Methods of sampling to most effectively measure inherent plant differences on a single plant basis in barley
by Wallace E Jones

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
Space-planted Betzes and Carlsburg II barley plants were selected and the first, second, and third heads to emerge from the boot were tagged and harvested separately from the remaining plant.

Counts were made of the number of seed per head in the,first and first three heads of each plant to emerge and the average number of seed per head on the total plant calculated. Weights of seed produced on the first three emerging heads were projected to their equivalent 100 seed weights and 100 seed weights for the total seed produced by the plant was calculated from the number and weight of seed produced.

Protein analysis was made of a composite sample of the first three emerging heads of each plant and a sample of the remaining seed of each plant. Percent protein for the total seed produced per plant was calculated from this information.

Either the first or the first three emerging heads were found 'to be an accurate sample of the total plant' performance for the characters studied. The means of characters between varieties were found to be significantly different in the first and first three emerging heads but not in the total plant.

Correlations were found between the characters studied. When the effects of varieties were removed by an analysis of variance and covariance, it was found that an increase in number of seed per head is accompanied by an increase in seed weight and a decrease in protein. An increase in seed weight is accompanied by a decrease in protein. - Coefficients of regression, applied to reduce the error -variance, were successful in reducing the number of replicated plants required to measure differences.

On the basis of this study, it is concluded that the most accurate individual plant differences may be measured: (1) in number of seed per head by an average of all heads produced by a plant, (2) in weight per 100 seed by the first three emerging heads adjusted for average number of seed per head, and (3) in percent protein on an as is basis by a composite sample of the first three emerging heads adjusted for their projected 100 seed weight.

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ABSTRACT

Space-planted Betzes and Carlsburg II barley plants were selected and the first, second, and third heads to emerge from the boot were tagged and harvested separately from the remaining plant.

Counts were made of the number of seed per head in the first and first three heads of each plant to emerge and the average number of seed per head on the total plant calculated. Weights of seed produced on the first three emerging heads were projected to their equivalent 100 seed weights and 100 seed weights for the total seed produced by the plant was calculated from the number and weight of seed produced.

Protein analysis was made of a composite sample of the first three emerging heads of each plant and a sample of the remaining seed of each plant. Percent protein for the total seed produced per plant was calculated from this information.

Either the first or the first three emerging heads were found to be an accurate sample of the total plant performance for the characters studied. The means of characters between varieties were found to be significantly different in the first and first three emerging heads but not in the total plant.

Correlations were found between the characters studied. When the effects of varieties were removed by an analysis of variance and covariance, it was found that an increase in number of seed per head is accompanied by an increase in seed weight and a decrease in protein. An increase in seed weight is accompanied by a decrease in protein.

Coefficients of regression, applied to reduce the error variance, were successful in reducing the number of replicated plants required to measure differences.

On the basis of this study, it is concluded that the most accurate individual plant differences may be measured: (1) in number of seed per head by an average of all heads produced by a plant, (2) in weight per 100 seed by the first three emerging heads adjusted for average number of seed per head, and (3) in percent protein on an as is basis by a composite sample of the first three emerging heads adjusted for their projected 100 seed weight.

INTRODUCTION

Montana, as a barley surplus state, must find additional markets for barley produced within the state if the accumulation of a surplus is to be avoided.

Increased interest of malsters and brewers in Montana as a potential source of malting barley, particularly two-row types, has led to considerable expansion of work with two-row varieties at the Montana Experiment Station. In a search for two-row varieties of good malting quality with better straw and yield, the Polish introduction, Betzes, although only intermediate in agronomic characters, is one of the most promising in quality. This barley appears to modify readily in malting, producing malts higher in extract and wort nitrogen ratio than Hannchen, is about equivalent in alpha amylase to Hannchen, but somewhat lower in diastatic power than Hannchen. It is also similar in kernel size and type to Hannchen.

Another European variety, Carlsburg II from Denmark, is equivalent to Hannchen in malt extract, but low in wort nitrogen ratio and amylase activity. This variety is relatively late and shows an undesirable frayed hull characteristic.

