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PROTEIN CONTENT OF CORN AS INFLUENCED BY LABORATORY ANALYSES AND FIELD REPLICATION

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Protein Content of Corn as Influenced by Laboratory Analyses and Field Replication

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CONSIDERABLE interest has been shown in recent years in the possible changes in crop-plant composition that may be brought about by alterations in cultural practices. An increase in the protein percentage by such means has particular interest to the farmer. A rate-of-planting test with corn, conducted at the Colorado Experiment Station, afforded an opportunity to study this problem through protein analyses** made on a composite shelled-corn sample from each replicate.

Aside from the information obtainable on the influence of different rates of planting, the data offered an excellent opportunity to study the variations between duplicate chemical analyses made on each sample in the laboratory and the variations between the different replicates of the same treatment. This is particularly useful to the investigator who is interested in the reliability of the samples he analyzes. Nitrogen, being highly variable, proved to be especially valuable for this purpose.

MATERIALS AND METHODS

Nitrogen determinations were made on shelled corn from each plot of an experiment conducted on rate of planting corn each year during a 3-year period, 1931 to 1933, inclusive.

Two yellow-dent varieties, Golden Glow and Pride of the North, were used in the experiment. The two varieties grown each year were planted to give 3, 4, and 5 plants per hill in 42-inch rows. The hills were 36 inches apart in the row. These same varieties were also planted in drill rows, with individual plants spaced 12, 9, 6, and 3 inches apart in the row (1).

The test was planted in three-row plots, with the center row harvested for yield. A sample from the composite shelled corn of the center row was used for the protein determination. A random arrangement of plots was used each year except in 1931, when a systematic arrangement was followed. There were two replications; i. e., three plots for each rate of planting for each variety and for each year.

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(1) Leonard, Warren H., and Robertson, D. W., 1935, Rate of Planting Corn Under Irrigated Conditions, Colo. Exp. Sta. Bul. 417.

The nitrogen determinations were made by the Gunning method (2). The sample from each plot was run in duplicate, while the nitrogens were converted to proteins by use of the factor 5.7. The data were analyzed statistically by the analysis of variance (3). The method for estimating the variation between duplicate laboratory samples, as compared with the variation due to replicates, was similar to the ones used by Tippett (4) and Immer (5).

EXPERIMENTAL RESULTS

INFLUENCE OF RATE PLANTED ON PROTEIN CONTENT.—The summarized data on the protein content of corn planted at different rates are given in table 1. The average protein percentages for the years 1931, 1932, and 1933 were 9.42, 9.70, and 8.90, respectively.

TABLE 1.—PROTEIN IN YELLOW-DENT CORN PLANTED AT DIFFERENT RATES OVER A 3-YEAR PERIOD, 1931 TO 1933, INCLUSIVE

Variety	Method planted	Rate planted	Plants per acre	Protein[3]			
				1931	1932	1933	Mean
			Number	Percent	Percent	Percent	Percent
Golden Glow	Hills	3[1]	12,446	10.31	9.86	9.10	9.76
Golden Glow	Hills	4	16,594	9.46	9.67	9.24	9.46
Golden Glow	Hills	5	20,743	9.24	9.83	9.00	9.36
Pride North	Hills	3	12,446	9.87	10.21	9.16	9.75
Pride North	Hills	4	16,594	9.22	9.42	8.80	9.15
Pride North	Hills	5	20,743	8.95	9.26	8.36	8.86
Golden Glow	Drills	12[2]	12,446	10.29	10.18	9.68	10.05
Golden Glow	Drills	9	16,594	9.58	9.86	8.98	9.47
Golden Glow	Drills	6	24,891	8.92	9.17	8.50	8.86
Golden Glow	Drills	3	49,783	9.17	9.26	8.52	8.98
Pride North	Drills	12	12,446	9.87	9.98	9.41	9.75
Pride North	Drills	9	16,594	9.13	9.86	8.79	9.26
Pride North	Drills	6	24,891	9.06	9.71	8.61	9.13
Pride North	Drills	3	49,783	8.84	9.55	8.38	8.92
Average per year				9.42	9.70	8.90	

[1]Plants per hill.

