AN INVESTIGATION INVOLVING THE SAMPLING OF MORPHOLOGICAL CHARACTERS FOR USE IN ESTIMATING DISEASE RESISTANCE AND SAMPLE SIZE IN HOPS

by

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INTRODUCTION

The hop research project at the Oregon Agricultural Experiment Station was revised in 1948. One of the principal objectives was the investigation of resistance to downy mildew, Pseudoperonospora humuli (Miy. and Saka) Wils.

A large number of hybrid seedlings as well as new lines in the breeding project must be evaluated each year for resistance to downy mildew. The nature of the disease makes it impossible to depend upon natural field infection in the evaluation of resistance. It will be necessary therefore to utilize a method of producing infection on excised plant parts under controlled environmental conditions.

The effectiveness of this method will be determined largely by estimating the most efficient size and number of samples for this determination. The present investigation involves a determination of the efficiency of alternate apportionment of the size of the various components of the sampling error in estimating resistance.

The study of physical quality factors relative to commercial grading in hops has also received considerable attention. The accuracy of an analysis of physical quality factors was also recognized as being limited by the method of sampling. A part of the present study is devoted to a determination of the number of samples per lot necessary to evaluate these factors with a given degree of accuracy.

REVIEW OF LITERATURE

One of the principal objectives of hop research in Oregon since the beginning of the program in 1930 has been the breeding for resistance to downy mildew, Pseudoperonospora humuli (Miy. and Saka) Wils. (22).[1]

Smith (26) suggested a number of difficulties which may be encountered in the determination of plant reaction to downy mildew of hops. He presents data which indicate that it is almost impossible to rely on field notes in determining resistance, for even with mildew generally present many susceptible plants may escape infection. Hoerner (10) stated that it may be misleading to generalize in evaluating comparative resistance because of highly variable localized conditions that may favor or inhibit infection. Pathologists have overcome this difficulty by the development of methods whereby artificial infection may be produced under controlled environmental conditions.

Most of the downy mildews require relatively low temperatures and a high humidity for the production of infection. Hoerner (13) stated that low temperatures and high humidity are conducive to spore production, germination and infection but that once infection takes place, higher temperatures and other weather conditions favorable to rapid growth of the hop vines also favor rapid

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1. Figures in parentheses refer to "Literature Cites," pages 38-40.

development of the disease producing organism. The spread of the disease in relation to the humidity and temperature was pointed out by Newton (21). He stated that the slow spread of the disease in June, July and August was due to an inverse relationship between temperature and humidity since the periods of high humidity occurred when temperatures were rarely above 50°F. The optimum temperature for germination of the spores was given as 68°F. These environmental relationships have been reported by many other investigators for downy mildew of hops and other plants (6)(4). Hoerner (15) suggested that the optimum temperature for infection under artificial conditions was 65°F.

These environmental factors have been used in producing infection under laboratory and greenhouse conditions. Hoerner (10) described a technique for infecting seedlings in which a camel's hair brush was used in transferring conidia from the various host plants to the seedlings. The cotyledons were wet at the time of inoculation. High humidity was maintained for the necessary incubation period by placing flats and pots in an improvised tent or by enclosing individual pots or plants in bell jars. Hoerner (14) (15) inoculated excised leaves and cultured them in a petri dish moist chamber. Yarwood (31) also used excised leaves, but placed the petiole in a vial of H_2O and subjected them to conditions of high humidity and low temperature.

Yarwood (30) stated that only in the case of the diseases due to fungi with a swarm spore stage in the germination of their sporangia, such as mildew of hops and cucumbers, was the infection

markedly less when no water was added to the inoculated leaves than when the inoculated leaves were atomized. Hoerner (11) described the method in which European workers applied suspensions of motile zoospores to the leaves of hops.

Yarwood (31) indicated that sporulation took place at night or in the absence of light. His data indicated also that fruiting takes place from 5 to 10 days after inoculation and that time of day of inoculation had no effect on infection. He stated that no definite relationship could be established between sporulation and any specific factor because sporulation may be correlated with time of day, low temperature, high humidity or intensity of light. Hoerner (10) indicated that the incubation period for the production of conidia on hop leaves was from 2 to 12 days.

Information has not been presented relative to the existance of physiological races of the hop downy mildew fungus. Hoerner (10)(11) stated that cross-inoculation was easily effected by use of conidia from several different varieties of hops from different locality collections, indicating that physiological forms of the fungus, if they exist at all, are not of common occurrence. No attempt was made in the present study to obtain inoculum from any given variety or line and the possibility of the existence of physiological races of the pathogen was completely ignored. Although there is some evidence of resistance in certain varieties or lines of hops under field conditions, their resistance reaction under controlled environmental conditions is inconclusive (10)(12)(14).

A method for the rapid determination of comparative degrees of infection of hops by downy mildew has been described by Hoerner (15). Excised leaves were inoculated with zoosporangia and incubated in moist petri dishes at 65°F. A chart (Figure 2), showing increasing degrees of infection designated by the numerals 1 through 10, was used as the scale for the evaluations.

Yates and Zacopanay (32) have presented a very thorough discussion of the interpretation of the analysis of variance as applied to sampling in field experiments. Cochran (2) presented an example of the use of this technique and showed how it may be used to provide an estimate of the accuracy of the sample as well as the accuracy of alternate methods of sampling. Winsor and Clarke (29)(appendix) developed a method for estimating the separate components of variation in an experiment. They extended the suggestions of Fisher (5) in his development of the analysis of variance and presented a detailed description of the procedure. This method has been applied to experimental data by numerous workers to test the relative efficiency of the distribution of size and number of samples (3)(16)(17)(18)(23)(27).

Investigations for the development of hop grades have been conducted through the Oregon Agricultural Experiment Station since 1937 (20)(24)(8). Sather and Hill (20) presented data indicating the effectiveness of several methods of sampling from commercial lots. Tentative United States standards for hops were developed by Hill and Bullis (8) for the purpose of study in later investigations. The tolerances for the various measurable qualities were presented.

MATERIALS AND METHODS

The infection of the plants, in this study, was accomplished under controlled environmental conditions on excised plant parts. Hop plants sucker or sprout profusely from the crown and produce many branches or sidearms along the main vine during most of the growing season. Either the suckers or sidearms may be used for artificial inoculation. The suckers were used in the present study. These were cut at ground level when they were from 6" to 12" in length and had at least four leaves that were large enough with which to work. They were placed in half pint milk bottles of water. The bottle caps were perforated in order to receive the bases of the stems. They were taken into the greenhouse where they were placed in moisture chambers before being inoculated.

The inoculations were made in the afternoon after the plants had been in the moisture chambers long enough to become turgid. The source of inoculation was diseased plant parts from naturally infected hop fields (Figure 3). These were put into a moisture chamber over night to assure abundant fruiting. A spore suspension was obtained by immersing this plant material in water. This spore suspension was strained and sprayed on the underside of the leaves, under ten pounds pressure, by use of a small paint atomizer.

After being inoculated the plants were placed in the moisture chambers (Figure 4). High humidity was maintained by means of vegetable display spray-heads which maintain a fog-like mist in the

moisture chambers. At the end of seven days the plants were evaluated for resistance. The method described by Hoerner (15) was used with the exception of changing the comparative degrees of infection from 1 through 10 to 0 through 9. The use of one digit numbers provides for faster calculation.

Twenty-one lines or strains of hops selected from the breeding block of the U.S.D.A. hop breeding project were used in the study. These lines were selected so as to have as wide a variation between lines as possible in their resistance to downy mildew. It was not possible to predict their reactions accurately since the only information was from field notes which are usually unreliable in evaluating hop mildew resistance.

Each line in the breeding block is maintained in 5 hill plots, all 5 hills originating from clonal cuttings from one plant. Three hills from each line were selected at random and two sprouts from each hill were cut for the inoculations. These sprouts were arranged in a randomized complete block design in the greenhouse moisture chambers. Four replications were used, one complete replication being completed in each of four consecutive days.

It was found that in making relative evaluations of resistance the factor of personal bias could be great especially when the operator is aware of the identity of the plants. To overcome this to a great extent it was arranged to have the identity of the suckers unknown to the person making the evaluations. Leaves which had been placed in the various classes from 0 through 9, during the process

of evaluation, were preserved by pressing for further observation. Ten leaves from each class were selected at random and counts made of the number of spots per given area of leaf surface. The number of spots in three areas of .25 square inches in size were counted on each leaf. The number of spots were so numerous in the classes above class 7 that they coalesced making it impossible to obtain an accurate count.

