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For Oats And Barley

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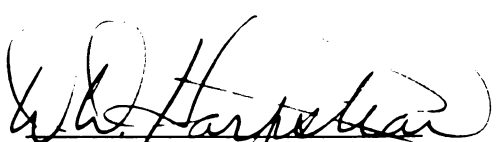

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AN ANALYSIS OF SELECTED BREEDING APPROACHES FOR OATS AND BARLEY

By

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ABSTRACT

AN ANALYSIS OF SELECTED BREEDING
APPROACHES FOR OATS AND BARLEY

by

James Laurence Nelson

Oats (Avena sativa) and barley (Hordeum vulgare) are studied by different means with respect to the value of the approaches to plant breeding. Chapter one includes an analysis of twenty-one oat genotypes grown in common in four locations for the years 1977 through 1979. Yield and the primary components of yield, as well as other agronomic data, were recorded and used to construct a new index of stability and superiority. Additionally, the oat population was studied by means of path coefficient analysis and three potential population parameters were noted. Chapter two contains an analysis of the oat variety Heritage analyzed by means of regressing the primary components of yield for Heritage against the mean components for the population of adapted cultivars. Seed number per panicle, Y, is the component which establishes Heritage as a superior variety. Chapter three tests the hypothesis that mean seed number per unit area, XY, for pure lines with respect to each other, is an effective predictor of the relative frequencies of each genotype after one generation of growth in bulk mixtures. Three barley genotypes and two oat genotypes constituted the pure lines and the bulk mixtures. The hypothesis was rejected and the explanation was differential seedling vigor in the

early stages of growth for several of the genotypes. Chapter four presents a method for parental selection such that two populations with similar yields can be seperated by regressing one yield component against another. Outliers are thus indentified as lying off the primary regression line and these genotypes may then be used in crossing combinations which produce unselected progeny whose grain yield exceeds that of each of the highest yielding parents. Chapter five details the work to date on the wide hybrid between H. vulgare and H. jubatum with respect to the introgression of wild germplasm from H. jubatum into the genome of H. vulgare. Additionally, the results of a series of experiments, intended to increase the frequency of haploid sectors of H. vulgare for the hybrid, are reported. A chemical called griseofulvin was demonstrated as inducing a higher grequency of haploidization -- both for H. vulgare and for H. jubatum -- from the hybrid. Griseofulvin was shown not to induce the same phenomenon in six diploid cultivars of barley. Therefore, an hypothesis was proposed that a degree of genomic instability is necessary for griseofulvin to induce sectoring and that this sectoring is a function of whole genomes and not of individual chromosome elimination.

This dissertation is dedicated to

L. S. Nelson, M.D., F.A.C.S., F.I.C.S. and
L. S. Nelson, Jr., M.D.

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PREFACE

This dissertation is appropriately divided into five chapters. Each is sufficiently different in terms of the research it reports to merit such a division. Chapters 1 through 4 are related insofar as the common thread running current throughout them is the use of the primary components of yield to study various phenomena.

This is appropriate in view of the unique set of yield component data which, but for the author, might well have remained unused. As the last graduate student of the late Dr. John E. Grafius and, to a considerable extent, his spiritual heir, it seemed the responsibility of the author to resurrect some of these data and apply them to contemporary problems in plant breeding. There is much, however, which remains to be done.

Chapter 1 introduces yield components and their possible use in a new index of stability and superiority in oat populations. This index is complemented by a path coefficient analysis of the same oat population with yield components. Chapter 2 introduces a slight modification of simple linear regression with yield components to assess the stability and superiority of performance of Heritage, a new oat variety. Chapter 3 attempts to relate the expression of yield components in pure stands of oats and barley to the performance of the varieties when grown as bulk populations. Chapter 4 details the use of yield components in parental selection for higher yield. These chapters will,

possibly, suggest the many uses of yield components, ranging from studies of the genotype-environment interaction (Chapter 1) through their effective manipulation in improving yield.

Chapter 5 is an anomaly only in the context of this dissertation. Of all the chapters, it represents the greatest effort on the part of the author and it represents his original research topic. The methods used in attempting to transfer genetic variability from a wild species to Hordeum vulgare and the results of these efforts are reported.

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CHAPTER 1

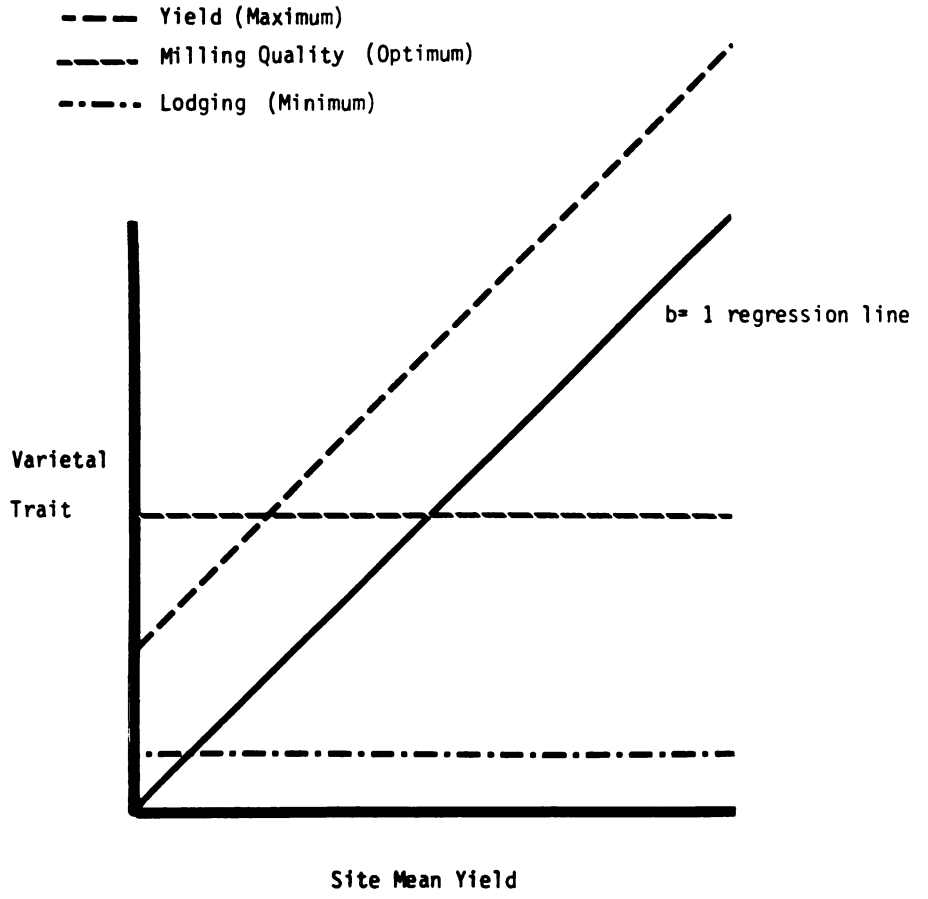
AN ALTERNATIVE APPROACH TO ESTIMATION OF STABILITY AND SUPERIORITY OF A COOL SEASON OAT POPULATION

Introduction

It is the author's view that all agronomic traits may be classified as either maxima, minima or optima, for the purpose of plant breeding. Yield components and test weight in cereals are examples of maximum traits, disease and lodging are minima and the various quality factors (malt and milling quality) are optima, for which some range of values exist, and within which the optimum traits should lie. These classifications are not rhetorical since they clarify breeding goals. In this light a plant breeder does not want absolute yield stability in a variety, for the consequence of this would be a variety buffered against all environmental variables, including those which would maximize yield. Simultaneous with breeding for maximum traits, the breeder seeks to impose through genetics a minimum expression of negative traits, of which disease is a ubiquitous example. Yet, over the range of environments, the breeder seeks to stabilize the expression of the many quality factors (optima) which are necessary constituents of a good variety. Figure 1 expresses the three categories as a function of an independent variable, site W (yield), for a hypothetical oat variety.

It had been suggested to the author by Dr. M. W. Adams that stability might also be broken into components, most logically those of yield.

FIGURE 1. Example Of Minimum, Maximum and Optimum Traits Regressed Against The Site Mean Yield (W) For A Hypothetical Oat Variety.



A normalizing transformation would standardize the traits and the correlation between the sets of yield components grown in different year-sites might offer some statistical measure of stability. It then occurred to the author that a combination of the two ideas, trait classification and the concept of stability as a function of multiple components, might be effected and tested on an appropriate data set. Additionally, the author desired to test another measure of stability, that resulting from path anlysis, and the yield model proposed by its author, Dr. George C. C. Tai.

Literature Review

It is not the purpose of this paper to present an exhaustive review of the many methods utilized by plant breeders to adjudge stability and superiority. In general, however, two watershed papers (1,2) presented methods which allowed plant breeders to compare many varieties over a range of locations and to establish some measure of statistical confidence in their judgements. Aside from the regression papers of Finlay et al. (1) and Eberhart et al. (2), other parameters have been suggested (3,4) which relate to stability of yield performance.

A possible limitation to all the preceeding methods is their reliance upon yield alone, which, as Grafius has shown, is an artifact resulting from the multiplicative interactions of its primary components (5). Just as yield has been broken into its components, perhaps stability, properly expressed as a function of components, might better explain varietal behavior in differing environments.

Path coefficient analysis does not provide direct measures of varietal superiority. In the agronomic literature, Dewey and Lu (6) first applied path analysis to yield components, albeit somewhat confusingly. Subsequently, Duarte and Adams (7) used path analysis to analyze both primary and secondary yield components in Phaseolus. Eventually, additional literature appeared which tied together the promise of path analysis with a more thorough understanding of yield components. Based upon the proposal of the sequential development of yield components in cereals (8), Tai (9,10) developed a method for resolving the individuals paths. Additionally, he proposed a model for yield, expressed in standard deviation units. Finally, Hamid and Grafius (11) proposed a developmental allometry for barley which was later modified by Grafius (12). These papers represent the entirety of literature familiar to the author which bears upon path analysis and crop yield expressed through its components.

Materials and Methods

I. Stability Index

The stability index which the author proposes utilizes only maximum traits and, for this data set, it includes the following traits:

X = panicles/ft^2

Y = seeds/panicle

Z = weight/seed

W = weight/seed

TW = pounds/bushel

It is of interest to note that the set of maximum traits could be

augmented with minimum traits, were the latter expressed as a differential between some upper limit (i.e., 100%) and their actual value (the author appreciates this suggestion from Dr. M. W. Adams).