[2]Inches between plants in the row.

[3]Protein is equal to nitrogen x 5.7. Average for three replications per year.

Considerable information can be realized immediately when the data are subjected to an analysis of variance (3). The results of this analysis appear in table 2. Significance is cal-

(2) Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 3d Ed., 1930.

(3) Fisher, R. A., 1934, Statistical Methods for Research Workers, 5th Ed.

(4) Tippett, L. H. C., 1931, The Methods of Statistics, pp. 90-94, 176-178.

(5) Immer, F. R., 1932, A Study of Sampling Technic With Sugar Beets, Jour. Agr. Res., 44:633-647.

culated from the "F" value of Snedecor (6) which is the ratio of the larger mean square to the smaller mean square. The treatments are subdivided into varieties, subtreatments (rates and methods of planting), and subtreatments x varieties, all of which appear to be significant at either the 5-percent or 1-percent levels. The analysis shows also that the protein content in corn has a highly significant difference from year to year. This is shown in table 1.

TABLE 2.—ANALYSIS OF VARIANCE OF PROTEIN IN RELATION TO VARIETY AND RATE OF PLANTING

Variation due to	Degrees of freedom	Sum of squares	Mean squares	Standard error	F
Years	2	28.0839	14.0420		57.31**
Blocks within years	6	4.0954	0.6826		2.79*
Treatments	13	33.9613	2.6124		10.66**
(Varieties	1	1.5921	1.5921		6.50*
(Sub-treatments	6	29.0721	4.8454		19.78**
(Varieties x sub-treatments	6	3.2971	0.5495		2.24*
Treatments x years	26	5.7628	0.2216		0.90
Error	78	19.1121	0.2450	0.4950	
Total for plots	125	91.0155			
Samples within plots	126	0.4073	0.0032		
Total samples	251	91.4228			

*Exceeds 5-percent point. **Exceeds 1-percent point.

The data may be arranged according to variety, method of planting, and number of plants per acre as shown in table 3. The protein percentage is observed to decrease as the number of plants per acre increases. The analysis of variance indicates that the varieties differ in protein content, Golden Glow containing the greater amount. However, protein content is significantly affected by the rate of planting. There is a strong tendency for a high protein content to accompany a thin stand. This tendency is exhibited by each variety with few discrepancies. Comparison of the protein content for the three-plant rate for hill-planted corn and the 12-inch intervals between plants for drilled corn indicates little variation when only method of planting is concerned. However, the protein content for the 12,446-plant rate is significantly higher than for all methods and rates that involve more plants per acre. The comparison of the five-plant rate per hill and the 3- and 6-inch intervals between plants for drilled corn indicates little variation in rates when close planting is involved. Thus individual rates differ from each other significantly only for the thinner plantings.

(6) Snedecor, G. W., 1934, Calculation and Interpretation of Analysis of Variance and Covariance, pp. 14-15, 88-91.

TABLE 3.—EFFECT OF DIFFERENT RATES OF PLANTING ON THE PROTEIN CONTENT OF SHELLED CORN, 1931 TO 1933, INCLUSIVE

	Method planted	Plants per acre	—Protein in shelled corn— Golden Glow	Pride of North	Average for rate and method
		Number	Percent	Percent	Percent
	12 inches between plants	12,446	10.05	9.75	9.900
1	3 plants per hill	12,446	9.76	9.75	9.755
	9 inches between plants	16,594	9.47	9.26	9.365
2	4 plants per hill	16,594	9.46	9.15	9.305
	5 plants per hill	20,743	9.36	8.86	9.110
	6 inches between plants	24,891	8.86	9.13	8.995
	3 inches between plants	49,783	8.98	8.92	8.950
	Average for variety		9.42	9.26	
	S. E. of mean for rates and methods of planting				0.0825
	Level of significance for rates and methods of planting (5-pct. point)				0.2322
	S. E. mean for varieties				0.0441
	Level of significance for varieties (5-pct. point)				0.1241

1 and 2 indicate comparisons of rates which differ significantly. In no case for a given rate do methods of planting differ significantly.