Some of the characteristics represented by Carlsburg II could well be combined with Betzes. Such a cross could result in; (1) a higher yield than Betzes under irrigation, (2) a hull subject to less fraying than that of Carlsburg II, (3) a stiffer straw than Betzes, and (4) malting quality equal to or better than Betzes.

The studies reported herein have been made with the objective of determining methods of sampling to most effectively measure inherent differences on a single plant basis the three characters, number of seed per head, weight per 100 seed, and protein content.

REVIEW OF LITERATURE

Using grain production as a measure of translocation, it has been shown by Williams (16) and Dungan (5) that tillers or "suckers" of corn under some conditions contribute to grain production of the principle stem. Bartel, Martin, and Hawkins (1) found evidence that plant juices in Dwarf Hegari move from the main stalk to the tillers or from the tillers to the main stalk depending upon where the nutritional deficiency occurred. Smith (14) has made observations on the physiological relations between tillers and main stem of wheat. When the spikes were removed from tillers, the de-spiked tillers contributed to grain production of the defoliated main stem, but when the spikes were not removed, the contribution was insignificant.

Labanauskus and Dungan (9) found that the undisturbed main stem of oats is not affected materially by tillers, although translocation of nutrients can take place under certain conditions; when blades are removed from the main stem or tillers and when panicles are removed and blades remain intact. Defoliated tillers benefited from an undistrubed main stem in order of their age. The performance of any stem appears to be influenced by the nature of conditions to which other stems of the same plant were exposed.

Engledow and Wadham (6) removed leaves and developing heads of barley in various combinations. On some plants all the heads were removed save one, which developed to no greater extent as judged by average size of kernel than did corresponding heads on untreated plants. Also, they found

that the head and straw of successive tillers -- T_o, T_1, T_2, ... -- show gradation in weight with T_o usually the heaviest and the others following in sequence. In addition, percentage of nitrogen in the tillers was usually found to be least both in grain and straw for T_o (main axis), and rose in sequence for T_1 (first side tiller), T_2, ... etc. They concluded that tillers may be independent units after early stages of growth.

Bonnett (2) reported that when stems of the barley plant producing heads pass into the second stage, i.e., jointing and spike differentiation, it is not long before all stems on the plant follow the same trend in rapid succession. An examination of a plant in head shows even the growing point of the tiller buds to be in the process of spikelet differentiation.

Pope (13) found a flattening of the dry-weight curve in the barley plant occurs at the time of rapid tiller development and is accompanied by enhanced growth of crown roots. The development and growth of the spike primordium into the full-sized flowering head is accompanied by a rapid elongation and increase in dry weight of the whole shoot. Up to the beginning of this period of rapid elongation ("jointing" or "shooting"), the amount of absorptive tissue evidently has been a factor limiting the growth of the plant. At the time of most rapid tiller growth, these young parts, growing much more actively than the main shoot, may compete sucessfully for the nutrients. The rapid growth of the tillers is accompanied by an increase in root growth until the tillers are nearly the size of the main shoot, when sufficient root development takes place to supply both kinds of stem.

Bonnett and Woodworth (3) working with close-drilled barley plants found that the average yield per head increases with an increase in the number of heads per plant and that there is a consistent tendency for average kernel weight to increase with the number of heads per plant. The average yield per plant is the result of a combination of average heads per plant and average yield per head. Average yield per head is determined by the average kernels per head and their average weight.

Numerous investigators have reported correlations of characters in barley. For the most part, they do not deal with individual tillers but are correlations of yield, test weight, height of plant, length of head, straw strength, etc.