The use of maximum traits affords both a measure of stability and of superiority. The measure of stability is the standard deviation of the vectors and the superiority lies in their length. The transformation can be found in Figure 2. In using this transformation, which is actually the Z transformation for normality, the different variables are converted to common units (standard deviations) with unit variances and means of zero. An example of non-transformed and transformed data are to be found in Table 1 for Heritage, entry number 3, of the twenty-one genotypes.

The data were derived from rod row oat experiments grown in four locations over three years, 1977-1979. Twenty-one genotypes were grown in these common experiments and one location was lost--East Lansing in 1979. The experiments were planted and analyzed as 5 x 5 square lattices with four additional varieties which varied from year to year. The entries were planted in four rows, eight feet in length, with eleven inch row spacings. Each entry was replicated four times. The following data were recorded: X (panicles/ft^2), Y (seeds/panicle), Z (seed weight), W (yield in bu/a), test weight (lbs/bu), height, lodging and heading date. These data were tabulated and means over locations each trait were calculated. Figure 2 details the transformation and the various statistics associated with the data.

The assumption behind this stability index is that varietal stability might be expressed through the correlation of a number of traits with the mean of each trait over year-sites. Referring to Table 1 and

FIGURE 2. Methods Used For The Calculation Of The Transformations And Associated Statistics Used In The Stability Index.

Transformation: $\frac{E_{ijk} - E_{ij}}{\sigma_{ij}}$

Where E_{ijk} = jth trait of the ith genotype in the kth year-site,

E_{ij} = mean of the jth trait of the ith genotype over year-sites,

σ_{ij} = standard deviation of the jth trait of the ith genotype over year-sites,

i = genotype (1-21),

j = trait (1-5 for X,Y,Z,W and TW, respectively),

k = year-site (1-11) (see Appendix 1).

Statistics: U_{ij} = trait mean of the ith genotype over year-sites,

$r_{(ijk)(U_{ij})}$ = correlation coefficient for traits 1,2,3 and 5

or r_{ik} = (n=4) between individual year-sites and trait means over year-sites for the ith genotype (W is excluded since XYZ=W),

$\Theta_{ik} = \cos^{-1} r_{ik}$ = direction of vector

W_{ik} = yield of the ith genotype in kth year-site,

= length of vector in standard deviation units,

$\sigma_{\theta i}$ = standard deviation of the ith genotypic vector.

TABLE 1. Example Of Data Transformation For Heritage Oat.

Non-transformed

Year-Site

Trait	1	2	3	4	5	6	7	8	9	10	11	U_{ij}
X	19.5	18.0	18.3	9.1	17.8	14.9	16.6	16.9	16.1	10.4	9.6	15.2
Y	73.8	84.6	87.3	70.1	88.4	84.6	97.7	71.3	79.2	105	90.6	84.8
Z (mg)	32.6	29.4	29.7	29.5	29.4	32.6	32.3	32.5	34.4	32.8	34.9	31.8
W	141	133	138	57	148	134	171	127	130	108	92	125
TW	35.1	33.0	32.6	30.6	33.6	36.0	34.8	31.8	34.8	33.3	37.3	33.9
HT (in)	35.0	35.8	40.2	29.7	43.9	36.4	44.5	34.0	37.1	42.2	36.4	37.7
LD (%)	8	25	2	---	20	---	0	---	---	25	---	13
HD	18	---	---	---	28	---	---	---	---	---	24	23

Transformed

Year-Site

Trait	1	2	3	4	5	6	7	8	9	10	11	U_{ij}
X	.26	-.60	-.13	-.94	-.04	-1.1	-.38	.89	1.38	-.88	-.81	-.12
Y	1.0	.82	.90	.38	1.05	1.72	1.41	.13	-.21	.53	1.00	.89
Z	.10	.04	-.14	0	-.69	.44	.04	.32	.57	.19	.46	.21
W	1.35	1.52	1.26	-.62	.75	1.37	1.59	1.35	1.04	-.10	.50	.47
TW	.22	-.06	-.12	.19	-.74	.57	.85	.08	1.24	-.50	1.04	.19
HT	-.73	-.37	-.49	-.49	-.83	-.94	.13	0	.26	-.70	-.59	.23
LD	.71	-.22	.45	---	.22	---	-1.04	---	---	.45	---	1.08
HD	1.12	---	---	---	.94	---	---	---	---	---	1.25	.59

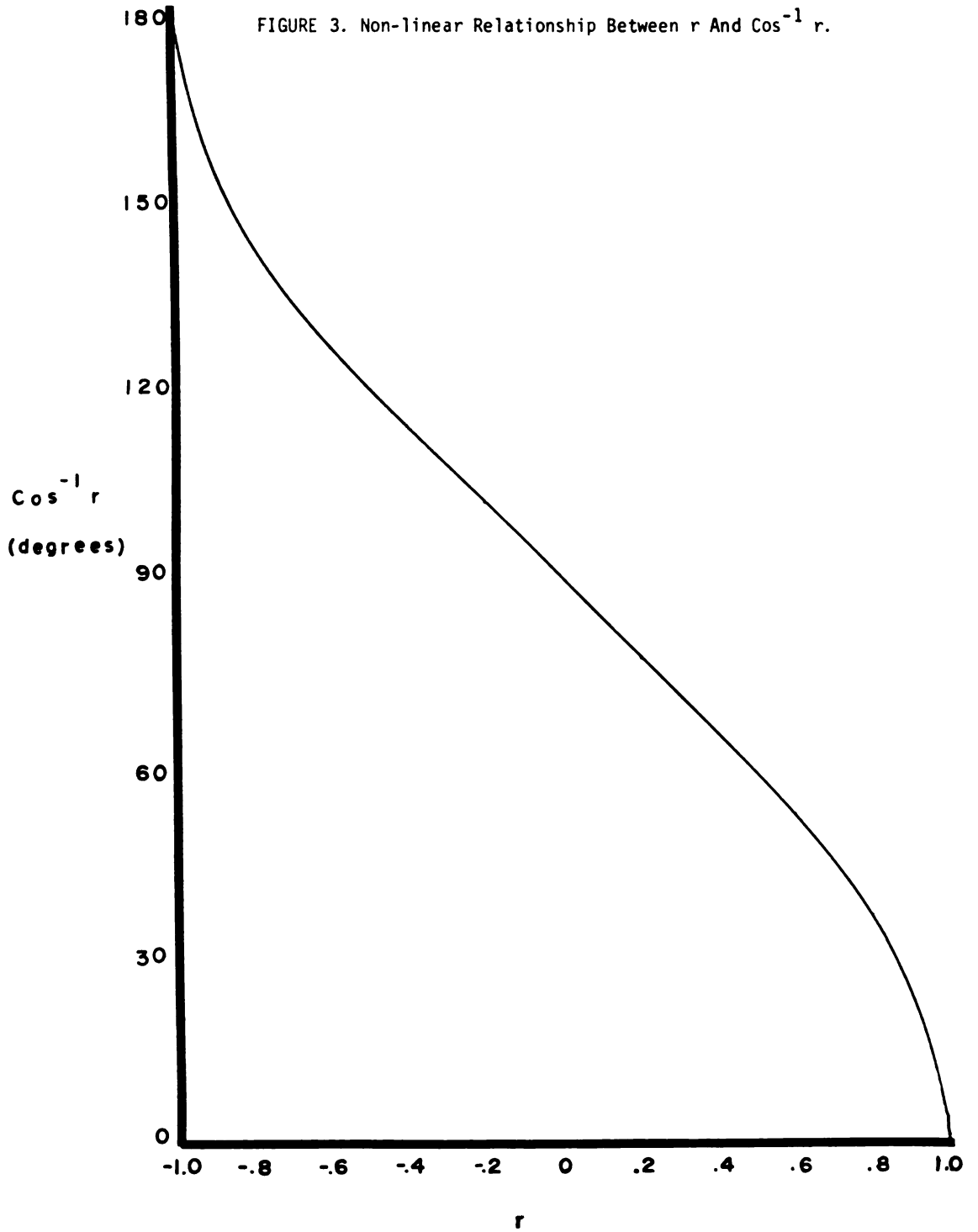
X= panicles/ft^2, Y= seeds/panicle, Z= weight/seed, TW= test weight (lbs/bu), HT= height in inches, LD= lodging and HD= heading date (days after 6/1).

Figure 2, this would involve calculating a pair-wise correlation coefficient, r_{ik}, for each year-site with the mean. This would generate eleven correlation coefficients whose distribution is a reflection of stability. This distribution can be represented graphically if one converts the correlation coefficients to angles, such that $\Theta = \cos^{-1} r_{ik}$. Figure 3 represents Θ as a function of r_{ik}. Any variety in this study, then, has eleven vectors with assigned directions (Θ). Each vector can be accorded a length by assigning the respective transformed yields, W_{ik}. These two data comprise the intravarietal stability and superiority measures since the more closely the vectors are clustered, the more stable their phenotypic expression. Their superiority is a function of their length and this allows comparisons between years and between locations. Having calculated these vectors it is possible to contrast varieties by comparing tabular values for theta (Θ_{ik}), the respective lengths of the vectors (W_{ik}), and the standard deviation for the genotypic Θ_{ik} ($\sigma_{\theta i}$). A set of correlation coefficients between the genotypic means and the mean over genotypes cannot be calculated since this comparison, or grand, mean is necessarily zero as a consequence of the normalizing transformation.

II. Path Analysis

The two underlying assumptions behind the path analysis used in this study are, first, that yield (W) is the product of the multiplicative interaction of the three primary components of yield, X, Y and Z and, secondly, the allometric plant development hypothesis of Grafius (8,12) is correct. These assumptions first allowed Tai (9) to develop a path coefficient system in which the paths are resolved by means of

FIGURE 3. Non-linear Relationship Between r And Cos^{-1} r.



simultaneous equations. Tai proposes that for any year and site there are essentially three different environmental resource groups, R_{ijk} (j = 1-3), which are exploited with varying degrees of efficiency by each genotype, through that genotype's yield components over time. Thus, paths from each resource may be calculated algebraically from the path coefficients connecting the yield components to one another and to yield. Figure 4 demonstrates the relationships.

