Effect of Harvest Area and Replication on Detection of Treatment Differences in Sugar Beet Field Experiments[1]

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Introduction

Yield and sucrose determinations from field plot trials are a vital part of sugar beet research programs concerned with breeding, varietal evaluation, soil fertility, disease, pest control, or other aspects of sugar beet production. Various plot sizes and harvest sampling techniques are used, most of which seem to be based on general experience and convenience rather than a systematic evaluation of the errors encountered in such experimentation. Surprisingly, the authors have been unable to locate any recent publications concerning optimum harvest areas, plot size, or the magnitude of differences one might expect to detect using various experimental techniques. In 1942, the ASSBT Standardization Committee recommended that sample areas two rows wide and 30 feet in length be harvested for demonstration strip trials, and for varietal trials that plots two to eight rows wide and 30 to 75 feet in length be used with the entire plot being harvested if possible (1)[3]. No justification for these recommendations was given nor were any references made to supporting experimental data. The most recent publications found specifically evaluating the effect of numbers of samples or sample areas on experimental error are those of Immer (3, 4, 5). This work represents one of the earliest applications of the analysis of variance to sugar beet research in this country. These papers essentially describe the results of uniformity trials, and while the results are still relevant, it would seem appropriate to evàluate the errors involved under present conditions.

The results reported here arose from an attempt to determine the optimum harvest areas for a series of sugar beet fertility trials located in eastern Colorado. Results from a set of trials conducted in 1972 were available, and were judged to be representative of the plots that might be encountered at sites located in the fields of farmer cooperators. In addition, the results of four variety trials conducted in eastern Colorado were available for similar analysis.

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[3]Numbers in parentheses refer to literature cited.

Methods

The statistical methods employed are based on the availability of weights or sucrose determinations on more than one subsample from each plot, allowing for the calculation of a within-plot or sampling error term as well as the between-plot experimental error. Methods of calculation of within-plot error and the partitioning of the plot error into the within- and between-plot components are given in many statistical methods texts. In general, we have followed the methods shown by LeClerg *et al.* (6).

The estimated variance components for the analysis of variance of a randomized complete block experiment with n_r replications (blocks), n_t treatments, and n_s samples taken from each plot can be represented as follows:

Source	Components of the estimated mean square
Replications	$s_s^2 + n_s s_p^2 + n_s n_t s_r^2$
Treatments	$s_s^2 + n_s s_p^2 + n_s n_r s_t^2$
Error (plots within reps)	$s_s^2 + n_s s_p^2$
Samples within plots	s_s^2

The sample, plot, replication, and treatment variances are s_s^2, s_p^2, s_r^2, and s_t^2, respectively. Thus, the error mean square, i.e. the error variance of an individual plot, is made up of two components. The first component, (s_s^2), is the sampling error within the plot. The second component, $(n_s s_p^2)$, represents the error arising due to differences among plots times the number of sampling units within each plot. From the relationship,

$$\text{Error Mean Square} = s_s^2 + n_s s_p^2 \qquad [1]$$

we obtain by simple rearrangement equation [2]:

$$s_p^2 = \frac{\text{error mean square} - s_s^2}{n_s} \qquad [2]$$

If more than one subsample is taken within a plot, the sample mean square provides an estimate of s_s^2, and by means of equation [2] it is then possible to estimate s_p^2, i.e., the error variance that arises due to true plot differences.

The standard error of a treatment mean is calculated by the usual relationship [3]:

$$s.e._{\bar{y}} = \left[\frac{\text{Error Mean Square}}{n_s n_r}\right]^{1/2} \qquad [3]$$

Substituting from [1] above, we obtain [4]:

$$s.e._{\bar{y}} = \left[\frac{s_s^2 + n_s s_p^2}{n_s n_r}\right]^{1/2} \qquad [4]$$

which can be rearranged to [5]:

$$s.e._{\bar{y}} = \left[\frac{1}{n_r}\right]^{1/2} \cdot \left[\frac{s_s^2}{n_s} + s_p^2\right]^{1/2} \quad [5]$$

Once estimates of s_s^2 and s_p^2 are obtained, equation [5] allows us to calculate an expected standard error of the mean for any combination of samples and replications.