The components of the variance which make up the sampling error are of particular interest in this study. The analysis of variance of the sampling data provided a means of comparing the magnitude of these various components. It also provided a means of comparing the relative efficiency of the various sizes and apportionment of the samples.

The original data from a previous investigation by Sather and Hill (24) were used in the study of sampling relative to commercial hop grading. The data comprised an analysis of twelve different lots of hops for two quality factors, leaf and stem content and seed content.

The method described by Harris, Horvitz, and Mood (7) was used in determining the number of samples necessary to estimate each of these quality factors with a known degree of accuracy. Before applying this method several conditions about the population had to be satisfied. The cumulative frequency method was used to test the normality of the original data as well as various transformations of the original data. The statistics g_1 and g_2 were calculated to test

for skewness and kurtosis (27).

The twelve lots of hops could not be combined for the study if their variances were not homogeneous or if their means were not equal. Bartlett's X^2 test of homogeneity of variances was used to test for homogeneity while the analysis of variance (F-test) was used to test the significance of the differences between the means.

It is also necessary that the leaf and stem content and seed content be randomly distributed in the population. The serial correlation method was used to test the randomness of the data.

These data also present an opportunity to analyze several other problems which may be of interest in sampling. The difference between sampling from the end and from the middle of the bale was discussed by Sather and Hill (24). Their data on the end and middle bale sampling was reanalyzed. The data on duplicate analyses of the same sample were also analyzed by application of the t-test.

Figure 1. A hop leaf showing typical secondary infection by downy mildew.

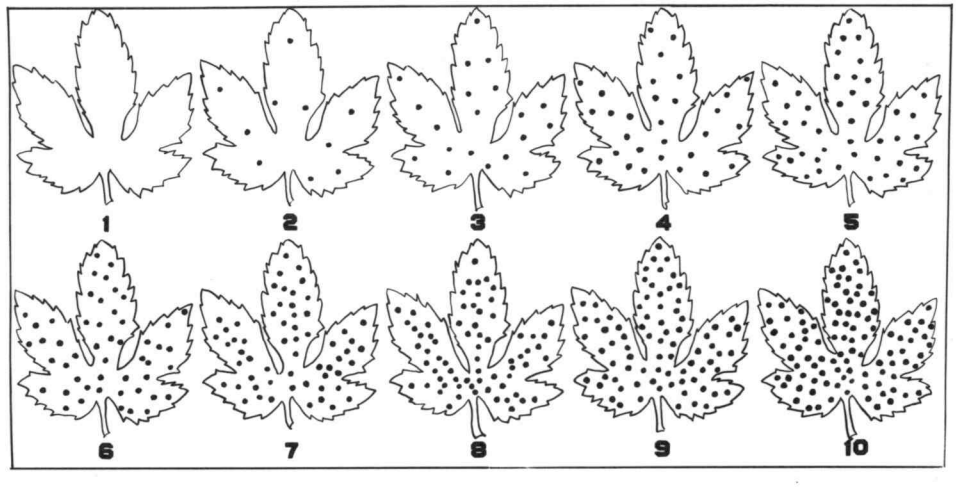

Figure 2. Scale showing comparative degrees of leaf infection.

Figure 3. Hop plant showing downy mildew infected suckers.

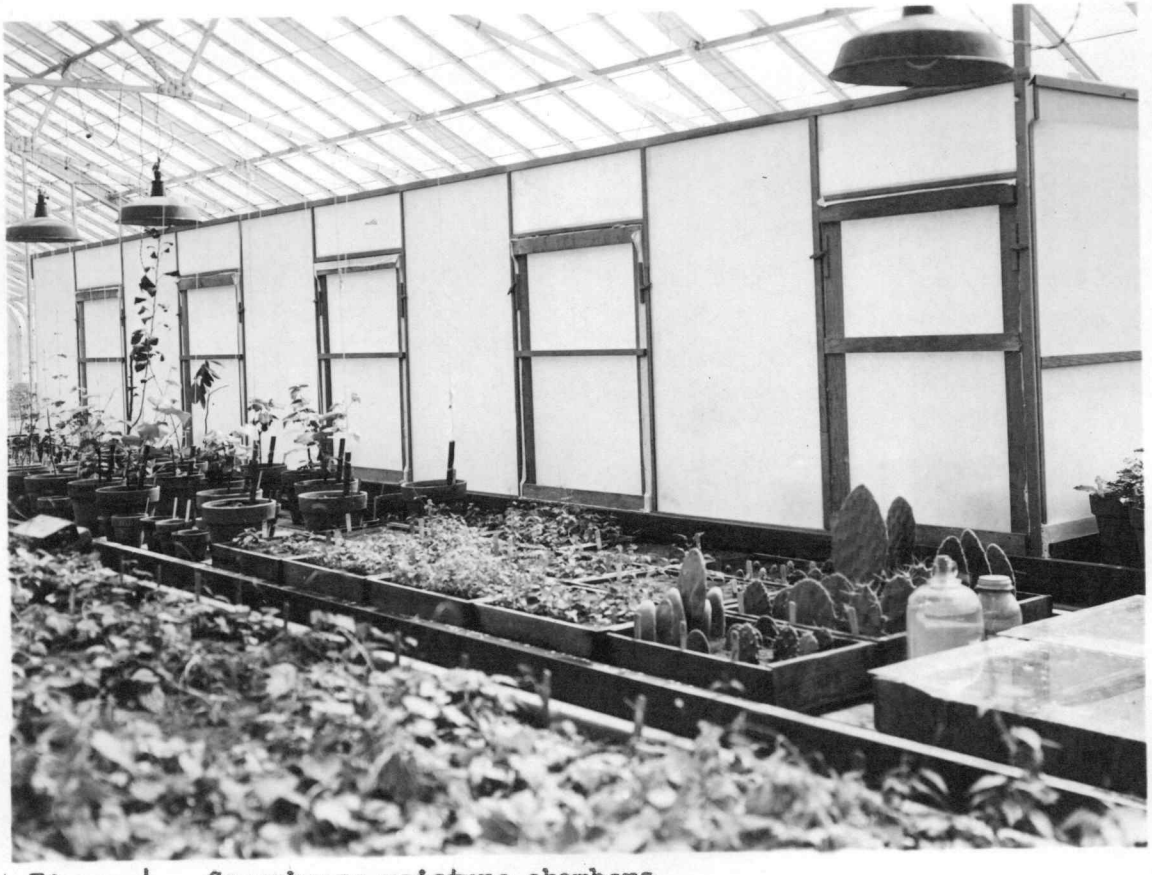

Figure 4. Greenhouse moisture chambers.

Figure 5. Leaf showing marginal infection.

Figure 6. Leaf showing irregular infection pattern.

EXPERIMENTAL RESULTS AND DISCUSSION

The described method of creating artificial infection has proved very effective. The infection was good in all moisture chambers. In making evaluations of resistance or infection the method described by Hoerner (15) was relatively easy to follow, however the total number of spots per leaf in each infection class was much higher in the present study than that shown in Figure 2. Hoerner's chart (Figure 2) was prepared by placing a definite number of spots on the leaf outlines to represent different degrees of infection. Figure 1 shows a typical example of secondary infection as it occurs in the field. Under greenhouse conditions the spots were generally more numerous. Another condition encountered was the occurrence of splotched (Figure 6) and marginal infection (Figure 5). Apparently an excess of free water collecting in these areas caused such patterns. The occurrence of these typical infection patterns was not so numerous as to invalidate the method.

The results of the test designed to evaluate the accuracy of the classification is presented in Table 1. The infection pattern did not appear completely uniform over the entire leaf, therefore it appeared justifiable to sample more than one area on each leaf. The individual observations in Table 1 show considerable variation. The analysis of variance Table 2 indicates that the hypothesis, that the class means are equal, is rejected. It is of primary interest in this study to know that each class mean is different from every other class mean. Tukey's method (28) was used in testing this

relationship. The test indicated that the means of classes 4, 5, 6, and 7 were different from each other. The means for classes 2 and 3 were not significantly different.