Tai (9) also proposed a yield equation, expressed in standard deviation units, which is composed of some value for mean yield of a genotype, three genotypic components derived from the respective paths, three environmental components associated with each genotypic component and an error deviate. Figure 5 details this system and the author's modifications. The author proposes that the grand mean and grand standard deviation (over genotypes and over year-sites) be substituted for Tai's genotype-specific values for the same statistics. The oat data set is more powerful than Tai's and, therefore, allows some estimates of population parameters associated with cool season oats. The use of the grand standard deviation relates each genotype to a population mean and therefore, affords comparisons between genotypes with respect to their genotypic strategies for exploiting the three groups of environmental resources. The solution to the yield equation is by least squares.

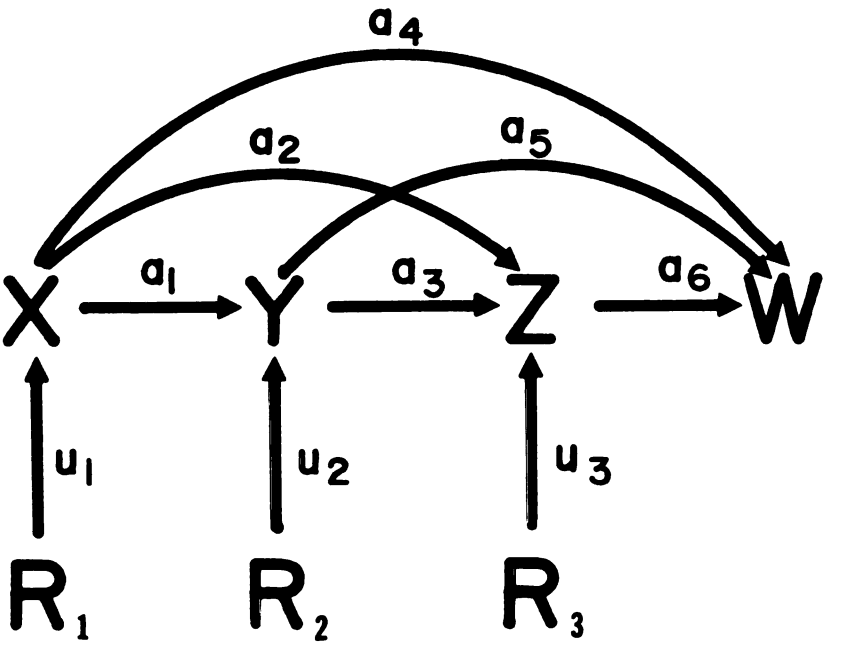
The same data set used in the stability index was used in the path analysis.

Results and Discussion

I. Stability Index

Table 2 contains the Θ_{ik} (eleven each for each of the twenty-one

FIGURE 4. Diagram Of Path Coefficients From The Primary Components Of Yield (X,Y,Z) To Yield (W) And From Environmental Resources (R_1,R_2,R_3) To The Components. (Tai, 1975)



$$r_{XY} = a_1$$

$$r_{XZ} = a_2 + a_1 a_3$$

$$r_{YZ} = a_3 + a_1 a_2$$

$$r_{XW} = a_4 + a_1 a_5 + a_2 a_6 + a_1 a_3 a_6$$

$$r_{YW} = a_5 + a_1 a_4 + a_3 a_6 + a_1 a_2 a_6$$

$$r_{ZW} = a_6 + a_2 a_4 + a_3 a_5 + a_1 a_3 a_4 + a_1 a_2 a_5$$

$$u_1 = \pm 1$$

$$u_2 = \pm (1 - a_1^2)^{1/2}$$

$$u_3 = \pm (1 - a_2 r_{XZ} - a_3 r_{YZ})^{1/2}$$

FIGURE 5. Yield Model Based Upon Path Analysis (Tai, 1975) And The Modification Proposed By Nelson.

Tai: $$\frac{W_{ij} - U_{wi}}{\sigma_{wi}} = v_{1i}r_{1j} + v_{2i}r_{2j} + v_{3i}r_{3j} + \varepsilon_{ij}$$

Nelson: $$\frac{W_i - U_w}{\sigma_w} = v_{1i}r_{1j} + v_{2i}r_{2j} + v_{3i}r_{3j} + \varepsilon_{ij}$$

$$W_i = U_w + \sigma_w (v_{1i}r_{1j} + v_{2i}r_{2j} + v_{3i}r_{3j}) + \varepsilon_{ij},$$

Where W_{ij} = yield of the ith genotype in jth environment,

U_{wi} = mean yield of the ith genotype over locations,

σ_{wi} = standard deviation of yield of the ith genotype over locations,

v_{1i} = genotypic component for yield component, X,

$= u_1(a_4 + a_1a_5 + a_2a_6 + a_1a_3a_6)$

v_{2i} = genotypic component for yield component, Y,

$= u_2(a_3a_6 + a_5)$

v_{3i} = genotypic component for yield component, Z, $= u_3a_6$

$r_{j(1-3)}$ = environmental components associated with the genotypic components,

W_i = yield of the ith genotype over all locations,

U_w = mean yield of all genotypes over all locations,

σ_w = standard deviation of yield of all genotypes over j,

i = genotype (1-21),

j = location (year-site) (1-11).

TABLE 2. Comparison Between Genotypes And Between Year-Sites For o_{ik}, o_i And σ_{0i}.

	Year-Site												
Entry	1	2	3	4	5	6	7	8	9	10	11	o_i	σ_{0i}
69-27-389	21.6	8.1	23.1	0	27.1	35.9	14.1	11.5	39.6	8.1	62.0	22.8	17.8
Menominee	13.8	26.7	36.8	53.3	22.9	60.5	41.4	69.0	91.9	10.3	84.2	46.4	27.7
Heritage	28.5	8.2	20.8	37.3	43.2	20.3	27.7	133.1	159.0	29.2	44.5	50.2	48.9
69-27-403	25.8	17.2	10.4	23.7	20.1	141.5	128.0	157.1	118.0	99.4	133.3	79.5	59.3
Orbit	12.3	18.9	8.2	22.4	19.1	33.5	37.9	21.4	1.0	11.8	50.1	21.4	14.3
Korwood	81.0	56.5	85.7	11.7	11.8	43.8	35.2	51.2	75.9	55.7	90.1	54.4	27.5
69-27-414	42.5	33.3	32.5	18.3	34.9	17.9	37.8	14.1	78.3	16.6	105.8	39.3	28.5
Garry	41.4	19.6	31.5	60.2	11.4	30.3	35.3	11.6	52.4	28.0	16.7	30.8	15.9
Ausable	18.2	31.8	49.0	19.3	10.5	28.6	39.3	26.6	98.2	35.3	32.2	35.4	23.4
Mariner	27.1	12.4	35.4	30.2	37.6	16.9	19.1	23.0	25.4	16.1	21.2	24.0	8.0
Moore	60.7	47.9	35.7	76.1	54.4	39.2	14.2	56.0	66.5	37.9	25.0	46.7	18.4
Portal	63.7	79.8	43.0	90.6	39.8	23.4	44.1	28.4	33.1	22.3	26.0	42.2	20.4
Mackinaw	26.6	30.6	29.2	29.9	33.3	26.4	33.3	7.5	30.7	27.9	25.4	27.4	7.1
Benson	20.0	19.4	9.6	36.8	16.5	21.5	8.3	15.5	27.1	28.8	21.0	20.4	8.3
Lang	31.6	27.9	13.7	22.5	32.8	24.8	19.9	37.6	28.8	19.2	34.4	26.6	7.3

TABLE 2, continued.

	Year-Site												
Entry	1	2	3	4	5	6	7	8	9	10	11	θ_i	$\sigma_{\theta i}$
Marathon	42.1	44.9	29.5	57.4	10.4	24.1	33.8	47.9	40.5	37.1	21.4	35.4	13.4
69-28-124	33.8	23.4	35.0	48.2	18.0	37.7	28.3	28.3	67.6	25.0	57.0	36.6	15.2
Noble	90.6	21.4	34.7	11.4	37.6	41.2	18.6	14.2	29.6	31.0	24.3	32.2	21.6
Dal	24.3	14.6	40.5	36.9	44.8	12.6	64.0	106.9	97.6	19.3	11.4	43.0	33.5
Cld 64	87.6	28.1	17.2	30.1	93.0	48.5	43.3	70.9	36.2	8.1	41.7	45.9	27.4
Wright	36.4	29.2	46.6	8.7	38.3	18.3	47.6	35.5	48.7	28.4	25.1	33.0	12.6

genotypes), their means over year-sites (Θ_i) and the standard deviations ($\sigma_{\theta i}$) associated with each set of eleven Θ_{ik}. The Θ_{ik} represent the divergence of the series of maximum traits, properly transformed, in each year-site, from the mean over year-sites. It is apparent that there is considerable variation, or plasticity, in a genotype's ability to form yield since the mean degree of correlation is low (high $\sigma_{\theta i}$) in many of the higher yielding genotypes. There would appear to be no correlation between stability as determined by this system and the origin of the genotypes, for the stabilities of Michigan lines range from 7.1 for Mackinaw to 59.3 for genotype 69-27-403.

It is of interest to note what changes are exacted through the use of the transformation. A sample of genotypes and their non-transformed values were correlated for the eleven year-sites in a manner identical to that used on the transformed data set. With only a few exceptions, the R_{ik} values were exclusively 1.0. The exceptions were few and none were less than $R_{ik} = .99$. By imposing the conditions of a mean of zero, unit variance and common units of measure (standard deviations), it is obvious that the integrity of the genotype has been severely disrupted. The transformation, however, does legitimatize comparisons since all traits in all genotypes are expressed in the same units. This was the intention when Gauss created the normalizing transformation in the 19th Century. The effect of the transformation can be seen in Table 3, which lists the various intra-genotypic correlation coefficients for both transformed and non-transformed data.