A series of soil fertility field trials on sugar beets was conducted on farmer cooperators' fields in 1972. These trials consisted of four treatments and four replications in a randomized complete block arrangement. Plots were 50 feet long and 8 rows wide with a row width of 22 inches. Yield subsamples were each two rows wide and 30 feet long, representing an area of 110 ft². Two subsamples were harvested from each plot. Data sets of this type were available from twelve locations.

In addition, data sets were available from four variety trials conducted over two years. Two of these were located at the Colorado State University Agronomy Research Center in Fort Collins and the others were on farmer cooperators' fields in eastern Colorado. These trials included either seven or eight varieties and five or six replications in a randomized complete block arrangement. Plots were 25 feet long and six rows wide with a 22-inch row width. Eighteen-foot harvest samples were taken from each of the six rows, representing an area of 33 ft² per row. Two of the samples taken from each plot were used for sucrose analysis. Beets were sampled with a rotating rasp mechanism and sucrose determined using a method similar to that outlined in A.O.A.C. (2).

Data were analyzed using standard analysis of variance techniques. In a few cases, particularly in the variety trials, some subsamples were missing. Analysis of variance methods allowing for nonequal subclass numbers were therefore necessary, and the appropriate n_s was calculated using the method given by Snedecor (7, p. 290).

Results and Discussion

The error mean squares and the plot and sample variances for root yield from the twelve fertility trials are shown in Table 1. The relationship between sample and plot variances is highly site dependent, but in eight of the twelve cases sample variance (s_s^2) is greater than plot variance (s_p^2). This is rather surprising considering that these sample variations are based on the relatively large experimental unit of 110 ft².

On sites 5 and 12 the sampling variance was larger than the error mean square, resulting in a negative estimate of (s_p^2). The most likely explanation is that this is simply due to random error as the differences

Table 1.—Yield error, plot, and sample variances for twelve sugar beet fertility experiments in eastern Colorado. The experimental unit for samples was 110 ft^2.

Site	Error mean square	Between-plot variance (s_p^2)	Subsample variance (s_s^2)
	$(T/A)^2$	$(T/A)^2$	$(T/A)^2$
1	2.943	0.328	2.287
2	9.986	4.217	1.552
3	5.231	1.896	1.440
4	2.024	0.209	1.605
5	2.410	–	3.143
6	13.434	5.146	3.141
7	6.440	1.538	3.364
8	1.772	0.388	0.997
9	2.075	0.771	0.533
10	3.512	1.151	1.209
11	6.261	1.471	3.696
12	2.247	–	3.312

are not significant at the .05 probability level as determined by the F test. However, in crops such as sugar beets where low yields in one row tend to be partially compensated by higher yields in adjacent rows, such a reversal is entirely possible. In such cases the interpretation would be that samples drawn from different plots, after adjusting for treatment and block effects, would be more similar than samples drawn from the same plot. If the larger within-plot variances are due to random effects and both values actually estimate the same variance, samples within a plot would vary the same as samples drawn from different plots. In this case, increasing the number of samples drawn would have the same effect as a proportionate increase in the number of replications.

From the s_p^2 and s_s^2 values shown in Table 1, predicted standard errors of the mean were calculated for varying numbers of subsamples per plot by means of equation [5]. Four replications were assumed. The n_s's were multiplied by the subsample area, in this case 110 ft^2, and the predicted s.e.$_{\bar{y}}$'s plotted as a function of area. These curves are shown in Figure 1. This and succeeding figures are based on the implicit assumptions that plot size is not changed by increasing harvest area, and that sampling error is dependent only on harvest area and is independent of the geometry of the harvested area. While these assumptions may not be entirely met, the figures should be reasonably valid if interpretation of extremes is avoided. Note that in all cases the predicted s.e.$_{\bar{y}}$ decreases rapidly as harvest areas increase up to about 100 ft^2. In addition, in most cases there is a substantial decrease in s.e.$_{\bar{y}}$ as harvest area is increased from 100 to 200 ft^2. There seems to be very little advantage in harvesting more than about 200 ft^2 or a total of 110 linear feet of 22-inch row, except perhaps for sites 5 and 12 mentioned above. The characteristic shape of these curves can be under-