Table 1. Number of spots per .25 sq. in. of leaf surface in various infection classes

Class	Areas	Leaves									
		1	2	3	4	5	6	7	8	9	10
2	1	4	5	9	4	9	7	5	4	10	4
	2	2	7	9	6	12	2	4	6	4	4
	3	3	9	11	9	8	4	6	10	2	6
3	1	12	10	9	16	8	25	11	12	8	9
	2	12	3	14	32	8	15	12	12	10	12
	3	11	9	15	11	14	12	7	6	10	20
4	1	28	20	36	10	31	55	36	38	37	36
	2	21	39	39	22	31	22	38	37	33	42
	3	14	36	42	27	28	37	29	32	22	34
5	1	47	119	57	77	97	128	114	126	79	101
	2	54	87	122	55	83	95	135	123	55	113
	3	69	76	61	49	166	152	129	119	97	79
6	1	183	135	142	73	158	122	150	32	189	179
	2	123	85	113	49	95	94	137	29	190	175
	3	134	170	143	60	176	106	121	54	136	156
7	1	204	224	258	236	114	256	209	164	131	260
	2	208	210	187	225	145	224	183	124	135	106
	3	200	261	248	254	75	264	224	93	109	118

Table 2. Analysis of variance of the number of spots per .25 sq. in. of leaf surface in six infection classes

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
Total	984,983.245	179	5,502.700
Classes	785,092.311	5	157,018.462**
Leaves within classes	146,156.934	54	2,706.610
Sampling Error	53,734.000	120	447.783

** Significant at the .01 level.

The formulae for estimating the various components of the variance are presented in Table 3. In addition to the theoretical variances where three leaves were used as a sampling unit, the formulae are given for variances where only one leaf was used as a sampling unit without decreasing the number of observations.

Table 3. Theoretical analysis of variance

Source of Variation	Degrees of Freedom	Parameters estimated by mean square
		3 leaves as sampling unit
Varieties	20	$\sigma^2 + 3\sigma^2_s + 6\sigma^2_p + 18\sigma^2_{plots} + 72\sigma^2_v$
Replications	3	$\sigma^2 + 3\sigma^2_s + 6\sigma^2_p + 18\sigma^2_{plots} + 3780\sigma^2_r$
Experimental Error	60	$\sigma^2 + 3\sigma^2_s + 6\sigma^2_p + 18\sigma^2_{plots}$
Sampling Error		
Plants	168	$\sigma^2 + 3\sigma^2_s + 6\sigma^2_p$
Suckers	252	$\sigma^2 + 3\sigma^2_s$
Leaves	1,008	σ^2
	-	1 leaf as a sampling unit
Varieties	20	$(\sigma^2 + \sigma^2_s) + 6\sigma^2_p + 18\sigma^2_{plants} + 72\sigma^2_v$
Replications	3	$(\sigma^2 + \sigma^2_s) + 6\sigma^2_p + 18\sigma^2_{plots} + 3780\sigma^2_r$
Experimental Error	60	$(\sigma^2 + \sigma^2_s) + 6\sigma^2_p + 18\sigma^2_{plots}$
Sampling Error		
Plants	168	$(\sigma^2 + \sigma^2_s) + 6\sigma^2_p$
Suckers	1,260	$(\sigma^2 + \sigma^2_s)$

σ^2 is the population variance component added by leaves
σ^2_s " " " " " " " suckers
σ^2_p " " " " " " " plants
σ^2_{plots} " " " " " " " leaves
σ^2_v " " " " " " " varieties
σ^2_r " " " " " " " replications

The hypothesis that the two variations, that due to leaves and that due to suckers, are equal was tested by the ratio of the estimated variance for suckers to the estimated variance for leaves.

The ratio of the estimated variance for plants to the estimated variance for suckers is used to test the hypothesis that the two variations, that due to plants and that due to suckers are equal. The ratio, variance for plots over variance for plants, tests the hypothesis that the two variations that due to plants and that due to plots are equal. The experimental error component or plot variance is used in testing the variance for varieties and replications. The latter tests are only of interest, in the present study, to the extent that they give us an indication of the variability of the varieties selected for the study.

The analysis of variance for resistance of the twenty-one varieties with four replications in the greenhouse moisture chambers is presented in Table 4.

Table 4. Analysis of variance of resistance to downy mildew of 21 varieties of hops

Source of variance	Sum of squares	Degrees of freedom	Variance	F
Total	3751.93	1511		
Varieties	506.99	20	25.35	2.16*
Replications	365.31	3	121.77	
Experimental error	702.52	60	11.71	2.96**
Sampling error	2177.11	1428		
Bet. Plants	665.78	168	3.96	1.28
Bet. Suckers	778.00	252	3.09	4.23**
Bet. Leaves	733.33	1008	.76	

* Significant at the .05 level
** Significant at the .01 level

The hypothesis that the variances for leaves and for suckers are equal is rejected since F = 4.23 with 252 and 1008 degrees of freedom. This indicates that there is more variation among suckers than there

is among leaves within suckers. Therefore, it would be preferable to take a smaller number of units of measurement (leaves) while taking a correspondingly larger number of sampling units (suckers). Thus we can increase the precision of the experiment without increasing the number of observations in the experiment.

The hypothesis that the variances for suckers and plants are equal is 1.28 with 168 and 252 degrees of freedom. Since the calculated value of F and the actual value at the 5% point of probability are approximately equal it was not known definitely that the variances are equal. The acceptance of the hypothesis was verified by another experiment specifically designed to measure the between plant differences. The results of this experiment are given in Table 5 which gives an F-value for plants of .18 with 4 and 12 degrees of freedom. This is not significant at the 5% level of probability. This indicates that there is no difference between the two variations, that among the plants within plots and that among the suckers within plants.

An F-value of 2.96 with 60 and 168 degrees of freedom is significant at the 1% level of probability, thus the hypothesis that the variance for plots is equal to the variance for plants is rejected. This indicates that the variation between plots is much larger than that between plants within plots. As a result of this relationship it is apparent that in a similar experiment it would be preferable to use more plots while sampling a smaller number of plants in each plot.

Table 5. Analysis of variance of resistance to downy mildew of 4 varieties of hops using one leaf as a unit

Source of variation	Sum of squares	Degrees of freedom	Mean square
Total	476.00	199	
Variety	122.00	3	40.67
Plants	12.55	4	3.14
Variety X plants	204.00	12	17.00
Sampling error	137.00	180	.76

The sample estimates from the analysis of variance Table 4 can be put on an equal or comparable basis by applying the component analysis, Table 3. For example, from Table 3 we see that the sucker mean square is an estimate of $\sigma^2 + 3\sigma^2_s$, the coefficient 3 meaning that each branch or sucker comprised 3 observations or leaves. To put this on a comparable basis the following equation, $\sigma^2_s = \frac{1}{3}[(\sigma^2 + 3\sigma^2_s) - \sigma^2]$, is used. In this manner we find the comparable variances as follows; the estimated value of $\sigma^2_s = .776667$, of $\sigma^2_p = .145000$, of $\sigma^2_{Plots} = .430556$, and of $\sigma^2 = .7600$. The efficiency of the various apportionments of the sampling components relative to the original experiment may be calculated by using these comparable variances. These are presented in Table 6. This verifies the conclusions that were made from the analysis of variance. The efficiancy of the sampling increases as the number of plants is increased. Increasing the number of suckers without increasing the number of plants also increases the efficiency. This is of interest in the breeding program since the first year seedlings normally

produce a large number of branches although it is not possible to increase these seedlings by clonal cuttings until after the second year.

Table 6. Relative efficiency of various distributions of sampling.

Line	Leaves	Branches	Plants	Plots	Estimated Variance	% Efficiency
1	3	2	3	18	11.71	100
2	1	3	6	18	9.72	120
3	1	2	9	18	9.58	122
4	1	1	18	18	9.43	124
5	1	6	3	18	10.16	115
6	1	9	2	18	10.59	111
7	1	18	1	18	11.90	98
8	2	1	9	18	10.35	113
9	2	3	3	18	10.93	107
10	2	9	1	18	12.67	92
11	3	1	6	18	11.28	104
12	3	3	2	18	12.14	96
13	3	6	1	18	13.45	87
14	6	1	3	18	14.04	83
15	6	3	1	18	15.78	74
16	9	1	2	18	16.80	70
17	9	2	1	18	18.11	65
18	18	1	1	18	25.10	47

The leaf and stem content for the combined data on twelve lots of hops did not exhibit a normal frequency distribution as indicated by the normal probability graph (Figure 7). The plotted cumulative frequencies do not follow a straight line but exhibit a definite curved appearance. The statistics g_1 and g_2, for testing skewness and kurtosis respectively, were calculated from this data. A value for g_1 of 6.938 indicates a frequency distribution curve with an excess of items smaller than the mean or to the left of the mean. A value of t for g_1 of 55.870 with ∞ degrees of freedom is significant

at the 5% level, indicating a departure from normality. A value for g_2 of .8201 indicates an excess of items near the mean causing a peaked curve. A t-value for g_2 of 3.309 with ∞ degrees of freedom is significant at the 5% level also indicating a significant departure from normal.