Table 4 lists the vector lengths, or the individual genotypic yields in standard deviations for the eleven year-sites. This may be construed as the measure of superiority, since yield is the single trait

Table 3. Comparisons of intravarietal component correlation coefficients between non-transformed and transformed data. Intravarietal Correlation Coefficients: $r_{..}$

Entry		xy	xz	xw	xtw	yz	yw	ytw	zw	ztw	wtw
69-27-389	NT	-.207	-.339	.787	.151	-.116	.330	.009	-.226	.689	.298
	T	.017	-.220	.707	.286	-.588	.584	-.332	-.648	.518	-.026
Menominee	NT	-.405	-.345	.839	.237	.048	.035	.104	-.083	.607	.427
	T	-.655	.218	.358	.363	-.286	.081	-.037	.428	.546	.795
Heritage	NT	-.195	-.267	.826	-.005	.146	.288	.352	-.006	.697	.297
	T	-.624	.133	.384	.199	-.246	.337	-.039	.078	.743	.216
69-27-403	NT	-.134	-.068	.859	.123	-.100	.249	.281	.176	.562	.374
	T	-.725	.659	.418	.599	-.848	-.048	-.332	.379	.417	.503
Orbit	NT	-.612	-.075	.817	.119	.197	-.119	.432	.122	.738	.443
	T	-.835	.018	.451	-.058	-.373	-.049	-.266	.055	.545	-.079
Korwood	NT	-.518	-.150	.759	.405	.352	.096	.166	.220	.537	.640
	T	-.790	.052	.056	.293	.055	.379	-.245	.678	.346	.505
69-27-414	NT	-.215	-.234	.807	.490	.116	.283	.181	-.028	.398	.523
	T	-.725	-.183	.620	.535	-.136	-.211	-.486	-.241	.025	.742
Garry	NT	-.524	-.363	.745	-.038	.097	.051	.352	-.170	.529	.288
	T	-.423	.247	.615	.061	-.513	.228	.182	.271	.508	.460
Ausable	NT	-.394	-.267	.695	.186	.022	.252	.133	.015	.576	.447
	T	-.649	-.208	.178	-.401	-.326	.362	-.034	-.199	.432	-.650
Mariner	NT	-.518	-.346	.755	.065	.282	.044	.449	.031	.307	.347
	T	-.447	.049	.585	-.082	-.484	-.060	.141	.419	.156	.510
Moore	NT	-.507	-.264	.706	-.040	.093	.141	.234	-.006	.748	.323
	T	-.708	.059	.286	-.295	-.163	.385	.267	.081	.557	.081
Portal	NT	-.421	-.022	.649	.267	.212	.343	.201	.334	.675	.429
	T	-.364	.304	.707	.283	-.823	.168	-.796	-.014	.852	-.091
Mackinaw	NT	-.336	.159	.779	.366	-.233	.235	.113	.165	.417	.445
	T	-.600	.154	.091	.077	-.496	.297	-.054	.312	.693	.524
Benson	NT	-.464	.062	.779	.241	.119	.038	-.120	.451	.865	.430
	T	-.467	.334	.159	-.050	-.638	.535	.086	-.369	.347	-.187
Lang	NT	-.469	.059	.808	.477	.503	.052	.148	.446	.696	.582
	T	-.426	.342	.723	.323	-.264	.237	-.280	.311	.240	.121
Marathon	NT	-.064	-.627	.702	-.101	-.027	.178	.227	-.196	.186	.257
	T	-.713	.265	.644	.826	-.154	-.914	-.332	-.029	.065	.378
69-28-124	NT	-.718	.050	.820	.338	.019	-.311	-.067	.271	.551	.450
	T	-.682	.038	.286	.170	.003	.318	.136	.536	.756	.787
Noble	NT	-.137	-.361	.731	.018	.746	.484	.434	.262	.705	.446
	T	-.504	.039	.784	.034	-.222	-.079	.257	.302	.374	.139
Dal	NT	-.378	-.477	.712	.080	.352	.220	.310	.076	.355	.412
	T	-.240	-.626	.562	-.063	-.282	.465	-.479	-.408	.396	-.426
CLD 64	NT	-.224	-.179	.843	.306	.049	.203	.018	.113	.695	.414
	T	-.282	-.482	.632	-.257	-.101	.453	.011	-.379	.760	-.219
Wright	NT	-.684	-.279	.744	.003	.411	-.101	.194	.126	.725	.299
	T	-.424	.024	.898	.215	-.469	-.125	.436	-.225	-.229	.476

TABLE 4. Comparison Between Genotypes And Btween Year-Sites For Vector Lengths, W_{ik} and $\bar{W}_i$.

	Year-Site											
Entry	1	2	3	4	5	6	7	8	9	10	11	$\bar{W}_i$
69-27-389	-.67	.46	.53	.02	1.45	.63	.36	.53	1.72	.93	-1.12	.31
Menominee	1.31	.74	1.07	.69	.58	1.25	.72	.63	1.27	1.37	1.53	.47
Heritage	1.35	1.52	1.26	-.62	.75	1.37	1.59	1.35	1.04	-.10	.50	.47
69-27-403	1.39	1.56	-.07	.95	1.38	1.84	1.84	.46	.93	1.23	1.53	.49
Orbit	.18	.29	1.65	-.29	.62	1.27	.50	1.21	.93	1.89	-.02	.34
Korwood	.98	.79	-.13	-.05	.63	.62	.11	-.73	.82	1.17	.65	.22
69-27-414	1.00	.20	1.11	-.75	.51	.81	1.59	1.24	.80	.51	1.52	.40
Garry	.34	.44	.55	.87	-.31	.65	-.79	1.53	.50	-1.46	1.21	.13
Ausable	.11	-.15	.79	.59	.34	-.46	-.06	-.85	.18	-.47	.39	.05
Mariner	.16	-.21	.11	.66	-.20	-.23	.16	-.97	.16	.04	.22	-.01
Moore	.49	.87	-.56	.95	.23	.68	-.85	.99	.03	-.03	.71	.12
Portal	-1.20	-1.34	-1.30	-.02	-.24	-.39	-.04	-1.08	-.26	.28	-.14	-.25
Mackinaw	.30	1.42	.11	-.83	.22	-.01	.62	-.61	-.30	-.49	-.68	0

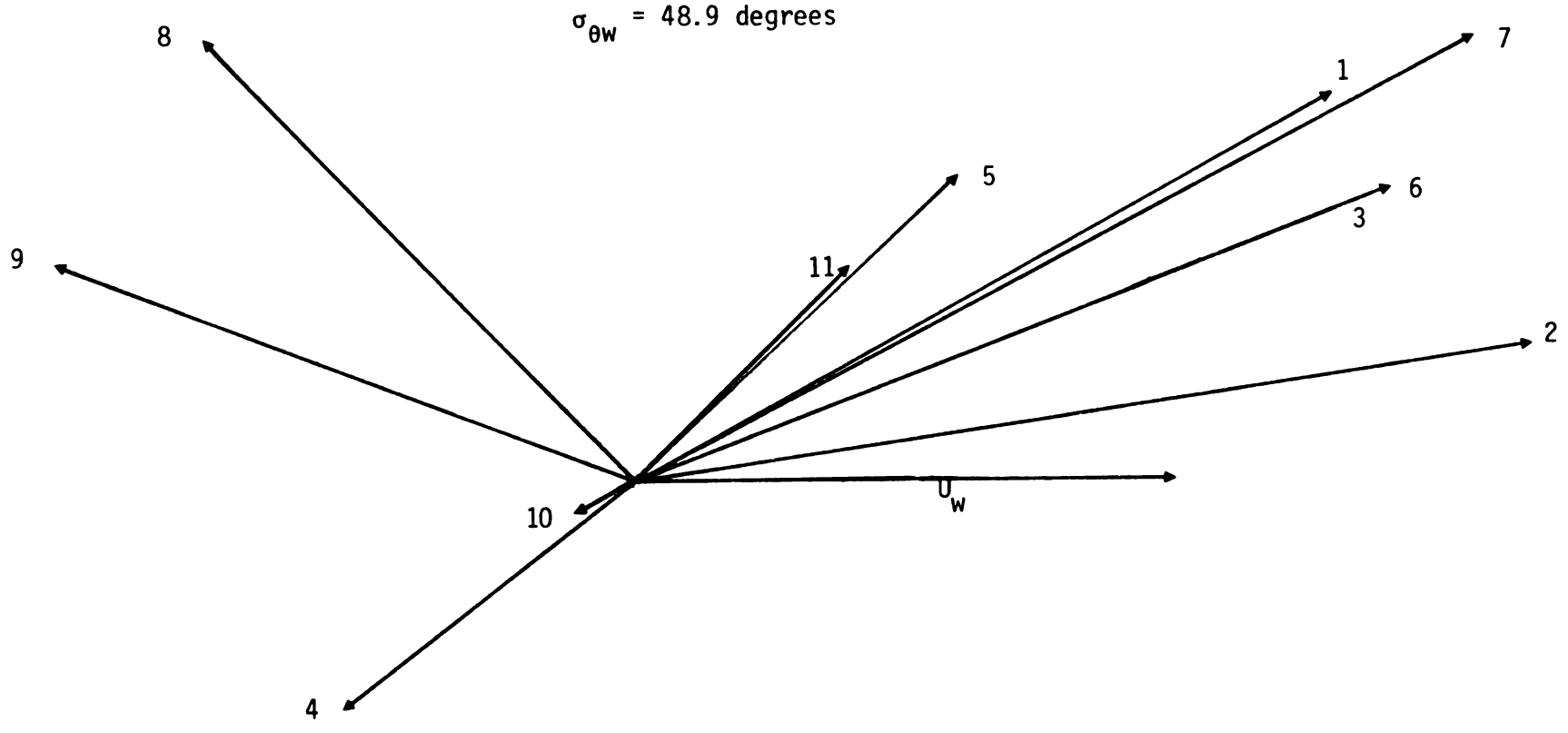
TABLE 4, continued.