COMPARISON OF FIELD REPLICATES AND DUPLICATE ANALYSES.—In the analysis of variance, the variance of a treatment mean (not to be confused with variance due to treatments), which may be considered to measure the precision of the experiment, is found by dividing the mean residual variance by the total number of individual measures which contribute to the treatment mean.

In the simple case where one sample is drawn from each plot with the treatment replicated for m plots, the variance of a treatment mean is simply $\frac{V_p^2}{m}$ where V_p^2, the mean variance between plots, approaches σ_p^2, the true variance of an individual plot, as m is increased indefinitely. However, when n samples are drawn from each plot the variance of a treatment mean is $\frac{V_p^2}{mn}$, where now $\frac{V_p^2}{n}$ estimates σ_p^2, the true variance of an individual plot, plus the true variance of an individual plot mean, or $\frac{\sigma_s^2}{n}$. This follows because a plot mean is now subject to variation due to more than one sample. It is evident that σ_s^2 is the true variance of an individual sample taken from a plot. The relationship may be shown as follows:

$$\frac{V_p^2}{mn} \longrightarrow \frac{\sigma_p^2}{m} + \frac{\sigma_s^2}{mn} = \frac{1}{m}\left(\sigma_p^2 + \frac{\sigma_s^2}{n}\right)^*$$

It is clear that σ_p^2 can be estimated from the above formula, since V_p^2 and V_s^2, the latter being an estimate of σ_s^2, are obtainable from the variance analysis.

*The sign $\longrightarrow$ is used to denote that the quantity on its left approaches the quantity on its right as the degrees of freedom are increased without limit.

In the present experiment $V_p^2=0.2450$, $V_s^2=0.0032$, and $n=2$. Therefore,

$$\frac{0.2450}{2} \longrightarrow \sigma_p^2 + \frac{0.0032}{2}, \text{ and } 0.1209 \longrightarrow \sigma_p^2$$

Thus the standard error of a plot mean can be estimated as $0.348 = \sqrt{0.1209} \longrightarrow \sigma_p$. In like manner $0.057 = \sqrt{0.0032} \longrightarrow \sigma_s$, the standard error of an individual sample for chemical analysis drawn from a plot. Here the ratio σ_p/σ_s is estimated as $0.348/0.057 = 6.1$, showing the variation *between plots* to greatly exceed that *within plots* or between samples.

It is of considerable importance to analyze how the precision of an experiment as measured inversely by $\frac{1}{m}(\sigma_p^2 + \frac{\sigma_s^2}{n})$ is affected by varying m, the actual plot replications in the field, on one hand; and n, the number of samples drawn from a plot on the other hand.* The most important inference to be drawn is that the precision is mainly controlled by m, the number of plot replications. Increasing the number of samples taken from the different plots can only appreciably affect the precision when σ_s^2 is *not* relatively small as compared with σ_p^2.

In the present problem 0.0032, the estimated value of σ_s^2, is small compared with 0.1209, the estimated value of σ_p^2. Hence, it must be concluded that to make more than one analysis on a sample from a plot was unwarranted by the small gain in precision resulting. A tabular arrangement of precision measures for the means of the important types of treatments involved in the present experiment is given to illustrate the negligible effect of making one, two, or three analyses from samples.