It is desirable, in the statistical tests applied in this study, that the data conform to a normal distribution or that the mean and standard deviation be independent of each other. Several transformations are available for correcting this relationship, but individual transformations are only applicable to certain types of data. The $\sqrt{x}$ transformation was applied to the combined data and the relative cumulative frequency plotted on normal probability paper (Figure 8). A value for g_1 of .3918 with a t-value of 3.1546 with ∞ degrees of freedom indicates some change in the distribution from the original data. The t-value, however, is significant at the 5% level, indicating that the distribution still deviates from the normal. The value for g_2 was 2.7826 with a t-value of 11.2292 with ∞ degrees of freedom indicating significant kurtosis.

The logarithmic transformation was expected to be appropriate for the data on leaf and stem content since the mean and standard deviation appear to be proportional. The relative cumulative frequency for the logarithmic transformations is presented in Figure 9. A value for g_1 of .1747 with a t-value of 1.4066 with ∞ degrees of freedom indicates that there is no significant skewness in the distribution. A g_2 of .3198 with a t-value of 1.29 with ∞

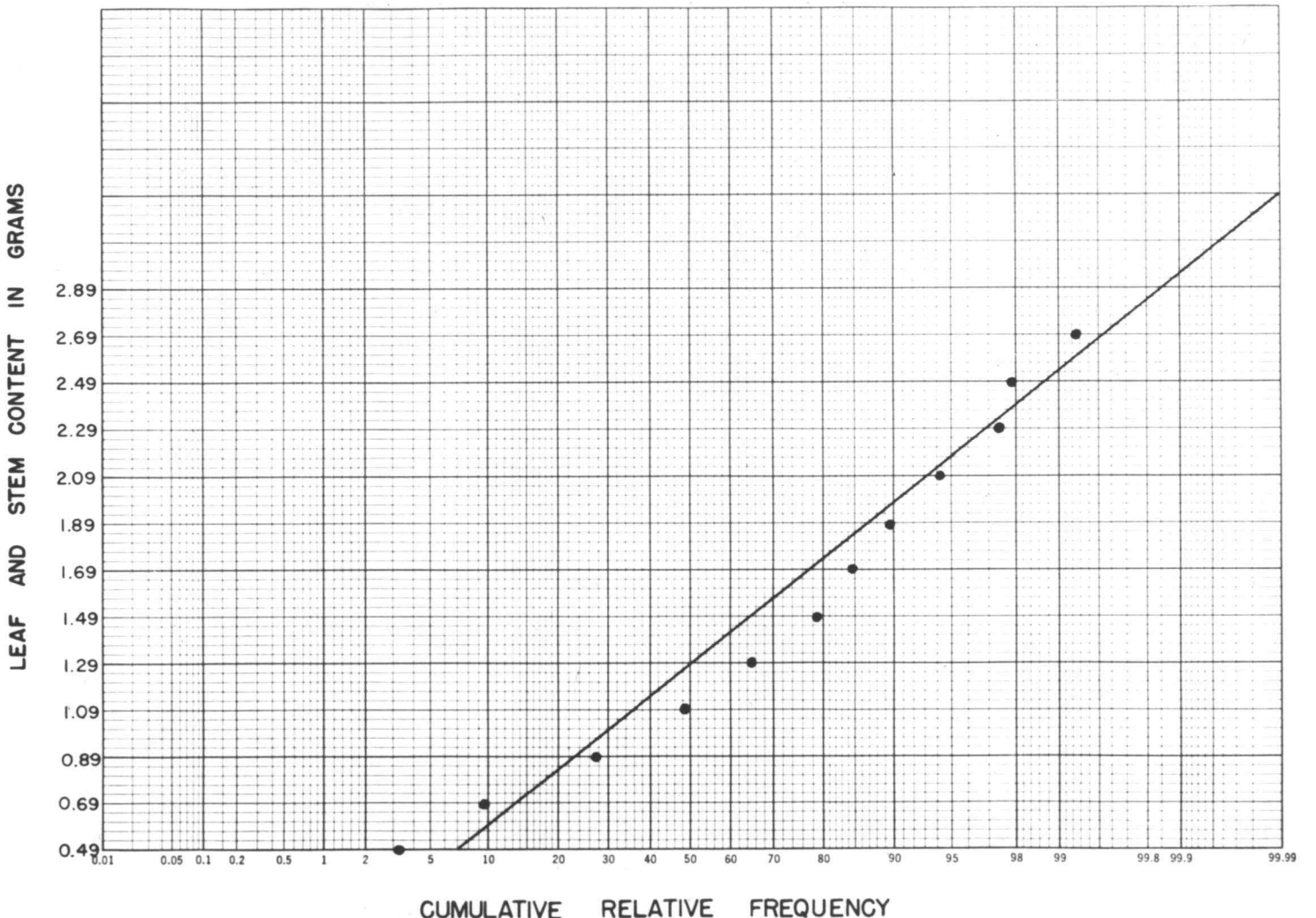

Figure 7. The use of probability graph paper for testing normality of leaf and stem content of 12 lots of hops.

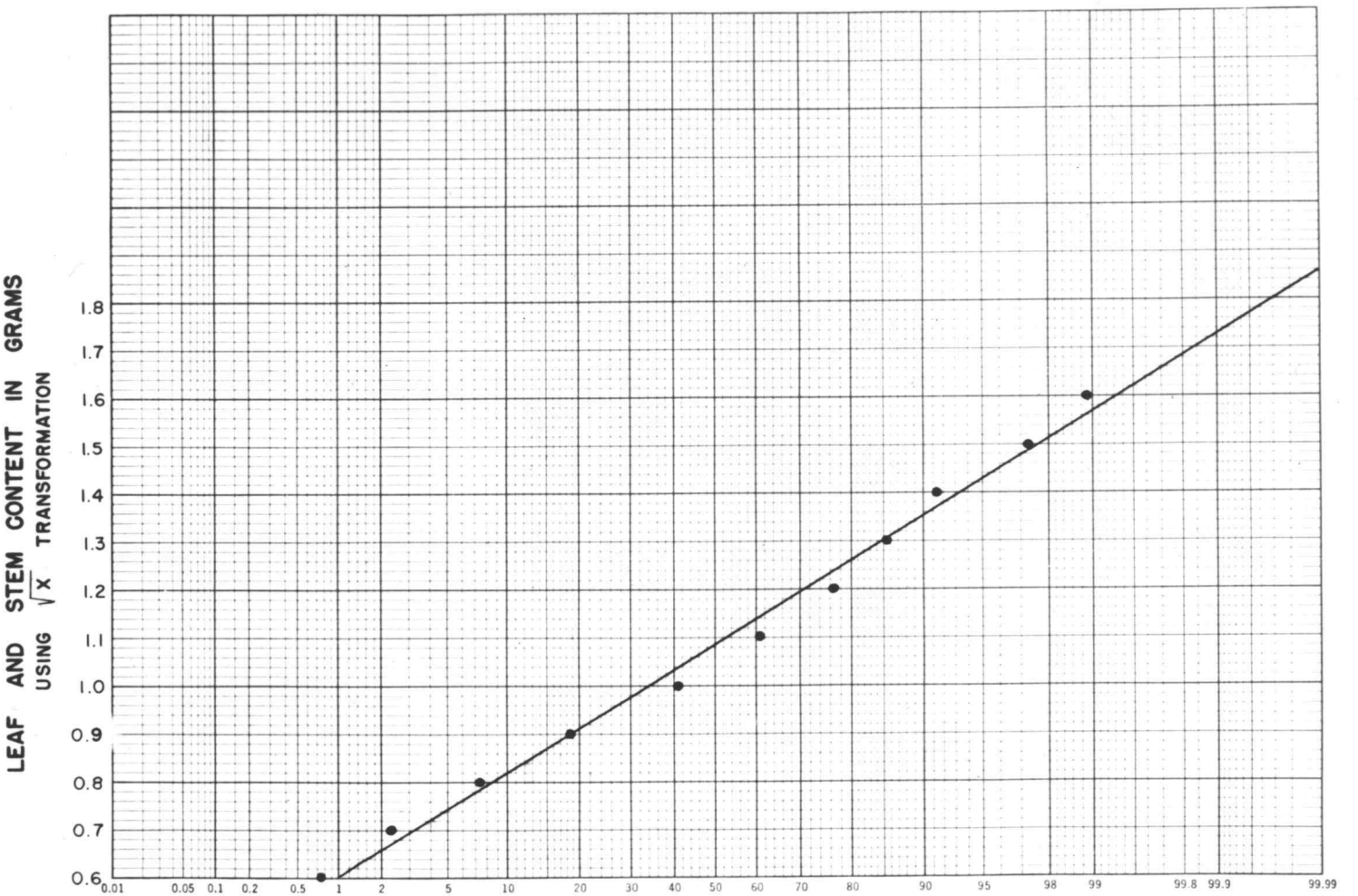

Figure 8. The use of probability graph paper for testing the normality of the $\sqrt{x}$ transformation of the leaf and stem content of 12 lots of hops.

degrees of freedom indicates that kurtosis is not significant. The distribution of the data transformed by use of logarithms, therefore, fits the normal distribution in spite of the fact that it shows a slight peak to the right of the mean.