Hodgskiss (7) found a positive correlation of .239 for which significance was not indicated between number of kernels per culm and average weight per kernel in the two-row variety Chevalier. Lambert and Liang (10) reported a highly significant negative correlation of -.305 between weight per 100 kernels and number of seed per head in the F_4 generation six-row segregates of crosses between Mars, a six-row, and Spartan, a two-row variety. Den Hartog and Lambert (4) noted a negative correlation of -.11 between average kernel weight and protein in F_5 lines of six-row segregates from crosses between Mars and several two-row varieties. A partial correlation of the fifth order showed a positive correlation of .11 between these characters. Hsi and Lambert (8) reported a negative correlation of .07 in the F_5 generation and a positive correlation of .06 in the F_6 generation between average kernel weight and protein in a continuation of the work by Den Hartog and Lambert. Partial correlations of

the second order of .15 and .12 in the F_5 and F_6 generations respectively were obtained when diastatic power was taken into account. The coefficients of correlation obtained by Den Hartog and Lambert and by Hsi and Lambert are too low to be of much use.

MATERIALS AND METHODS

Betzes (C. I. 6398) and Carlsburg II (C. I. 10114) barley varieties were used in this study. All Betzes plants were from a single plant selection made in 1955 while Carlsburg II plants were grown from seed randomly selected from a bulk population since no pure line selections had previously been made. No crosses were made in connection with this study.

Rows for study were selected from the 1956 breeding nursery and were distributed throughout the nursery. Rows were one foot apart and plants one foot apart in the rows. A total of 51 plants of Betzes were grown in five rows and 56 plants of Carlsburg II in six rows. Four plants of Carlsburg II were eliminated from this study because a portion of one of the first three heads to emerge was missing. Plants were observed daily and the first, second, and third heads on each plant to emerge from the boot were tagged and the tags dated. Heading date was taken at the time the first head appeared above the flag leaf. When two or more heads appeared above the flag leaf on the same date, the head with the greatest portion above the flag leaf was given the first number. In the event two heads were the same distance above the flag leaf, the taller tiller was given the first number.

Heads produced per plant of Carlsburg II ranged from a low of 7 to a high of 64 with a mean of 38 and Betzes ranged from a low of 11 to a high of 73 with a mean of 39.8 heads per plant.

Plants were harvested by hand, the first, second, and third heads to emerge being harvested separately and the remainder of the plant bulked

after counting the total number of heads producing seed. The number of seed per head produced by the first, second, and third heads to emerge were determined by actual count and the seed saved separately. The remaining heads were threshed by the plant and weighed. Three 100-seed samples were taken from the remaining seed of each plant and weighed and averaged. The total weight of the remaining seed was divided by the average weight of 100 seed and multiplied by 100 to give the number of seed produced by the remaining plant. The number of seed produced by the remaining plant was added to the number of seed produced by the first three heads emerging to find the total number of seed produced by the plant. The weight of the seed produced on the remaining plant was added to the weight of seed produced on the first three heads emerging to find the total weight of seed produced by the plant.

Seed from the first, second, and third heads to emerge on each plant were bulked into a composite sample and a sample was taken from the remaining seed of each plant. These samples were sent to the Montana Grain Inspection Laboratory for protein analysis by the Kjeldahl method, percent protein on an as is basis being calculated by percent nitrogen times 6.25. The weight of protein in the first three heads emerging was added to the weight of protein in the remaining plant to give the weight of protein in the total seed produced by each plant which was then divided by the total weight of seed produced by each plant to give the percentage of protein contained in the seed produced by each plant.

Analysis of variance of individual characters to obtain the standard error of a single plant was made according to the method outlined by

Snedecor (15) for subsamples with different numbers of observations.

Coefficients of correlation and regression were calculated for the characters studied and the coefficients of regression were used to adjust the sums of squares for reducing the standard error of a single plant.

EXPERIMENTAL RESULTS

Means and standard errors of a single plant were calculated for the first and first three emerging heads and for all heads produced for the characters number of seed per head and 100 seed weight. Means and standard errors of a single plant were also calculated for the character percent protein in the first three emerging heads and all heads produced. The results are presented in Table I and II.

Table I. Means of a single plant for the characters number of seed per head, 100 seed weight, and percent protein when measured by the first head emerging, first three heads emerging, and all heads of Betzes and Carlsburg II barley.