Entry	Year-Site 1	2	3	4	5	6	7	8	9	10	11	$\bar{W}_i$
Benson	-.68	-.93	-1.65	.02	-1.80	-.07	-.77	-.30	-.30	-1.68	-1.09	-.39
Lang	-.59	-.39	-1.75	-2.47	1.46	-.75	-.06	.42	-.40	.31	-.83	-.14
Marathon	-.27	-2.14	.31	.75	-.05	-.25	-.30	-.09	-.47	.05	.28	-.10
69-28-124	.11	.39	.51	1.79	.52	-.88	.13	-.24	-.65	-.98	-.43	0
Noble	-1.11	-.83	.46	-1.31	-.43	-1.74	.11	.40	-.96	.58	-.13	-.22
Dal	-.70	-1.11	-1.18	.39	-.65	-1.68	-1.72	-2.04	-1.11	-.84	-1.66	-.53
Cld 64	-2.37	-.52	-1.45	-1.62	-2.83	-1.69	-1.08	-1.35	-1.09	-1.42	-.90	-.77
Wright	-1.40	-1.01	.08	.35	-1.08	-.46	-2.00	-1.20	-2.03	-.93	-1.64	-.54

of greatest importance. Any positive value represents above average yield with respect to the zero mean of all twenty-one genotypes. Superior varieties, superior in their yield, are readily apparent in this table. It is of interest to note that Heritage yield was below the mean in only two year-sites, those of Kalamazoo County in 1977 and Lenawee County of 1979. A discussion in greater depth on Heritage may be found in Chapter 2 of this dissertation. Lest the success of the Michigan oat program be questioned, observation of the yield performance of Clintland 64 should dispell any doubts. Clintland 64 was a recommended variety until 1970 when newer varieties, considerably superior to Clintland 64 in yield, were introduced. For the eleven year-sites in this study, Clintland 64 had become the poorest variety of the twenty-one genotypes included. Appendix 1 lists the specific year-sites with the numbers under which the years and locations may be found.

Based upon Table 2 and 4, it is possible to visually represent each variety as a cluster of vectors (eleven each) graphed with respect to a standard (U_x or $\bar{W}_i$) lying along the x-axis. Figure 6 is an example, using Heritage as the genotype. The standard, U_w, is the x-axis and it equals .91 standard deviation units of yield in length. Each year-site for Heritage is thus plotted relative to the standard and their lengths are simply the $\bar{W}$ for each year-site. Any yield superior to the mean (=0) of all twenty-one genotypes must lie in the first two quadrants. This is a function of the $\cos^{-1} r_i$ which produces Θ_i of between 0 and 180^o (see Figure 3). This places all angles within the first two quadrants and any yield which is positive insures a positive direction. Quadrant 3 has two year-sites (4 and 10) expressed. The angles (37.3^o and 29.2^o, respectively) are positive, but the lengths are negative, since the

FIGURE 6. Intravarietal Distribution Of Vectors For Heritage For Eleven Year-Sites. (Length = Yield In Standard Deviation Units)



yield of Heritage in these two year-sites was below the population mean. The overall stability of this vector cluster is represented in the value of 48.9^{o}, the second highest such value among all entries. It is interesting to note that if all twenty-one genotypes were similarly graphed and their respective vectors summed for each year-site, the entire matrix of vectors would collapse to a single point. Properly manipulated, there is, indeed, symmetry in nature other than the five perfect solids!

Table 5 lists the means from Tables 2 and 4. For mathematical etiquette, this is a better mode of expression since the mean vectors for each entry are arranged such that each has an assigned directional stability ($\sigma_{\theta i}$) and a corresponding length ($\bar{W}_i$). This table facilitates inter-genotypic comparisons. The author has no reservations concerning this measure of superiority; however, the measure of relative stability is more difficult to interpret since high degrees of instability are correlated ($r_{\theta W}$= .38) with superior yield. Testing the hypothesis, $H_o: r_{\theta W} = 0$ with t = 1.79, the probability is: $P < .1$. The most logical interpretation of this phenomenon is that genotypes which are superior in their yield (maximum traits, for this study), are superior due to their plasticity of response via their yield components, or component compensation. A stress manifested at one point in their development is compensated by a concomitant improvement in the remaining components when constrasted with more poorly performing genotypes, unless the stress occurs during the development of Z.

There is no final statement to be made concerning this new system for measuring and presenting cultivar stability and superiority. Further review and possible additional manipulation of the data are very likely.

TABLE 5. Intervarietal Paired Observations For Stability And Superiority.

Entry	Stability ($\sigma_{\theta i}$) (in degrees)	Superiority ($\bar{W}_i$) (in standard deviations)
69-27-389	17.8	.31
Menominee	27.7	.47
Heritage	48.9	.47
69-27-403	59.3	.49
Orbit	14.3	.34
Korwood	27.5	.22
69-27-414	28.5	.40
Garry	15.9	.13
Ausable	23.4	.05
Mariner	8.0	-.01
Moore	18.4	.12
Portal	20.4	-.25
Mackinaw	7.1	0
Benson	8.3	-.39
Lang	7.3	-.14
Marathon	13.4	-.10
69-28-124	15.2	0
Noble	21.6	-.22
Dal	33.5	-.53
Clintland 64	27.4	-.77
Wright	12.6	-.54

Since the purpose was to analyze genotypes by a different method for multiple traits, enormous complexities arise which must be interpreted with care lest the conclusions be nothing more than specious nonsense.

II. Path Analysis

Table 6 lists the six path coefficients and the correlational matrix from which they were derived. Calculation of u_1, u_2 and u_3 provide the necessary entries for computing the three genotypic components, v_1, v_2 and v_3 for each genotype. The equations are:

$$v_1 = u_1(a_4 + a_1a_5 + a_2a_6 + a_1a_3a_6) = u_1r_{xw}$$

$$v_2 = u_2(a_3a_6 + a_5)$$

$$v_3 = u_3a_6,$$

and the results are detailed in Table 7, accompanied by the genotypic mean yields. The results are remarkable in view of the highly variable results of Tai (9,10). First, all v_1, v_2 and v_3 are positive for each genotype. Secondly, the values are tightly clustered within each v_j and thirdly, the mean values descend sharply from v_1 to v_3:

$$\bar{v}_1 = .770, \sigma_{v1} = .057$$

$$\bar{v}_2 = .504, \sigma_{v2} = .127$$

$$\bar{v}_3 = .217, \sigma_{v3} = .084$$

This strongly supports the hypothesis of component compensation and, further, these results again indicate that the most important yield component toward the formation of yield is X, panicles/unit area, followed in importance by Y and Z. Tai concluded from his positive v_3 for potatoes that Z, or tuber bulking, was the most important component in

TABLE 6. Primary Paths And Correlation Coefficients From Transformed Data.

	Path Coefficients						Correlation Coefficients					
Entry	a_1	a_2	a_3	a_4	a_5	a_6	xy	xz	yz	xw	yw	zw
69-27-389	-.207	-.379	-.194	.954	.546	.160	-.207	-.339	-.116	.787	.330	-.226
Menominee	-.404	-.389	-.109	1.131	.479	.284	-.404	-.345	.048	.839	.035	-.083
Heritage	-.195	-.248	.097	.964	.450	.186	-.195	-.267	.146	.826	.288	-.006
69-27-403	-.134	-.083	-.111	.932	.402	.280	-.134	-.068	-.100	.859	.249	.176
Orbit	-.612	.073	.242	1.184	.588	.096	-.612	-.075	.197	.817	-.119	.122
Korwood	-.518	.045	.376	1.098	.605	.172	-.518	-.150	.352	.759	.096	.220
69-27-414	-.215	-.219	.069	.940	.469	.138	-.215	-.234	.166	.807	.283	-.028
Garry	-.524	-.431	-.129	1.144	.632	.184	-.524	-.363	.097	.745	.051	-.170
Ausable	-.394	-.306	-.098	1.024	.649	.274	-.394	-.267	.022	.695	.252	.015
Mariner	-.518	-.273	.141	1.136	.558	.266	-.518	-.346	.282	.755	.044	.031
Moore	-.507	-.292	-.055	1.112	.684	.224	-.507	-.264	.093	.706	.141	-.006
Portal	-.421	.081	.246	.947	.698	.207	-.421	-.022	.212	.649	.343	.334
Mackinaw	-.336	.091	-.202	.953	.590	.150	-.336	.159	-.232	.779	.235	.165
Benson	-.464	.149	.188	.965	.446	.338	-.464	.062	.119	.779	.038	.451

TABLE 6, continued.

	Path Coefficients						Correlation Coefficients					
Entry	a_1	a_2	a_3	a_4	a_5	a_6	xy	xz	yz	xw	yw	zw
Lang	-.469	.379	.681	1.005	.441	.164	-.469	.059	.503	.808	.052	.446
Marathon	-.064	-.632	-.068	.988	.254	.430	-.064	-.627	-.027	.702	.178	-.196
69-28-124	-.718	.131	.113	1.204	.549	.200	-.718	.053	.019	.820	-.311	.271
Noble	-.137	-.264	.710	.890	.385	.296	-.137	-.361	.746	.731	.484	.262
Dal	-.378	-.401	.201	1.101	.484	.431	-.378	-.477	.352	.712	.220	.076
Clintland 64	-.224	-.177	.009	.983	.410	.269	-.224	-.179	.049	.843	.203	.113
Wright	-.684	.005	.414	1.269	.686	.198	-.684	-.278	.411	.744	-.101	.126

Approximate Critical Values for r not equal to zero: r= .37 for $P \leq .1$

r= .44 for $P \leq .05$

r= .55 for $P \leq .01$

r= .66 for $P \leq .001$

TABLE 7. Mean Yield (W_i) And The Three Genotypic Components Of The GxE Interaction For The Twenty-one Genotypes.

Entry	Yield (bu/A)	V_1	V_2	V_3
69-27-389	121.4	.787	.504	.152
Menominee	125.2	.839	.410	.264
Heritage	125.2	.826	.460	.180
69-27-403	125.7	.859	.368	.277
Orbit	122.2	.817	.483	.091
Korwood	119.3	.759	.573	.153
69-27-414	123.6	.807	.467	.134
Garry	117.2	.745	.518	.167
Ausable	115.4	.695	.572	.260
Mariner	114.0	.755	.509	.235
Moore	117.1	.706	.579	.209
Portal	108.4	.649	.679	.200
Mackinaw	114.1	.779	.527	.150
Benson	105.0	.779	.451	.339
Lang	110.8	.808	.488	.136
Marathon	111.9	.702	.224	.421
69-28-124	114.1	.820	.398	.204
Noble	109.0	.731	.590	.192
Dal	101.7	.712	.528	.373
Clintland 64	96.0	.843	.402	.263
Wright	101.6	.744	.560	.158

potato yield. This, as can be concluded from Table 7, is the exact opposite of oats, and by inference, of all cereals. Additionally, it might also be concluded that despite the apparent dissimilarities of the oat genotypes included in this study for the many traits and with respect to their different origins, they are, nevertheless, a highly homogeneous species.