The means for the twelve lots must be equal before it can be assumed that the lots come from the same population. The analysis of variance of the transformed data from the twelve lots is presented in Table 7. The analysis indicates that the lot means are different therefore the lots sampled must be from different populations.

Table 7. Analysis of variance of leaf and stem content of 12 lots of hops.

Source of variation	Sum of squares	Degrees of freedom	Mean square
Total	12.99295	385	
Lot means	6.08065	11	.55279**
Samples of same lot	6.91230	374	.01848

** Significant at the .01 level

A difference between the variances of the different lots would also indicate that the lots sampled were not from the same population. Bartlett's Chi-squared test of homogeneity of variances was used in testing the hypothesis that the variances were equal. A Chi-squared value of 31.96 with 11 degrees of freedom indicates that the hypothesis would be rejected. The variances are not equal and the lots are from different populations. The combined data

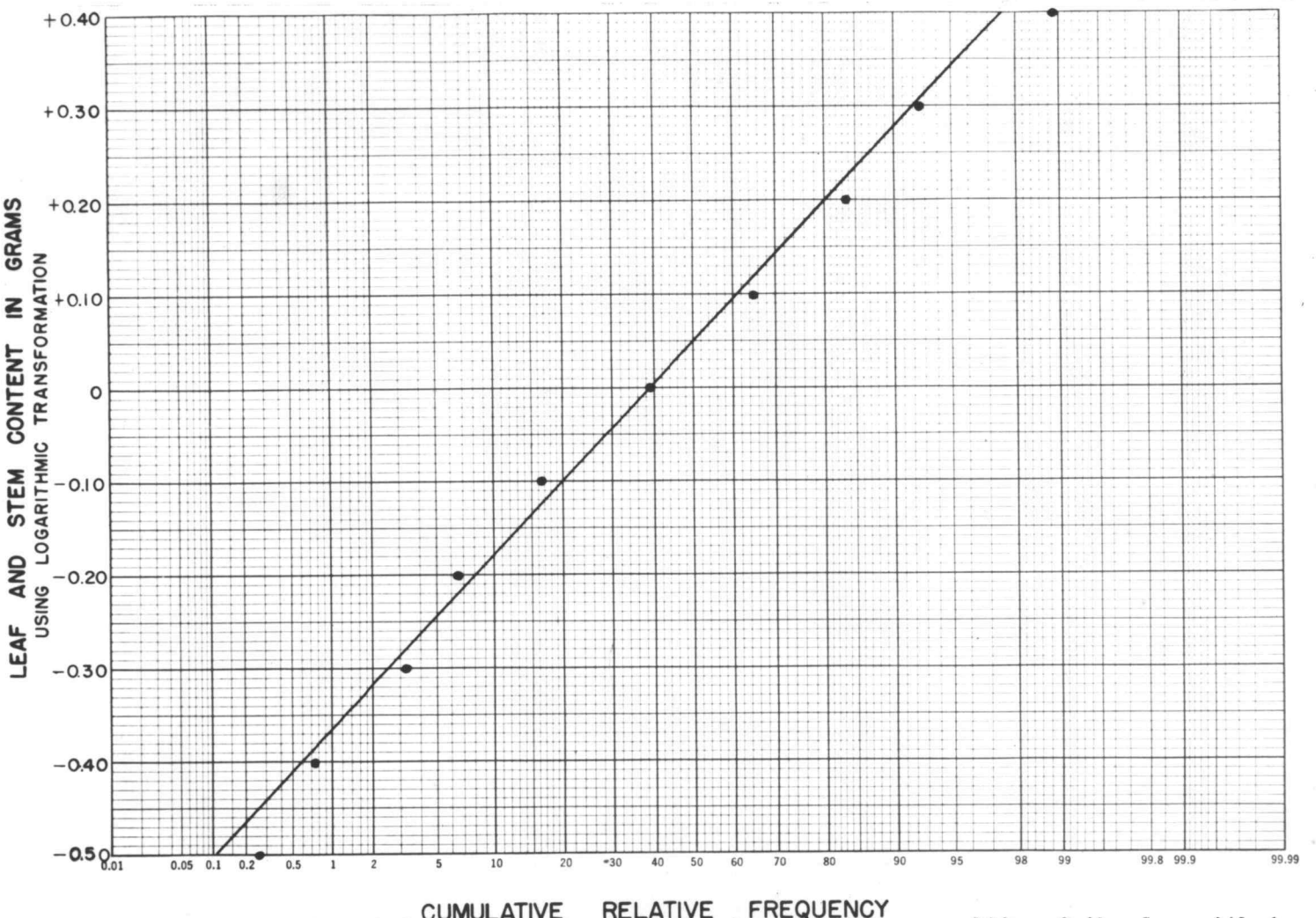

Figure 9. The use of probability graph paper for testing the normality of the logarithmic transformation of the leaf and stem content of 12 lots of hops.

from the twelve lots therefore cannot be used in the sampling experiment.

The number of samples in one of the individual lots was large enough to be used in the investigation of the number of samples necessary to measure leaf and stem content with a given accuracy. The lot with 104 samples was tested for normality by plotting the cumulative relative frequencies on probability graph paper (Figure 10). The plotted frequencies exhibit little deviation from a straight line, therefore no further test was made for normality.

Serial correlation was used in testing the randomness of the data on the leaf and stem content in the 104 samples. The effectiveness of the method used here for determining sample size depends upon a random distribution of the quality factor. The serial correlation coefficient for the 104 samples from one lot was calculated from the formula

$$R = \frac{\Sigma X_i X_i + 1 - (\Sigma X)^2/N}{\Sigma X^2 - (\Sigma X)^2/N}$$

The serial correlation coefficient was found to be -.13140. The existing tables for the distribution of the serial correlation coefficients are limited to 75 observations. Dixon as reported by Li and Keller (19) showed that for N greater than 25 the statistic $U = [R (N-1) + 1] \sqrt{(N+1)[N(N-3)]}$ approximates the normal distribution with mean equal to zero and standard deviation equal to one. The U-value for N = 104 with R = -.13140 is -1.45342 which falls within the five percent point for the one-tail table of U. This