Character	Variety	
	Betzes	Carlsburg II
Number of seed per head, No.		
First head emerging	30.0	27.1**
First three heads emerging	28.9	26.7**
All heads	21.3	21.6
Weight per 100 seed, Gms.		
First head emerging	4.49	4.82**
First three heads emerging	4.48	4.82**
All heads	4.13	4.01
Protein, %		
First three heads emerging	15.16	14.19**
All heads	14.83	13.33

** Significantly different from Betzes at 1 percent level.

Since the standard error of a single plant is the best estimate of the standard deviation of a single plant, it is desirable to discover to what extent the estimated standard deviation of a single plant estimates the unknown standard deviation of the population. Leonard and Clark (11)

Table II. Standard errors of a single plant for the data of Table I and standard errors of the estimated standard deviation of a single plant.

Character	Betzes		Carlsburg II		Combined	
	Estimated Standard Deviation	Standard Error of Standard Deviation	Estimated Standard Deviation	Standard Error of Standard Deviation	Estimated Standard Deviation	Standard Error of Standard Deviation
Number of seed per head, No.						
1st head emerging	2.32	.242	2.29	.239	1.74	.129
1st 3 heads emerging	1.89	.197	1.85	.193	1.50	.111
All heads	1.29	.135	1.69	.176	1.40	.104
Weight per 100 seed, Gms.						
1st head emerging	.447	.047	.379	.039	.376	.028
1st 3 heads emerging	.374	.039	.357	.037	.319	.024
All heads	.389	.041	.336	.035	.361	.027
Protein, %						
1st 3 heads emerging	1.249	.130	1.198	.125	1.067	.079
All heads	1.336	.139	1.692	.176	1.511	.112

state that it has been found that the best estimate of the hypothetical distribution of standard deviations derived from a large number of samples is $s/\sqrt{2n}$. Standard errors of the estimated standard deviations were calculated and are shown in Table II.

The lowest standard error of a single plant, when measuring number of seed per head, was obtained when all heads produced are taken into consideration. When measuring weight per 100 seed, the lowest standard error of a single plant in Betzes was found in the measurement of the first three heads to emerge, while in Carlsburg II a measurement of all heads produced gave the lowest standard error. However, when data were combined and the effects of varieties eliminated by means of an analysis of variance and covariance, a measurement of the first three heads to emerge produced the lowest standard error of a single plant. The first three heads to emerge had a lower standard error of a single plant than did a measurement of all heads produced for percent protein.

It is also desirable to discover if the measurements of the first and of the first three heads to emerge for a particular character are an accurate measurement of the performance of all heads produced for these characters. Differences were analyzed by means of "Students" pairing method, Ostle (12). The results are listed in Table III.

It may be seen from the data in Table III that the measurement of the characters, number of seed per head, 100 see weight, and percentage of protein may be measured as the same value in early heads as compared to all heads produced by the plant as evidenced by the insignificant t values in every case.

Table III. Analysis of differences of plant portions of Betzes and Carlsburg II barley by means of "Students" pairing method.

Character	Betzes			Carlsburg II		
	diff	s	t	diff	s	t
Number of seed per head						
1st head emerging vs						
1st 3 heads emerging	1.1	.912	.172	.35	1.000	.048
All heads	8.72	2.334	.534	5.53	2.269	.338
1st 3 heads emerging vs						
all heads	7.62	1.675	.729	5.18	1.807	.424
Projected 100 seed weight						
1st head emerging vs						
1st 3 heads emerging	.007	.023	.006	.002	.127	.003
100 seed wts. all heads	.353	.316	.156	.809	.365	.307
1st 3 heads emerging vs						
100 seed wts. all heads	.346	.231	.210	.807	.268	.417
Percent protein						
1st 3 heads emerging vs						
all heads	.331	.509	.091	.139	.448	.040

The data presented in Table I show that the early heads of these two varieties belong to different populations in respect to the characters studied and that measurements taken on a total plant basis are not significantly different. Since the varieties are known to differ in the three characters measured, and in the direction indicated by the early emerging heads for 100 seed weight and percent protein when grown in rows (Table IV), it could be assumed that the first head or the first three heads emerging were a better measure of genetic differences.

