive effects between strains are present. This can be taken care of by proper plot arrangement as described below and a different plot arrangement to estimate competitive effects is described in section 9. Now if the experimenter has reason to believe that competing effects between strains are present, then allot 1/kth of each plot to each of the k strains with the border between strains being minimized.

For example, in forage crop experimentation, a common harvesting procedure is to cut a 3' swath from the center of a 5' X 16' plot. For k = 4 strains (say A,B, C,D), the plot arrangement could be of the following form:



where the strains A,B,C, and D are to be randomly allotted to the subplots. Also, it may be desirable to add extra material on the ends of the plots and to trim the ends prior to harvesting. The above layout would tend to minimize the competitive effects between strains. It should be noted that small strips (e.g., 6") could be removed where the subplots intersect.

At first sight, it might appear that the above procedure introduces enough complexities in the planting arrangement to compensate for the sampling and hand-separation now practiced. In this connection it should be noted that

(i) the potential biases are eliminated and

(ii) the variance of a difference is 1/t of that of the present method, resulting from the use of a more efficient procedure.

8. PROCEDURES FOR COMPARING STRAIN EFFECTS

If the experimenter is interested in differences between pairs of strains rather than in estimating the alfalfa weights of individual strains as is possible with spring balance designs, the following alternative designs will be useful. Suppose that a balanced incomplete block (BIB) design (see Federer [1955], Cochran and Cox [1957], e.g.) exists with parameters $v = b$, $r = k$, λ, and suppose that we are interested in differences between estimated means of pairs of alfalfa strains. Then, we form v treatments by taking the ith block of the design to be grass plus the alfalfa strains occurring in this block. For example, suppose that there are 4 alfalfa strains arranged in the following BIB design:

(A B C)
(A B D)
(A C D)
(B C D)

with parameters $v = 4 = b$, $k = 3 = r^*$, $\lambda = 2$. The four treatments are:

Treatment					
1	= grass	plus	strains	A,B,C
2	= "	"	"	A,B,D
3	= "	"	"	A,C,D
4	= "	"	"	B,C,D

The <u>hay</u> yields for each strain are estimated as:

$$\hat{H}_A = \left(\text{treatments } 1 + 2 + 3\right) - 2\left(\text{treatment } 4\right)/3r$$
$$\hat{H}_B = \left(\quad '' \quad 1 + 2 + 4\right) - 2\left(\quad '' \quad 3\right)/3r$$
$$\hat{H}_C = \left(\quad '' \quad 1 + 3 + 4\right) - 2\left(\quad '' \quad 2\right)/3r$$
$$\hat{H}_D = \left(\quad '' \quad 2 + 3 + 4\right) - 2\left(\quad '' \quad 1\right)/3r$$

But $\hat{H}_A - \hat{H}_B = \hat{Y}_A - \hat{Y}_B$, etc., which is the difference between alfalfa yields in the experiment. After the v treatments are formed, they may be laid out in an appropriate design. If a randomized complete block design with r replicates is used, the design will be analyzed in the usual manner to obtain the error mean square and the standard error of a difference between any two $H_i/r(r^* - \lambda)$ of $\sqrt{2EMS/r(r^* - \lambda)}$.

It should be noted that any balanced incomplete block design for $v < b$ may be used but the use of the additional $b - v$ treatments does not result in any gain in information and does result in an additional $r(b-v)$ plots being used.

9. PROCEDURE TO ESTIMATE COMPETITIVE EFFECTS

In the event that there are competitive components such that the mixture or blend yields more (less) than the sum of the individual yields when grown alone, this would mean there could be an advantage (disadvantage) attained by growing mixtures or blends. Evidence for a plus yield has been obtained for wheat (see Jensen and Federer [1964, 1965]) and there is some indication that the same results may be obtained for specific alfalfa and soybean blends.

Now let us consider a method for ascertaining whether or not competitive effects are present. Consider the following three methods for the plot arrangement for a plot of size $\ell' \times w'$ (say $16' \times 5'$) with $\ell' \times h'$ (say $16' \times 3'$) being harvested from the center of the plot.

I. Standard method for the ith strain overseeded with grass (see section 3).

II. k strains in each experimental plot overseeded with grass and with 1/kth of the area being planted to one of the k strains in plot (see section 7).

III. k strains in each experimental plot with the seeds being randomly mixed prior to seeding.

The general competitive effect of the ith strain is estimated as the difference between strain yields from plot arrangements II and III. This difference measures how well the strain performs in competition as compared to its performance when planted alone. A comparison of yields from plot arrangements I and II measures the bias in the sampling procedure presently being used.

Hence, for a first experiment the forage crop researcher may wish to lay out the following experiment, keeping a record of all times and costs involved for each of the three plot arrangements. It is suggested that all three procedures be included in a given experiment and in order to obtain more precise contrasts on the three plot arrangements, one could use a split plot design with the $v = 4t-1$ blends or mixtures as the whole plots and with the three plot arrangements as the split plots in the ratio of t of I to one of II to one of III. Alternatively, one could use an incomplete block design such that the block size was small enough to retain relative homogeneity within the block and to use the $(v+1)(t+2)$ treatments as the number of treatments in the incomplete block design.

Alternatively, the experimenter may wish to replace the present plot arrangement with II, let the proposed procedure become the standard, and then to

(i) plant r/2 complete blocks to II and the other r/2 to III for v+1 treatments under consideration,

(ii) use the 2(v+1) treatments of II and III in the same complete or incomplete block design, or

(iii) use the II and III arrangements as the split plots and the v mixtures as the whole plots.

If there is as much interest in comparing the strains as there is in comparing methods II versus III, the experimenter should use (ii).

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