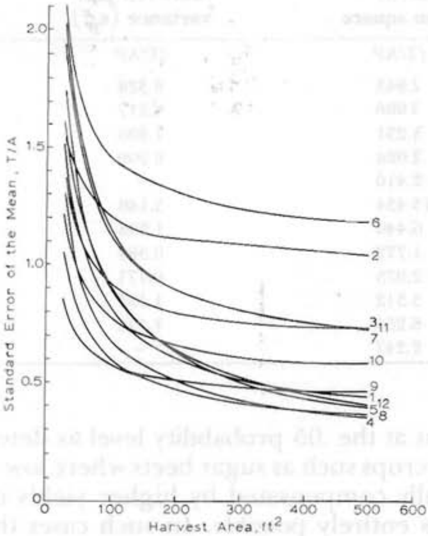

Figure 1.—Predicted standard error of the mean yield as a function of harvest area for twelve soil fertility experiments. Four replications are assumed.

stood from equation [5]. If n_s, i.e. harvested area, becomes large, s.e.$_{\bar{y}}$ approaches $\left[\frac{1}{n_r}\right]^{1/2} \cdot \left[s_p^2\right]^{1/2}$. Further decreases in s.e.$_{\bar{y}}$ can only be attained by increasing n_r.

Figure 2 was constructed by pooling the s_s^2 and s_p^2 estimates from ten sites. The highly variable sites 2 and 6 were not included as the ten locations probably more accurately represent the errors that might be expected on farmer cooperator sites where no particular. cultural problems or unusual soil variation are encountered. The curves in Figure 2 show the expected s.e.$_{\bar{y}}$ values for varying numbers of replications. While actual detectable treatment differences vary with the number of treatments the Least Significant Difference at the .05 level of probability (assuming t $.05 \approx 2.0$) provides a useful basis of comparison. From Figure 2, with a harvest area of 200 ft² and four replications, we expect a s.e.$_{\bar{y}}$ of 0.7 ton per acre, corresponding to a $LSD_{.05}$ of about 2 tons per acre. Three locations shown on Figure 1 had a predicted s.e.$_{\bar{y}}$ of about 0.5 tons per acre with a 200 ft² harvest area, so with four replications we would not expect the $LSD_{.05}$ to be below 1.4 tons per acre. Further reduction in LSD at these sites would require increased replication. From the pooled variances used in Figure 2 we would expect a s.e.$_{\bar{y}}$ of about 0.5 with eight replications and 200 ft² harvest area, or a $LSD_{.05}$ of 1.4 tons per acre. Similarly, from the least variable

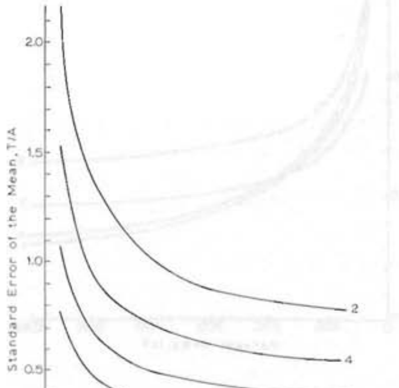

Figure 2.—Predicted standard error of the mean yield as a function of harvested area and number of replications as calculated by pooling variances from ten soil fertility experiments.

location shown on Figure 1 we might expect a $LSD_{.05}$ of about 1 ton per acre if eight replications were used.

The error mean squares and plot and sample variances for the four variety trials are shown in Table 2. In this case the experimental unit for subsamples is only 33 ft², and as might be expected the (s_s^2) is much larger than the (s_p^2). In two cases (sites 1 and 4) the error mean squares are slightly smaller than the sample mean squares (s_s^2). Figure 3 shows the predicted s.e.$_{\bar{y}}$ as a function of harvest area for the variety trials assuming four replications. With an estimated zero (s_p^2) on sites 1 and 4, the effect of increased harvest area on these sites would be expected to be equivalent to a similar increase in replication. However, for sites 2 and 3 there is little effective decrease in s.e.$_{\bar{y}}$ as harvest areas are increased above 200 ft².