Table IV. Average number of seed per head, 100 seed weight, and percent protein in drilled rows at several locations in Montana in 1955 and 1956.

Character	No. of Comparisons Averaged	Variety	
		Betzes	Carlsburg II
Number of seed per head	2	17.1	19.0
Weight per 100 kernels	10	3.99	4.07**
Percent protein	22	11.9	11.2**

** Significantly different from Betzes at the 1 percent level.

Coefficients of Correlation and Regression

Coefficients of correlation and of regression were calculated from analysis of variance and covariance using the error term mean square and products for the three characters studied using the first emerging head, first three emerging heads, and all heads produced of each variety. The results are presented in Table V.

When the deviations of seed weight about the mean are considered, it will be noted that they are associated with the deviations of protein about the mean protein percentage, and deviations of number of seed per head about the mean number of seed per head, which was expressed by highly significant correlations between all characters studied. In other words, environmental variation of the characters studied was related. There is an increase in 100 seed weight for each increase in number of seed per head, a decrease in protein for each increase in number of seed per head, and a decrease in protein with increase in 100 seed weight. These relationships do not necessarily hold true for the varieties Betzes and Carlsburg II, except for the regression of percent protein on 100 seed weight.

An increase in number of seed per head is accompanied by an increase in seed weight and a decrease in protein. In line with this, an increase in seed weight decreases protein. In all characters studied, the increase and decrease was lowest in the initial head or heads.

The amount of variation due to regression of 100 seed weights on number of seed per head was calculated to be 9 percent in the first emerging head, 17 percent in the first three heads to emerge, and 21 per-

Table V. Coefficients of correlation and regression between characters studied in the first emerging head, first three emerging heads, and all heads produced of Betzes and Carlsburg II barley and combined data.

Characters Studied	Coefficient of Correlation=(r) Betzes	Carlsburg II	Combined	Coefficient of Regression=(byx) Betzes	Carlsburg II	Combined
No. of seed per head (x) & 100 seed wt. (y)						
1st head emerging	.003	-.040	.395**	.005	-.007	.085**
1st 3 heads emerging	-.010	.215	.566**	-.002	.041	.118**
All heads	.226	.604**	.497**	.080	.121**	.128**
No. of seed per head (x) & % protein (y)						
1st 3 heads emerging	.455**	-.292*	-.298**	.273**	-.234*	-.208**
All heads	-.104	-.493**	-.359**	-.107	-.493	-.387**
100 seed wt. (x) & % protein (y)						
1st 3 heads emerging	-.492**	-.584**	-.411**	-1.522**	-1.963**	-1.373**
All heads	-.612**	-.656**	-.673**	-2,101**	-3.286**	-2.820**

* Denotes significance at the 5 percent level.
** Denotes significance at the 1 percent level.

cent for all heads. The amount of variation due to regression of percent protein on number of seed per head was 5 percent in the first three emerging heads and 10 percent for all heads. Regression of percent protein on 100 seed weights in the first three emerging heads accounted for 10 percent of the variation and for all heads 36 percent. Also, the coefficients of regression are highest when all heads produced are considered, perhaps again indicating that the early emerging heads are less influenced by the environment.

Reduced Standard Errors of a Single Plant

Where the coefficient of regression was found to be significant (Table V), it was used to adjust the sums of squares and reduce the standard error of a single plant by means of the formula $b^2(x-\bar{x})^2 - 2b(x-\bar{x})(y-\bar{y}) + (y-\bar{y})^2$.

It is seen that the standard error of a single plant may be reduced where the regression coefficient is found to be significant (Table VI.) In the instances where the regression coefficient was not found to be significant, the standard error of a single plant was sometimes increased, as might be expected.

Table VI. Standard errors of a single plant unadjusted and when adjusted for number of seed per head and for 100 seed weight.