Figure 7 is a plot of the predicted mean yields graphed against actual mean yields. The expectation here is that the values would be closely centered around the b = 1 regression line (ascending at 45^{o} from the origin). The predicted $\bar{W}_i$ are actually almost randomly distributed. Based upon the r^2 values from Table 8, and as adjudged visually from Figures 7 and 8, it is apparent that the model of Tai (9,10) might require modifications to predict yield in oats. The author places considerable confidence in the genotypic components (Table 7) since they are resolved algebraically from path coefficients. These paths are, themselves, derived from the correlation coefficients within each genotype. Insofar as the correlations are correct, the v_j will be correct. The clustering of genotypes within the respective v_j classes suggests that the v_j are, in fact, population parameters of benefit to biologists.

What is especially disconcerting to the author, however, is the fluctuations in correlations which he has noted in using data recorded by the late Dr. John E. Grafius over the past twenty years. These data are sound insofar as one can assay the correlational matrix for many varieties for as many as twenty year-sites (see Chapter 2, Appendix 1). Comparing the correlations of Heritage in Appendix 1 of Chapter 2 with the non-transformed set from this chapter (Table 3), the differences are startling. If these discrepancies are generalized across crops and

TABLE 8. Three Environmental Components Of The GxE Interaction For The Eleven Environments, The Coefficients Of Determination For The Yield Equations And The Mean Fitted Yields In Standard Deviation Units.

Year-Site*	r_1	r_2	r_3	Coef. Det.: r^2	U_{wk}
1	1.58	-1.60	-2.16	.144	.26
2	3.74	.94	-.69	.249	-2.94
3	-1.82	-2.43	-3.01	.270	3.72
4	-1.33	-.61	.54	.155	-.92
5	-3.49	-3.09	-5.43	.275	6.28
6	.52	-1.40	-2.22	.219	1.00
7	2.78	-1.49	-2.35	.300	.46
8	-1.27	-2.69	-3.79	.351	3.04
9	1.09	-.94	-2.15	.136	.17
10	-.04	-.67	-1.59	.140	.48
11	-.45	-1.30	-1.18	.074	.17

*See Appendix 1 for explanation of years and locations.

FIGURE 7. Predicted Mean Yields (Standard Deviations) Plotted Against Actual Mean Yields.

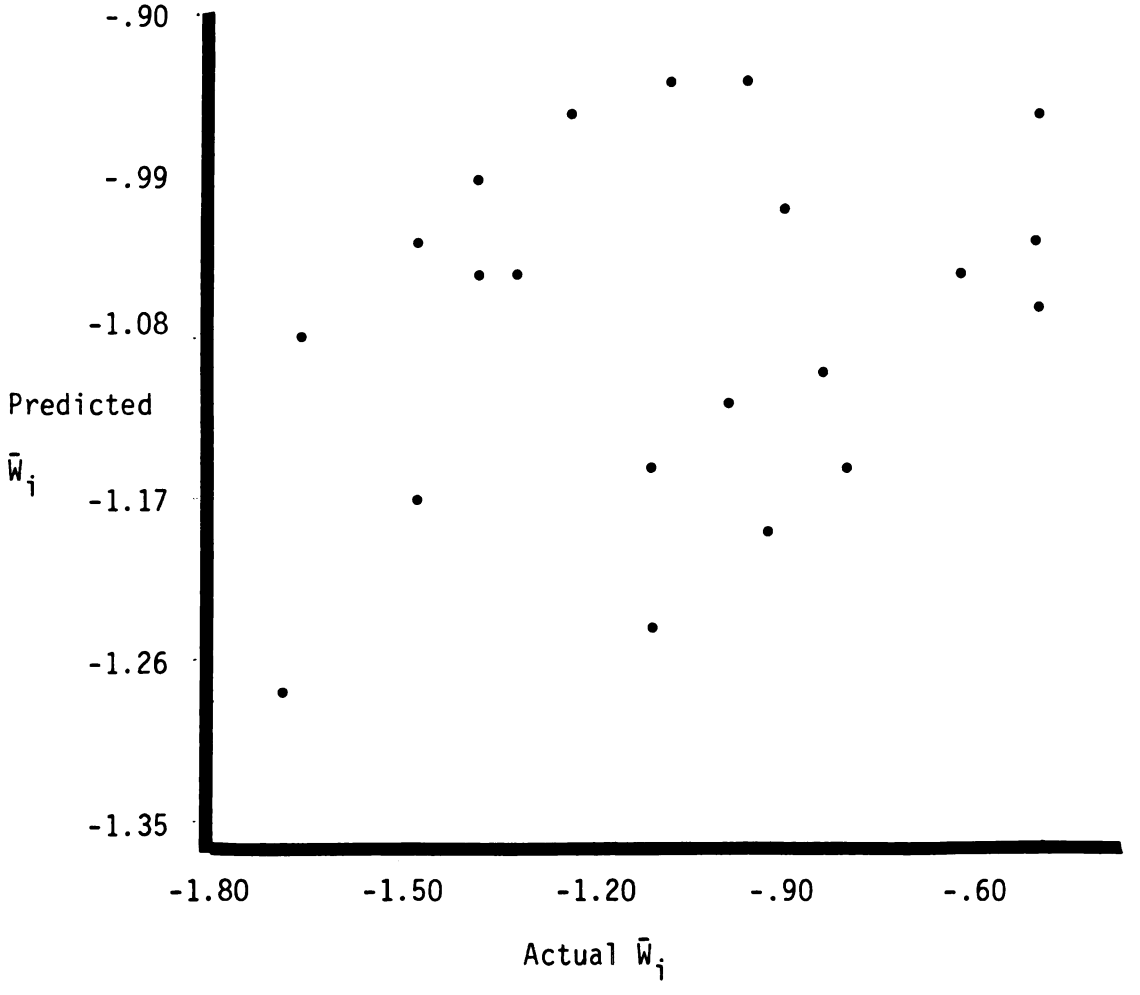
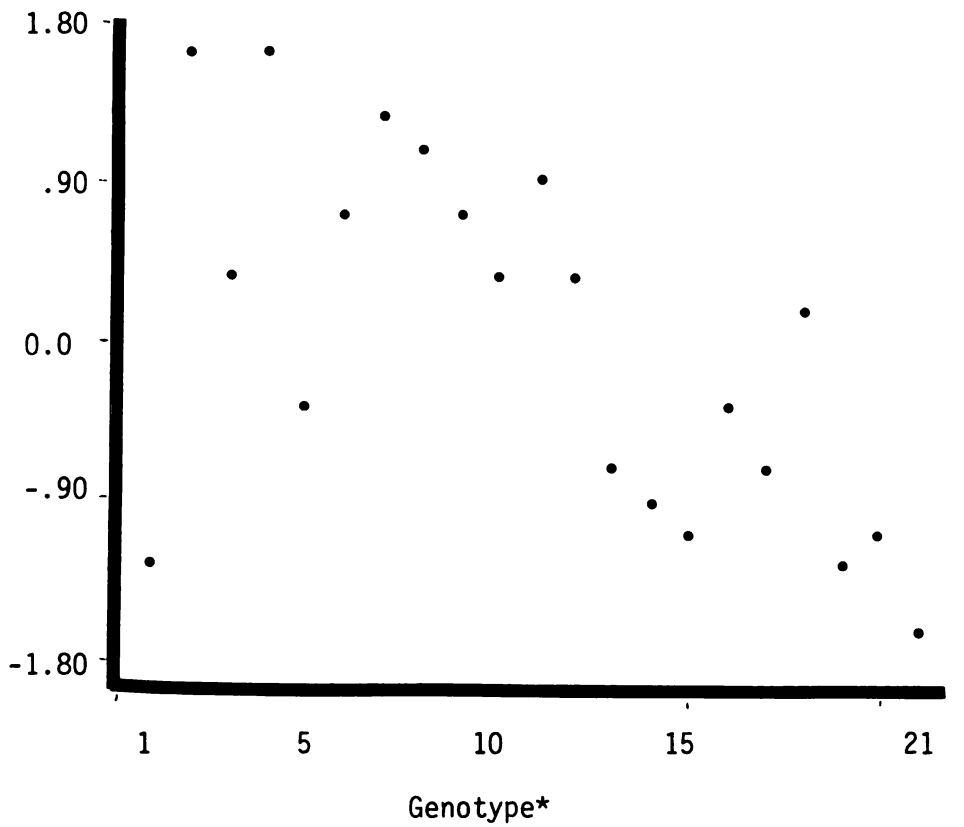


FIGURE 8. Residuals Plotted Against The Twenty-one Genotypes.



*See Appendix 2 for a listing of genotypes.

environments for different years, it means that most, if not all, of the published component correlations are too narrow in scope to draw conclusions from for population parameters. The implications for plant breeders is that extreme care must be taken when basing a breeding strategy upon component correlations drawn from a narrow group of environments and years. This conclusion would hold true of the author's work detailed in Chapter 4.

The environmental components of the GxE interaction, as resolved by least squares regression, are extremely heterogeneous (Table 8). It should be recalled that the matrix of observations which comprised this aspect of the study were transformed by using the grand mean and grand standard deviation (Figure 5), intended to relate each genotype and year-site to a mean of zero and a unit variance. The low coefficients of determination (Table 8) would indicate that there is insufficient information used to predict yield by this method. The adjusted r^2 values (adjusted for degrees of freedom) lower the r^2 values considerably (not listed). Figure 8 is a plot of the residuals as a function of genotype. Were the r^2 values high, one would find the residuals centered around zero on the ordinate and this tight band moving to the right as each genotype's residuals are recorded. What is the case in this study is that the residuals are skewed strongly in the directions of both higher and lower yielding genotypes. Observation of the mean fitted yields (the constant terms in multiple regression), U_{wk}, in Table 8 also suggest a bimodal distribution with the smallest terms clustered around year-sites 1-3 and 9-10. This might indicate an artifact stemming from the nature of the original transformation (Figure 5).

In conclusion, the author is generally pleased with the stability index and the path coefficient study which was so painfully accomplished. It should be noted that the data set is amenable to further analysis by modifications of the techniques herein used. Additionally, it would seem wise to apply other methods of analysis--in particular, factor analysis--in an attempt to empirically determine the commonality of the various statistics which result.