Table 2.—Yield error, plot, and sample variances for four sugar beet variety trials in eastern Colorado. The experimental unit for samples was 33 ft².

Site	Error mean square	Between-plot variance (s_p^2)	Subsample variance (s_s^2)
	$(T/A)^2$	$(T/A)^2$	$(T/A)^2$
1	6.510	–	6.879
2	7.879	0.722	3.598
3	14.547	1.426	5.994
4	6.656	–	7.637

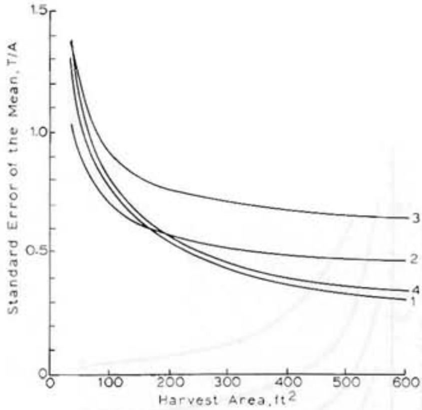

Figure 3.—Predicted standard error of the mean yield as a function of harvest area for four variety trials. Four replications are assumed.

Figure 4 was constructed from the pooled variances of the variety trials, and shows the effect of harvest area and replication on the expected s.e.$_{\bar{y}}$. Predicted standard errors are slightly lower for the variety trials than for the fertility trials, probably reflecting a more intensive level of management applied to these experiments. With four replications the s.e.$_{\bar{y}}$ is predicted as about 0.6 tons per acre, resulting in a LSD$_{.05}$ of 1.7 tons per acre. With eight replications this is reduced to a s.e.$_{\bar{y}}$ of 0.4 and a LSD$_{.05}$ of 1.13 tons per acre.

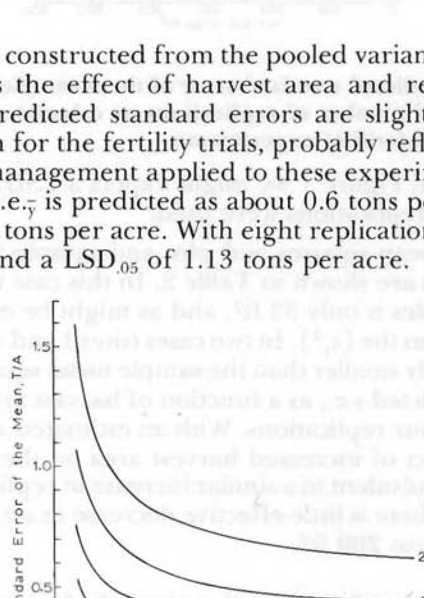

Figure 4.—Predicted standard error of the mean yield as a function of harvested area and number of replications as calculated by pooling variances from four variety trials.

The interpretations from the two sets of trials agree very well, especially considering that they represent quite different experimental techniques. The experimental unit was 33 ft^2 for the variety trial and 72% of the total plot area was harvested. Only 30% of the area was harvested in the fertility trials and the experimental unit was 110 ft^2.

If we wish to decrease the final s.e.$_{\bar{y}}$, we have the option of either increasing harvest area or replication, assuming (s_s^2) and (s_p^2) values as found in these trials. Per unit of material harvested, the decrease in s.e.$_{\bar{y}}$ will usually be slightly greater if replications are increased than if harvest area is increased. Increasing harvest area from 100 ft^2 to 200 ft^2 will apparently decrease the s.e.$_{\bar{y}}$ about the same as will increasing from four to six replications, assuming the relationship between (s_s^2) and (s_p^2) does not change. The data presented do not allow us to examine possible changes in these parameters but a consideration of the geometry should be helpful. In the variety trial technique where all rows are harvested and border effects are considered to be negligible, a decrease in number of rows harvested will give a proportionate decrease in plot size, thus decreasing (s_p^2). Thus, increasing replication would generally be more effective per unit harvested than increasing plot size. If cultural practices and field methods favor larger plots, it would not be advisable to use plots much larger than required for a 200 ft^2 harvest area.