	1st head emerging		1st 3 heads emerging		All heads	
	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted
	Basis of Adjustments Number of Seed Per Head					
100 seed weight						
Betzes		.447		.374		.389
Carlsburg II		.378		.357	.272	.336
Combined	.347	.376	.264	.319	.342	.361
Percent protein						
Betzes			1.049	1.249		1.336
Carlsburg II			1.129	1.198	1.489	1.692
Combined			1.023	1.067	1.418	1.511
			Basis of Adjustment, 100 Seed Weight			
Percent protein						
Betzes			1.020	1.249	1.069	1.336
Carlsburg II			.983	1.198	1.292	1.692
Combined			.978	1.067	1.124	1.511

Number of Replicated Plants Required to Measure Differences

The designer of an investigation that requires sampling is always confronted with a decision about the size of a sample and methods of sampling. Using the mean and error variance of a single plant, tables were prepared according to the formula of Snedecor (15) $n = t^2s^2/(\bar{x}-m)^2$ to find the minimum number of replicated plants required to measure designated differences for each character studied at the 5 percent and 1 percent levels of significance. Data from the combined analysis of Betzes and Carlsburg II were used.

Table VII. Number of replicated plants required to measure differences in number of seed per head.

Portion of Plant Measured	Difference to be Measured -- No. of Seed Per Head								
	At 1% Level of Sig.					At 5% Level of Sig.			
	1	2	3	4	5	1	2	3	4
	No. of Plants Required								
1st emerging head	21	6	3	2	1	12	3	2	1
1st 3 emerging heads	16	4	2	1		9	3	1	
All heads	14	4	2	1		8	2	1	

It may be seen in Table VII that the number of replicated plants required to measure a difference of one seed per head is lowest for all heads produced; this is in line with a lower standard error of a single plant for all heads produced as opposed to the standard error of a single plant for the first and the first three heads emerging. However, an excessive number of plants would not be required to measure a difference of two or more seeds per head at either the 5 percent or 1 percent levels of significance for the first or the first three heads emerging.

Table VIII. Number of replicated plants required to measure differences in 100 seed weights unadjusted and when adjusted for number of seed per head.

Portion of Plant Measured	Difference to be Measured -- 100 Seed Weights At the 1% Level of Significance									
	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
	No. of Plants Required									
1st emerging head										
Unadjusted	98	25	11	7	4	3	2	2	2	1
Adjusted	84	21	10	6	4	3	2	2	2	1
1st 3 emerging heads										
Unadjusted	71	18	8	5	3	2	2	2	1	
Adjusted	49	13	6	4	2	2	1			
All heads										
Unadjusted	90	23	10	6	4	3	2	2	2	1
Adjusted	69	18	8	5	3	2	2	2	1	
	At the 5% Level of Significance									
1st emerging head										
Unadjusted	56	14	7	4	3	2	2	1		
Adjusted	48	12	6	3	2	2	1			
1st 3 emerging heads										
Unadjusted	41	11	5	3	2	2	1			
Adjusted	28	7	4	2	2	1				
All heads										
Unadjusted	52	13	6	4	3	2	2	1		
Adjusted	39	10	5	3	2	2	1			

Adjustment of the error variance was effective in lowering the number of replicated plants required to measure a difference in 100 seed weight (Table VIII). The first three emerging heads offer the least number of replicated plants required to measure differences of 0.1 gram in 100 seed weights and are as efficient as using all heads produced and for adjusting for number of seed per head.

Adjustments for both number of seed per head and 100 seed weights were applied in an attempt to reduce the number of replicated plants required to measure differences in percent protein (Table IX). Adjustments for 100 seed weights offer a greater reduction than adjustment for number of seed per head in the total number of replicated plants required to measure differences in protein. The first three heads emerging require fewer plants than do all heads produced, adjusted or unadjusted. Little, if any, benefit by adjusting the protein on the basis of the first three heads emerging was noted. The futility of attempting to measure small genetic differences in protein content is evident.

Multiple correlation and regression may offer further possibilities in reducing mean squares and standard errors of a single plant.