APPENDIX 1. Notation For The Eleven Year-Sites.

Number	Year	Site
1	1977	E. Lansing
2	1977	Tuscola County
3	1977	Lenawee County
4	1977	Kalamazoo County
5	1978	E. Lansing
6	1978	Tuscola County
7	1978	Lenawee County
8	1978	Kalamazoo County
9	1979	Tuscola County
10	1979	Lenawee County
11	1979	Kalamazoo County

APPENDIX 2. Notation For The Twenty-one Genotypes.

Number	Genotype (Variety)
1	69-27-389 *
2	Menominee *
3	Heritage *
4	69-27-403 *
5	Orbit
6	Korwood *
7	69-27-414 *
8	Garry
9	Ausable *
10	Mariner *
11	Moore
12	Portal
13	Mackinaw *
14	Benson
15	Lang
16	Marathon
17	69-28-124 *
18	Noble
19	Dal
20	Clintland 64
21	Wright

* Michigan lines

CHAPTER 1--BIBLIOGRAPHY

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CHAPTER 2

ANALYSIS OF A NEW MICHIGAN OAT VARIETY BY YIELD COMPONENT REGRESSION

Introduction

An important aspect of a plant breeding program for agronomic crops is the methodology used in evaluating potential varieties. Aside from such important traits as disease and lodging resistance and the various quality factors necessary to a superior cultivar, evaluation typically centers around measures of yield. In most cases yield is considered as an holistic trait and it is ranked by contrasting a specific variety with other, presumably adapted, cultivars. There is no question that such systems are effective in that superior yield is easily identified in any given experiment. Comparisons between experiments over years and locations is confounded, however, since the genotype by environment interaction (GxE) is usually prevalent. Because the variability induced by different environments is so extreme various systems have evolved to identify individual cultivars which are both stable and superior in their yield. The primary method used by the Michigan State University oat and barley breeding programs is that of Pedersen et al. (1). The system is a modification of those proposed by Finlay and Wilkinson (2), Eberhart and Russell (3) and Schmidt (4).

The author proposes a modest change in Pedersen's system which is simply to regress an individual cultivar's yield components against

the mean component of the representative population. By this method it is hoped that the superiority (or inferiority) of a cultivar can be ascribed to one or more of its components, along with some measure of its stability. An explanation of the yield components may be found in the Materials and Methods section.

Literature Review

Pedersen, Grafius and Everson (1) proposed their system to simplify analysis of many genotypes grown in different locations over a number of years. It is very much a hybrid approach, but it is nonetheless elegant for this. Beginning with the concept of regressing varietal yield against the mean of all varieties grown in a common location (first proposed by Yates and Cochran (2)), Finlay and Wilkinson (3) placed reliance on the regression coefficient, b, to determine stability. A value of $b = 0$ was interpreted as absolute phenotypic stability, $b = 1$ as average response over environments and $b < 1$ as inferior performance. Eberhart and Russell (4) proposed an environmental index constructed by subtracting the specific environmental mean yield from the mean over every environment for yield. The mean square deviation from regression is their stability parameter. Schmidt et al. (5) introduced the coefficient of determination, r^2, into consideration for winter wheat evaluation. They showed that newer cultivars performed considerably better than the checks and that new cultivar response to improving environments is both superior to the checks and highly predictable. Hence, the coefficient of determination was suggested as a stability parameter.

The utility of the system is amply demonstrated by its use in evaluating Michigan oat, barley and wheat cultivars since the regression

graphs are comprehensible to a variety of people, including cereal growers. The method was applied to the traits yield and test weight for a collection of twenty-four oat varieties routinely grown in Michigan and covering the years 1968 through 1978 (6). The response of each cultivar was regressed upon the mean response of all twenty-four for the traits being evaluated. The number of tests ranged from seven (for older, low acreage varieties) to forty-four (more highly adapted and newer varieties). For yield the r^2 values were clustered as follows: 8 varieties > .90, 13 varieties > .80 and 3 varieties > .70. r^2 values for test weight were: 3 varieties > .90, 8 varieties > .80, 5 varieties > .70, and the other 8 varieties ranging down to $r^2 = .47$. With respect to yield, high coefficients of determination reflect the high degree of linearity associated with cultivar response; in other words, the site mean is an excellent predictor of performance. Test weight had a higher degree of variability, but the system identifies inherently poor cultivars and, as one might expect, the single variety with the lowest r^2 was Lang, which is poorly adapted to Michigan growing conditions and is not extensively planted in this state.

Materials and Methods

The regression method of Pedersen *et al.* is exceedingly simple and easily identifies varieties which are superior. Essentially, a variety is considered a constituent of an adapted population of genotypes developed in a common region. For Michigan oats this region is roughly inclusive of all cooperating stations in the Uniform Mid-season Oat Performance Nursery. More specifically, this population, or gene pool,

is of more northerly states within the UMOPN. Thus, the collection of cultivars developed and released in this region can be viewed as a gene pool which reflects average oat response. Regressing individual cultivar response upon the mean response of a collection of adapted genotypes for such traits as yield and test weight is an excellent measure of superiority. Plotting $\hat{y}$ values for a given cultivar with respect to the b = 1 regression line provides a visual method for determining response in different environments. The statistics which accompany regression analysis are the "y-intercept," the regression coefficient, b, the sample size, n, and the coefficient of determination, r^2. A superior genotype should have a positive "y-intercept," a large "n" to insure adequate sampling and a regression coefficient greater than or equal to one. A superior genotype which is stable in its superiority (stably superior) should have a high coefficient of determination, in addition to the above-mentioned statistics.

This paper proposes a further use of regression analysis, based not only on yield and test weight, but upon the primary components of yield as reflected in oats, where X = number of panicles/unit area, Y = number of seeds/panicle, Z = weight/seed, and the combined component, XY, or number of seeds/unit area. The individual cultivar which will serve as the tester is Heritage, formerly Michigan line 64-152-47. The data are derived from thirty separate experiments grown over the years 1972 through 1980. The experiments were rod-row nurseries which include a collection of from 25 to 36 adapted varieties developed for the aforementioned UMOPN region. For twenty site-years detailed yield component data for X, Y, and Z were computed. For all thirty site-years Z, W (yield) and TW (test weight) were recorded.

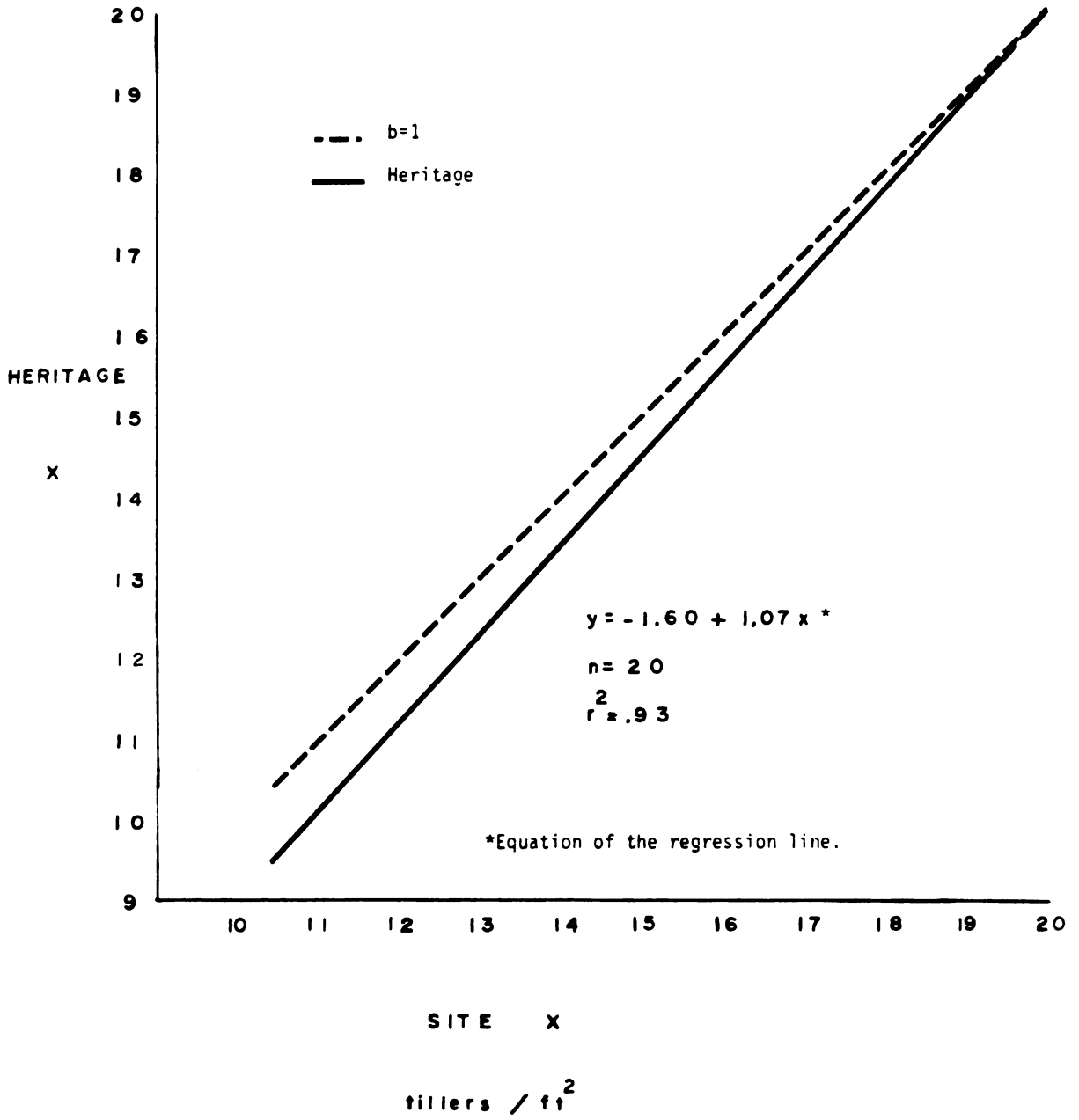
Thus, an estimate of the pooled component, XY, was computed since W/Z = XY. By regressing cultivar yield components upon site mean components it should be possible to analyze in greater depth the mode of response of the components (and, hence, of yield) to varying environments.