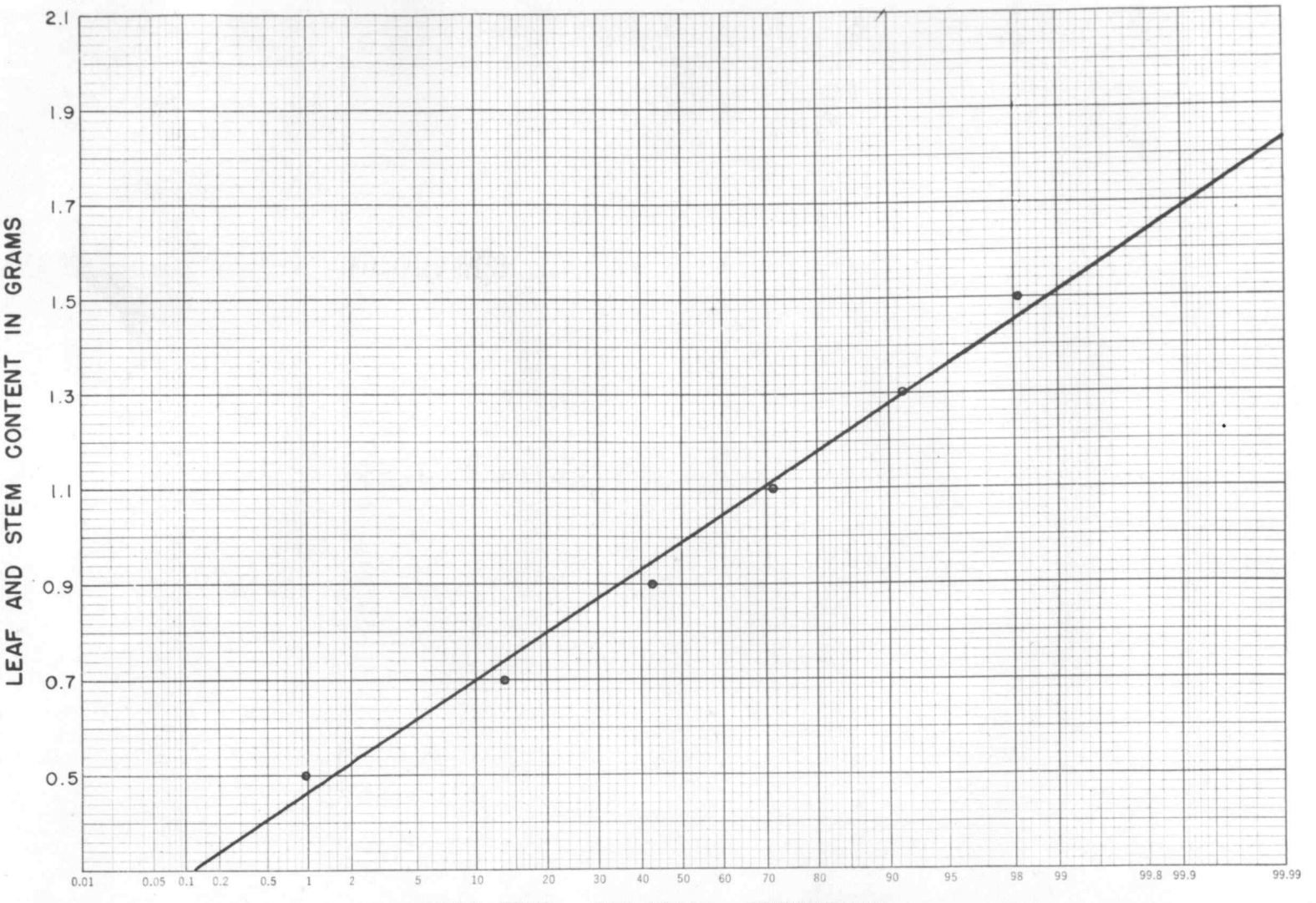

Figure 10. The use of probability graph paper for testing normality of the leaf and stem content of a single lot of hops.

indicates that the data is random.

The method described by Harris, Horvitz and Mood (7) for determining the number of samples for a specified confidence interval was used in this study. The length of the 99% confidence interval of the mean of the 20 gm. sample was specified to be .60 gms. which was considered sufficiently small according to the tentative hop standards (8)(9)(25). A sample size of 38 was determined from the formula

$$(d/s_1)^2 = t_1\text{-}(N)F_1\text{-}(N_1M)/(N \neq 1)$$

where $s_1^2 = 1.048$. The meaning of the symbols in the formula are clearly explained by Harris, Horvitz, and Mood (7). The sample size 38 determined by this method means that in sampling from commercial hops, it would be necessary to sample 38 bales per lot in order to determine leaf and stem content of the lot with a .99 confidence interval of 3% around the mean. The tentative standards set by Hill and Bullis (8) allow a tolerance of 3% between grades for leaf and stem content.

The investigation of the number of samples in determining seed content of commercial hops was conducted in much the same manner as that for leaf and stem content. The samples analyzed were the same samples used in the study of sampling for leaf and stem content.

The combined data for the twelve lots did not follow a normal distribution as illustrated by the cumulative frequency graph (Figure 11). The class marks or class intervals were transformed to logarithms and then plotted on normal probability paper (Figure 12). The transformations did not appear to change the distribution.

The twelve lots apparently all have their own independent distribution.

The analysis of variance of the twelve lots for seed content is presented in Table 8. The mean square for the lot means is highly significant indicating that the lot means are different.

Table 8. Analysis of variance of seed content of 12 lots of hops.

Source of variance	Sum of squares	Degrees of freedom	Mean square
Total	538.5484	385	
Lot means	527.9061	11	47.9915**
Samples of same lot	10.6423	374	.0285

** Significant at the .01 level

The variances of the twelve lots were not homogeneous. Bartlett's Chi-squared test of homogeneity of variances resulted in a Chi-squared value of 231.47 with 11 degrees of freedom.

The twelve lots are evidently from different populations and cannot be combined for the size of sample study.

The single lot of 104 samples, used in the previous test on leaf and stem content was also used for seed content. The frequency distribution of the original data (Figure 13) as well as the logarithmic transformation (Figure 14) were plotted on probability graph paper. The original data appears to approach the normal distribution more closely than the transformed data. The Chi-squared test was used to test the goodness of fit of the frequency distribution of the original data to the normal frequency distribution. A Chi-squared

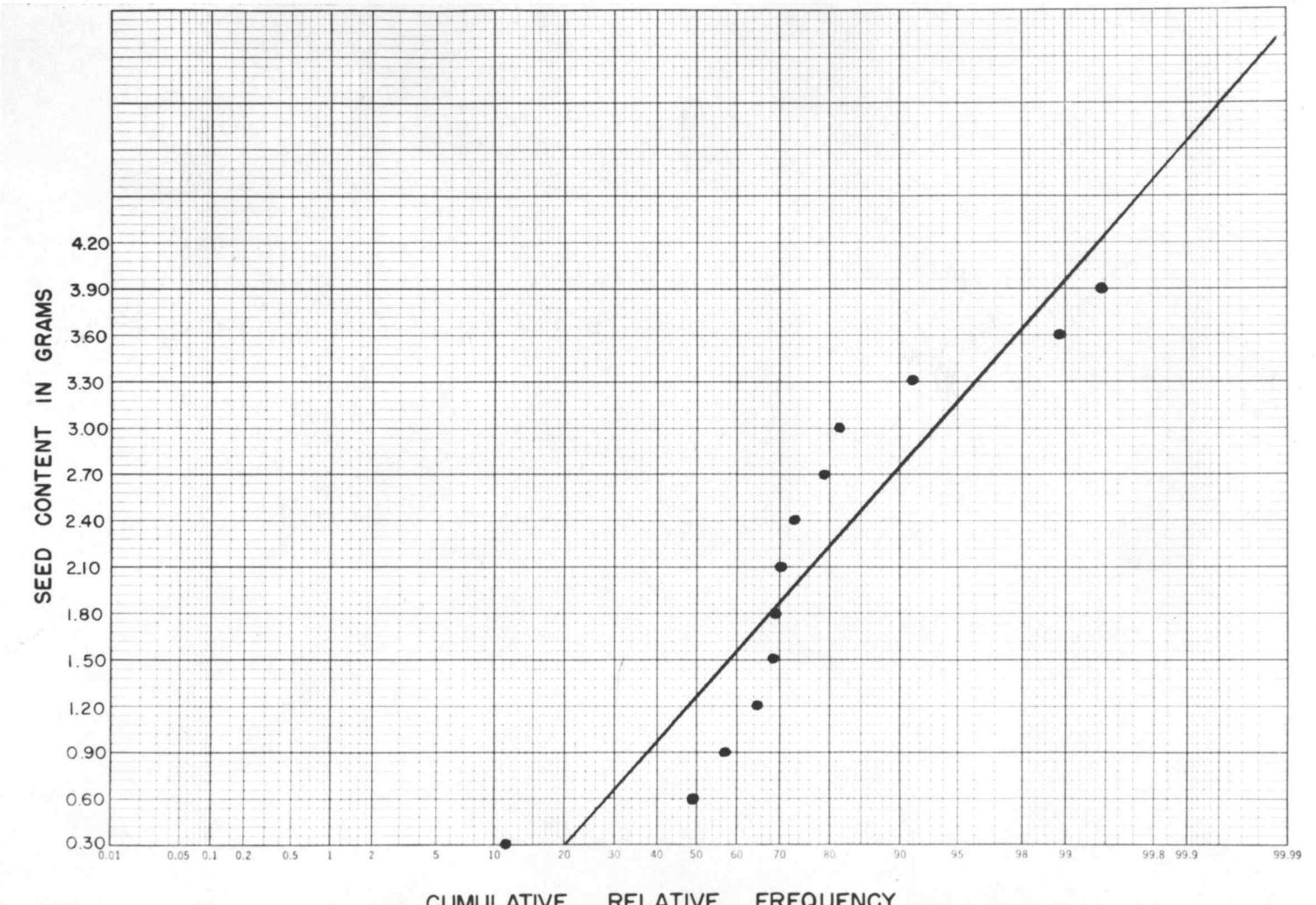

Figure 11. The use of probability graph paper for testing the normality of the seed content of 12 lots of hops.