The soil fertility trials are quite different. With many preplant application techniques a minimum of two border rows 22 inches in width are required. Thus, a plot that allows two harvest rows is only 25% smaller than one that allows for harvest of four rows. Increases in (s_p^2) as a result of increased plot size would probably be small in this case, and it would seem that a harvest area of about 200 ft^2 would be desirable.

In examining results from other trials we find a few $LSD_{.05}$ values lower than those we have suggested above. These trials were usually located on an experiment station where the soils were uniform and careful control of thinning, water management, and other cultural practices could be exercised. More than six or eight replications are usually impractical, both from an operational standpoint and because of the relatively small changes that result in s.e.$_{\bar{y}}$ as replication is further increased. Harvest areas above 200 ft^2 are apparently not advantageous and may even be detrimental due to increased plot size and possible increases in (s_p^2). Thus, it would seem that careful site selection and management offer the greatest potential for further precision.

The relationship of s.e.$_{\bar{y}}$ to number of samples analyzed for sucrose from the four variety trials is shown in Figure 5. Here again, we have assumed four replications. In this case the sampling error includes errors involved with the chemical analysis and with the sampling of individual beets as well as field sampling errors. All beets

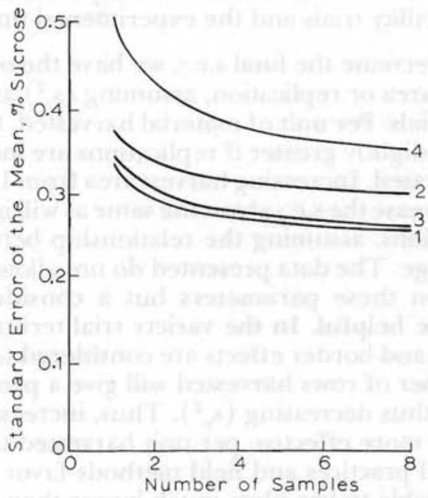

Figure 5.—Predicted standard error of the mean sucrose percentage from four variety trials as a function of number of samples analyzed. Samples consisted of all beets harvested from 18 feet of row.

in an 18-foot row were sampled. Under these conditions it would appear that there is a definite advantage to the use of at least two samples, but there would be little point in taking more than three samples per plot. These data would indicate that using four replications we might expect an s.e.$_{\bar{y}}$ of about 0.30% or a LSD$_{.05}$ of 0.85%. With eight replications we should expect a s.e.$_{\bar{y}}$ of about 0.21% and a LSD$_{.05}$ of 0.60%.

Summary and Conclusions

The between-plot and within-plot components of the yield error mean squares were calculated for twelve sugar beet fertility and four variety experiments. From these variances and the subsample areas, curves were constructed relating predicted standard errors of the mean to harvest area and replication. Agreement between prediction from the two sets of trials was satisfactory. Predicted s.e.$_{\bar{y}}$ values decreased rapidly as harvest areas increased up to about 100 ft^2 per plot and at most sites further decreases were observed between 100 and 200 ft^2. Further changes in s.e.$_{\bar{y}}$ were generally small for harvest areas greater than 200 ft^2.

These data would indicate that using four replications on an average site we might expect a LSD$_{.05}$ of about 2 tons per acre, with perhaps 1.5 tons per acre on the least variable sites. On sites with low variability and eight replications a $LSD_{.05}$ of about 1 ton per acre might be achieved.

Similar curves for number of sucrose samples per plot indicate that where single samples consist of the beets from 18 feet of 22-inch wide rows there is a considerable advantage in taking at least two samples per plot but very little improvement in $s.e._{\bar{y}}$ when more than three samples are taken. Using two samples per plot we would expect a $LSD_{.05}$ of about 0.85% with four replications, dropping to 0.60% using eight replications.

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