The values, estimating $1/m\ (\sigma_p^2 + \sigma_s^2/n)$ the variance for treatments, are as follows when the number of analyses per sample is varied:

	Rate of planting $m = 18$	Variety $m = 63$	Year $m = 42$
$n = 1$	0.0689	0.0197	0.0295
$n = 2$	0.0680	0.0194	0.0291
$n = 3$	0.0677	0.0193	0.0290

In the design of an experiment, then, with nothing known regarding σ_p^2 and σ_s^2, the experimenter can only be certain of the effect of plot replicates on the precision of the experiment. It is evident that only in the trivial situation where $\sigma_p^2 = 0$, will

*The present discussion applies whether replicate samples are actually taken from the different plots or, as in the case of the present experiment, a single sample per plot is subjected to replicate analyses.

the precision as measured by $\frac{1}{m}\left(\sigma_p^2 + \frac{\sigma_s^2}{n}\right)$ be affected by a variation of n to the same extent as by a proportionate variation of m. In practical work it is false argument to reason that inadequate plot replication can be compensated for completely by an increase in the number of samples per plot. The ideal design is one with sufficient plot replication, with but one sample drawn per plot.

Technical difficulties often prevent plot replication beyond a certain degree. In such cases it is frequently worthwhile to strengthen the precision of the experiment by drawing replicate samples from the different plots. The number that should be drawn depends upon several factors. These factors are: (a) The variation between plots as measured by σ_p^2 in relation to σ_s^2, and (b) the cost of growing a plot as compared with the cost of obtaining and analyzing replicate samples per plot. The time factor instead of the cost factor, or the combination of the two, should be considered in many types of experiments.

It is proposed to investigate how these relative costs determine a balance between plot replicates, m, and sample replicates, n, in order that a stated precision for an experiment may be obtained at a minimum expense. Let C represent the cost per plot replicate and c the cost per sample replicate in the conduct of an experiment. For a given treatment the total cost of plot replications will be mC, while the total cost of sample replications will be mnc. Hence E, the total expense per treatment, is given by:

$$E = mC + mnc$$

A certain criterion of precision to be obtained may be represented by

$$K = \frac{1}{m}\left(\sigma_p^2 + \frac{\sigma_s^2}{n}\right), \text{ where } K = \text{required variance of}$$

the mean for a treatment. The total cost will be made a minimum when $n = \sqrt{\frac{C}{c} \cdot \frac{\sigma_s^2}{\sigma_p^2}}$. Thus, n, and hence m, are determined to afford a most economical design. Furthermore, it is worthwhile to note that n is determined to be independent of K, the precision desired.

In the present experiment, substituting $n = 2$, $\sigma_p^2 = 0.1209$, and $\sigma_s^2 = 0.0032$, the ratio of costs $\frac{C}{c} = \frac{0.1209}{0.0032} \cdot (2)^2 = 151$. From the standpoint of expense the analysis of a duplicate sample from each plot would have been justifiable to produce the most economical design only if the cost per additional plot

had been 151 times the cost per analysis. In the determination of the cost per plot replicate, to divide the total cost of the experiment by the number of plots will usually give too high a cost. There is usually a definite overhead cost in the conduct of the experiment which should first be subtracted.

CONCLUSIONS

Shelled corn from Golden Glow was found to contain more protein than that from Pride of the North.

The protein content of shelled corn showed a marked variation from year to year.

The protein content of shelled corn was significantly affected by the rate of planting, the thinner rates resulting in the highest protein percentage. The method (hills or rows) of planting had no effect on the amount of protein.

Plot replication gave the larger error when the standard error due to plot replicates and that due to duplicate samples for protein analysis were compared. The ratio between the two was 6.1:1.

Two samples for protein did not perceptibly increase the precision of the experiment. As far as chemical analyses were concerned, a single sample per plot would have been sufficiently accurate when the error of duplicate analyses is considered in the light of the error of the experiment.

The cost ratio of plot replicate to sample replicate was computed. The analysis of a duplicate sample from each plot would have been justified in producing the most economical design only if the cost per plot had been 151 times the cost per analysis.