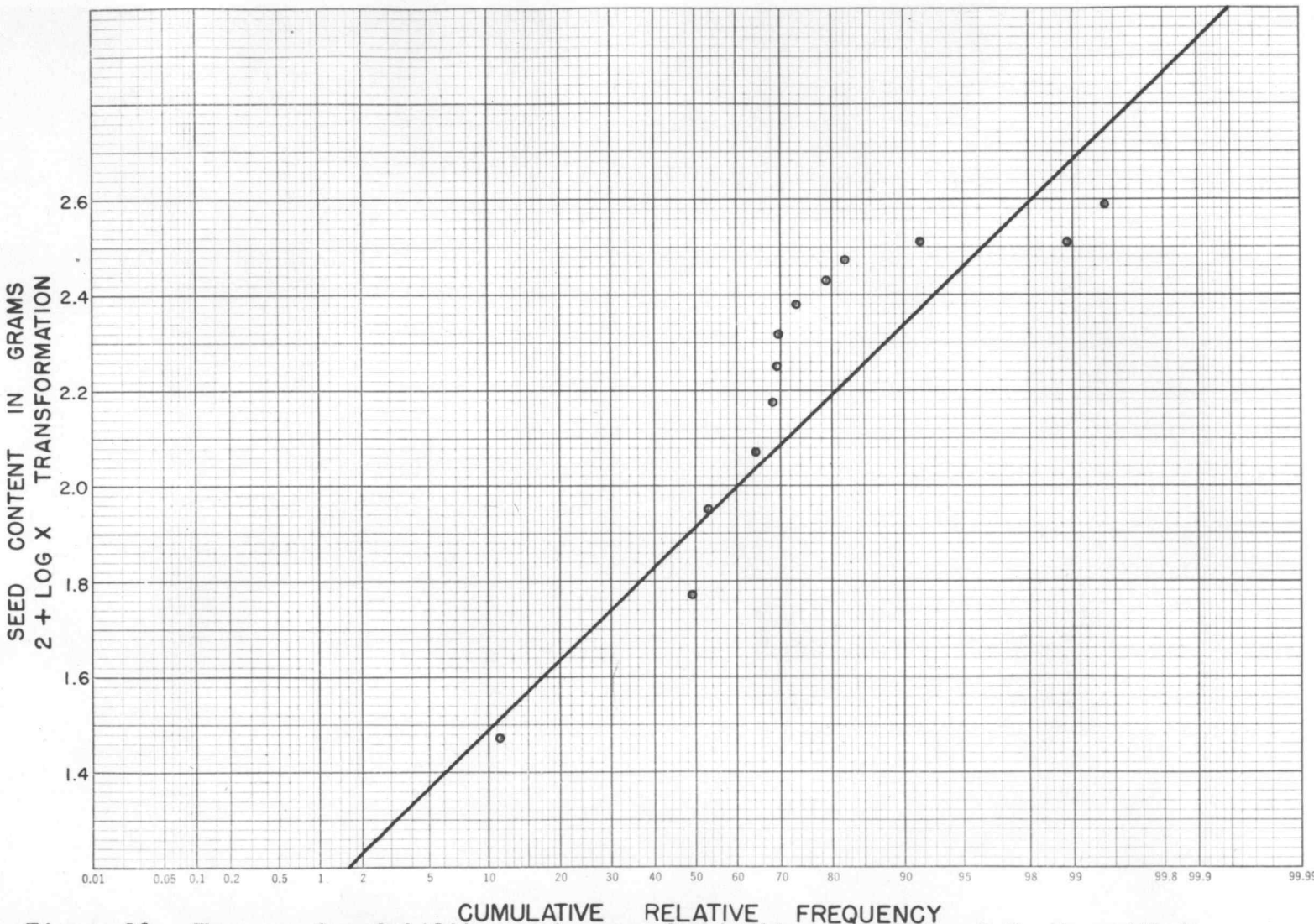

Figure 12. The use of probability graph paper for testing normality of the logarithmic transformation of the seed content of 12 lots of hops.

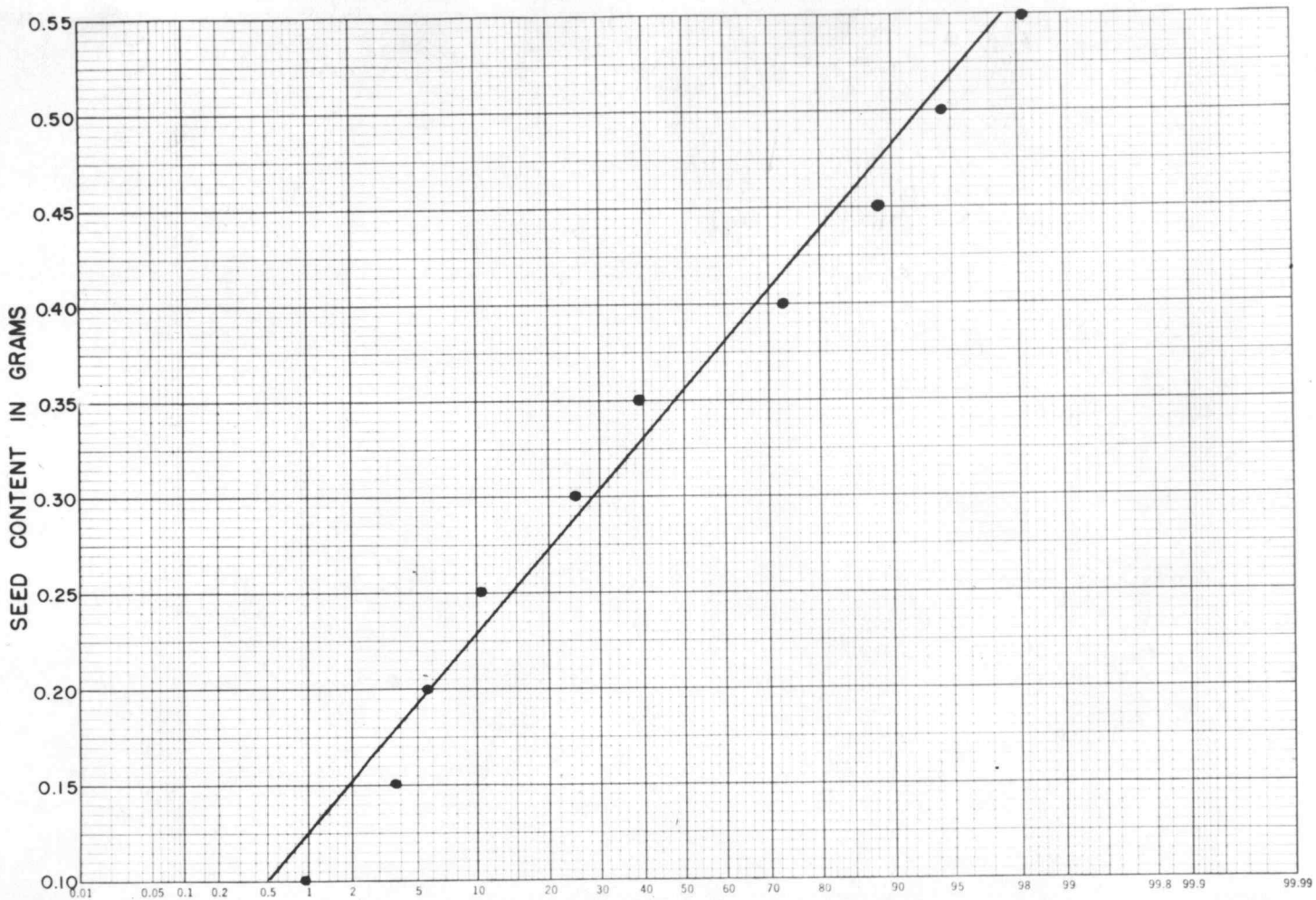

Figure 13. The use of probability graph paper for testing normality of seed content of a single lot of hops.

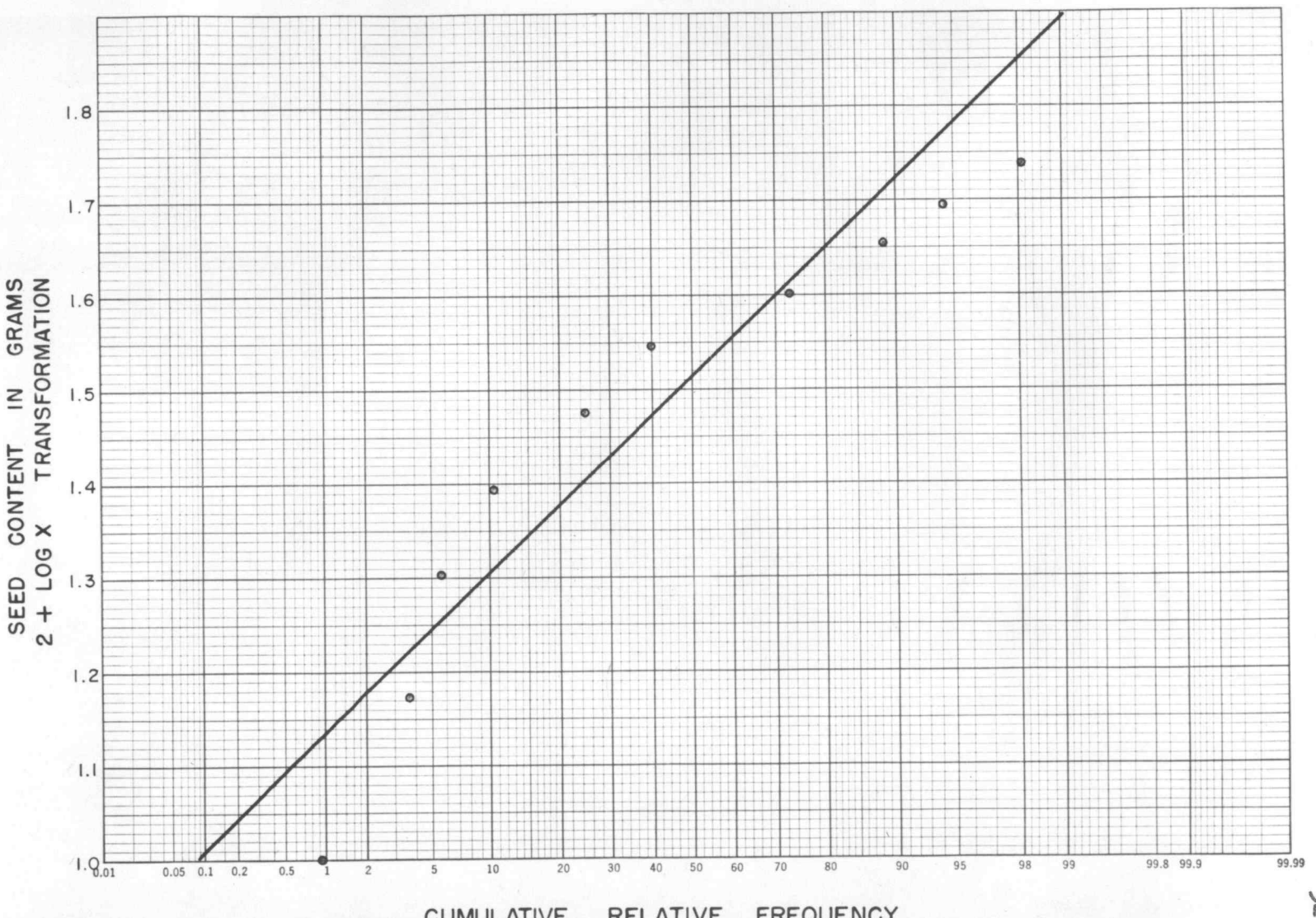

Figure 14. The use of probability graph paper for testing normality of the logarithmic transformation of the seed content of a single lot of hops.

value of 10.871 with 7 degrees of freedom indicates that the original data fits the normal distribution.

The serial correlation coefficient, R, was determined for the 104 samples by the formula presented in the previous section. An R of -.0492 was obtained for N = 104. A U-value of -.6062 for an R of -.0492 with N = 104 was obtained. This indicates that the set of data is random.

The application of the method of Harris, Horvitz and Mood (7) for the determination of number of samples was the same as in the previous section on leaf and stem content. The confidence interval for seed content would be the same as for leaf and stem content. σ^2 for seed content was .0085. The number of samples determined was 35.

Sather and Hill (24) presented data on duplicate analyses for seed content of the same samples. They also give a comparison of seed content of samples from the end and middle of the same bale. They have indicated that there are measurable differences between these duplicates and also between the end and middle bale sampling.

The original data from their study is presented in Table 9 and Table 10. The t-values calculated from this data are presented. A t-value of .0038 with 21 degrees of freedom is smaller than $t_{.05}$ of 2.080 with 21 degrees of freedom. This indicates that there is no difference between the duplicate analyses. The t-value of .0803 with 21 degrees of freedom for end vs. middle bale samples is smaller than $t_{.05}$ of 2.080 with 21 degrees of freedom. This indicates that there is actually no difference in the seed content between end and middle

bale samples. The position of sampling in the bale has been regarded as an important factor therefore it is interesting to see that no difference actually existed. The data in Table 10 does seem to indicate differences, but when the magnitude of the variance is considered this indication no longer exists.

Table 9. Comparison of duplicate analyses for seed content of the same sample.*

Bale Number	1st Analysis	2nd Analysis	Difference
10	3.3	3.5	0.2
20	4.4	4.6	0.2
27	3.4	3.1	0.3
30	3.5	3.3	0.2
40	4.9	4.8	0.1
49	4.5	4.3	0.2
50	2.7	2.9	0.2
60	3.5	3.6	0.1
70	2.8	2.6	0.2
80	4.2	5.4	1.2
90	4.7	4.0	0.7
98	3.2	3.1	0.1
100	3.0	2.7	0.3
110	3.8	4.0	0.2
117	2.8	2.6	0.2
120	2.8	3.6	0.8
130	4.0	3.3	0.7
140	3.1	3.0	0.1
150	2.3	2.8	0.5
160	3.0	3.5	0.5
163	2.3	2.8	0.5
165	3.5	2.9	0.6
	3.44	3.45	0.01

$t = .0038$ with 21 d.f.; $t_{.05} = 2.080$ with 21 d.f.

* Courtesy of Sather and Hill

Table 10. Comparison of seed content of end and middle bale samples.

Bale Number	Average Analysis End	Single Analysis Middle	Difference
10	3.4	3.0	-0.4
20	4.5	3.5	-1.0
30	3.4	4.5	+1.1
40	4.8	2.8	-2.0
50	2.8	4.5	+1.7
60	3.5	3.5	0.0
70	2.7	6.0	+3.3
80	4.8	5.1	+0.3
90	4.3	4.7	+0.4
100	2.8	3.2	+0.4
110	3.9	3.3	-0.6
120	3.2	4.8	+1.6
130	3.6	4.5	+0.9
140	3.0	4.0	+1.0
150	2.5	4.0	+1.5
160	3.2	3.2	0.0
165	3.2	3.7	+0.5
	3.52	4.02	+8.7

$t = .0803$ with 21 d.f.; $t_{.05} = 2.080$ with 21 d.f.

SUMMARY AND CONCLUSIONS

An investigation was conducted on twenty-one varieties or strains of hops to determine the effect of the size and distribution of samples on the efficiency of a method of evaluating the resistance of hops to downy mildew, Pseudoperonospora humuli (Miy and Saka) Wils.

The method of sampling used in this study introduced three variables or components in the sampling error namely, leaves, suckers, and plants. A comparison of these components was made by the analysis of variance and the relative efficiency of varying apportionments of the components is presented.

The data indicates that the efficiency is increased much more rapidly by increasing the number of plants per plot sampled while taking a correspondingly smaller number of suckers per plant. The gain in information obtained by increasing the number of leaves per sucker is apparently very small. As is very often the case in the breeding program, the number of plants of a given variety or line may be limited. The efficiency then may be increased considerably by increasing the number of suckers per plant with a minimum number of leaves per sidearm.

A method of producing infection under controlled environmental conditions is presented. The method is apparently very satisfactory where it is desirable to evaluate the relative resistance of a large number of lines each year. The method of evaluation provides for ten different classifications of resistance. The plant breeder is interested only in the very small percentage of the most resistant

plants. The primary interest therefore would be in the ability to determine the difference between the very resistant plants and the susceptible ones. The data indicates that the method of evaluation described in this study will satisfy these requirements.

A part of the present investigation was devoted to a study of sampling of commercial hops for quality determinations. The data on twelve commercial lots of hops was used in the investigation.

A preliminary analysis of the data indicated that it would not be desirable to combine the twelve lots for the sampling investigation. Their means and variances were different indicating that the lots were from different populations.

One lot of 104 samples was used in the determination of sample size. The method of Harris, Horvitz and Mood was used in determining the number of samples necessary to determine leaf and stem content and seed content. The method indicated that it would be necessary to sample 38 bales per lot for leaf and stem content and 35 bales per lot for seed content. This number of samples is necessary in order to determine these respective quality factors with a given degree of accuracy.

Two sampling factors discussed by Sather and Hill (24) were reanalyzed. It was found that the differences between duplicate analyses of the same sample were not significant. Samples taken from the end of the bale appeared to differ from those taken in the middle of the bale. The analysis indicates that these differences are not significant.

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