Since regression analysis is well known and its application is facilitated by the readily available use of small and inexpensive calculators, I will not detail the methodology. All experiments were planted in lattice designs with four replications and analyzed as such. The data for Heritage were extracted from tables of adjusted means and the site data were simply the means of all entries included in the rod-row experiments.

Results and Discussion

The utility of the simple linear regression approach, as modified for the primary components of yield, is demonstrated in Figures 2-5. Figure 1, the regression for Heritage tiller number on site X, shows a suppression of panicle production in the poorer sites, since the regression line lies below the b = 1 regression line. The b = 1 regression line represents average response to the environment and Heritage responds more favorably as the site mean improves. Low site X is correlated with Kalamazoo County, one of the four testing sites for Michigan oats. The soils are sandy and this frequently results in heat and drought stress in the crop. For those regions, therefore, in which stress occurs early in the season, Heritage might not be the variety of choice since the consequence might be a yield reduction through poor tiller initiation.

FIGURE 1. Regression Of Heritage X Onto Site X.



The expression of seed number strongly influences yield in Heritage (Figure 2), for the regression for Y lies considerably above the b = 1 regression line throughout the range of site values. The question arises, however, whether this Y-superiority is sufficient to offset the yield reductions imposed by low X in the poorer, more stressed, sites. Figure 3, the regression for the pooled component over thirty year-sites, shows that X is compensated by the consistently high values for Y. The regression coefficient is nearly 1.2, which demonstrates the strong and favorable response of Heritage to improving environments. At the poorest locations, the compensation is sufficient to insure that Heritage responds at least as well as the average for most oat cultivars grown in these locations. The high value for r^2 provides strong confirmation that the predicted response is reliable.

Figure 4 indicates that Heritage Z is little different from the site seed weight, except at the extremes of the range of values resulting from the different environments over the eight years of testing. Considering the inferior expression of X at the poorer sites, it is possible that the slight inferiority of Z, coupled with poor X in Heritage, would reduce yield. This would be the consequence only if the locations in which low site Z was prevalent were highly correlated with the same locations which manifest a reduced X. An observation of the data and the correlation coefficients between X and Z, for both the site and Heritage, would indicate that they are poorly correlated. r_{xz} (Heritage) = -.13 and r_{xz} (site) = -.11 would imply that seed weight is randomly distributed. The entire set of correlation coefficients and their respective t-statistics may be found in Appendix 1.

FIGURE 2. Regression Of Heritage Y Onto Site Y.

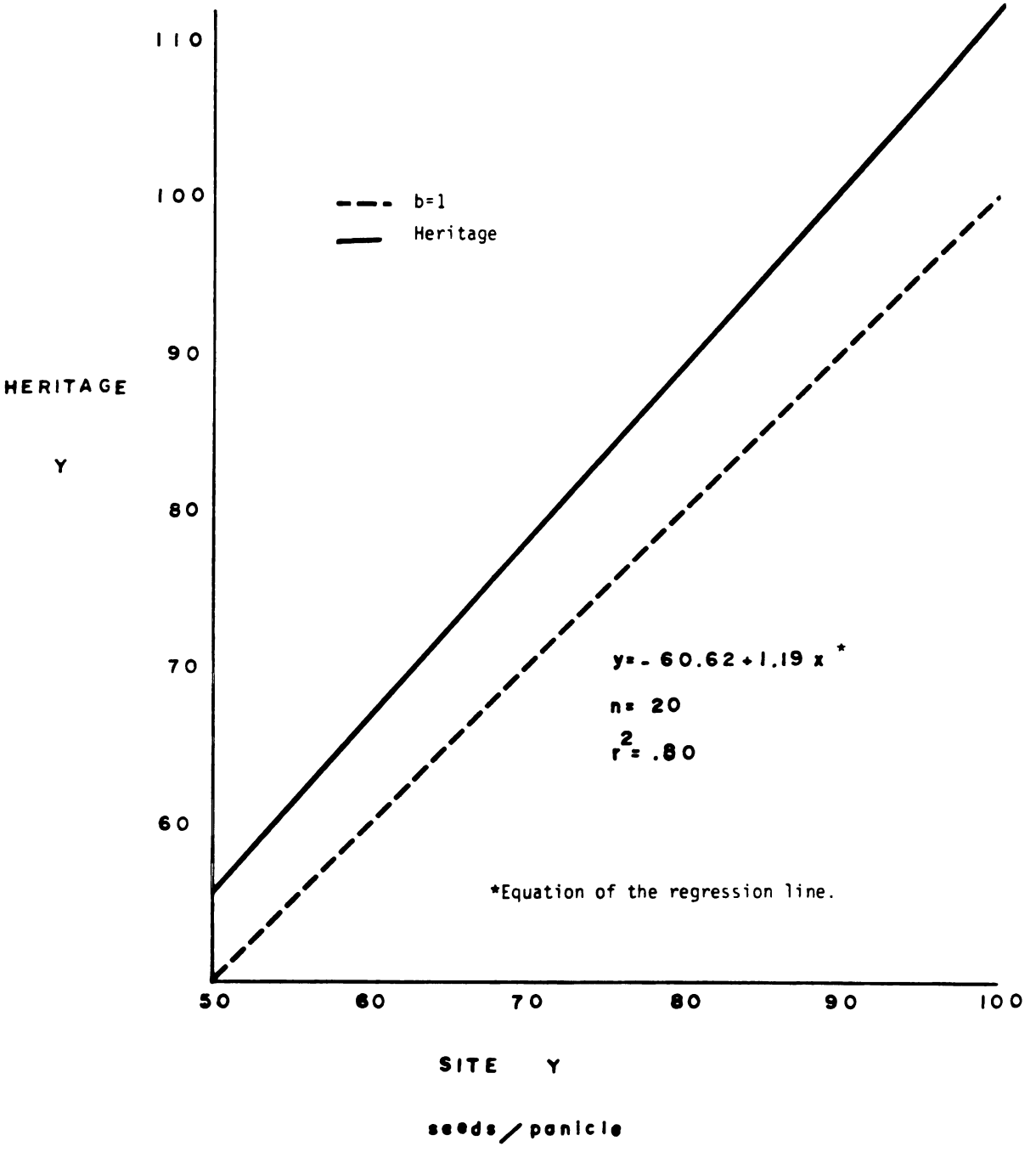


FIGURE 3. Regression Of Heritage XY Onto Site XY.

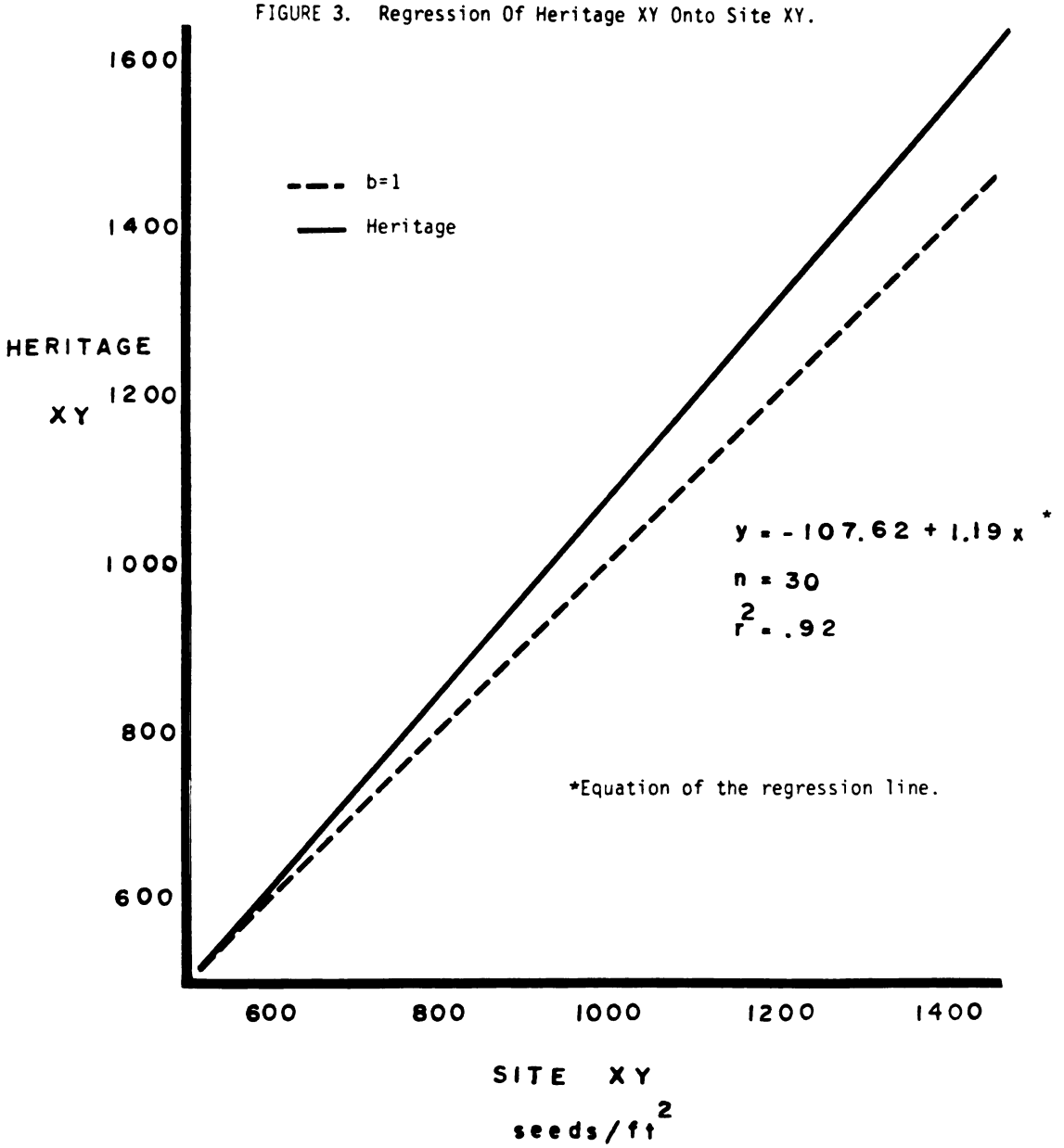


FIGURE 4. Regression Of Heritage Z Onto Site Z.

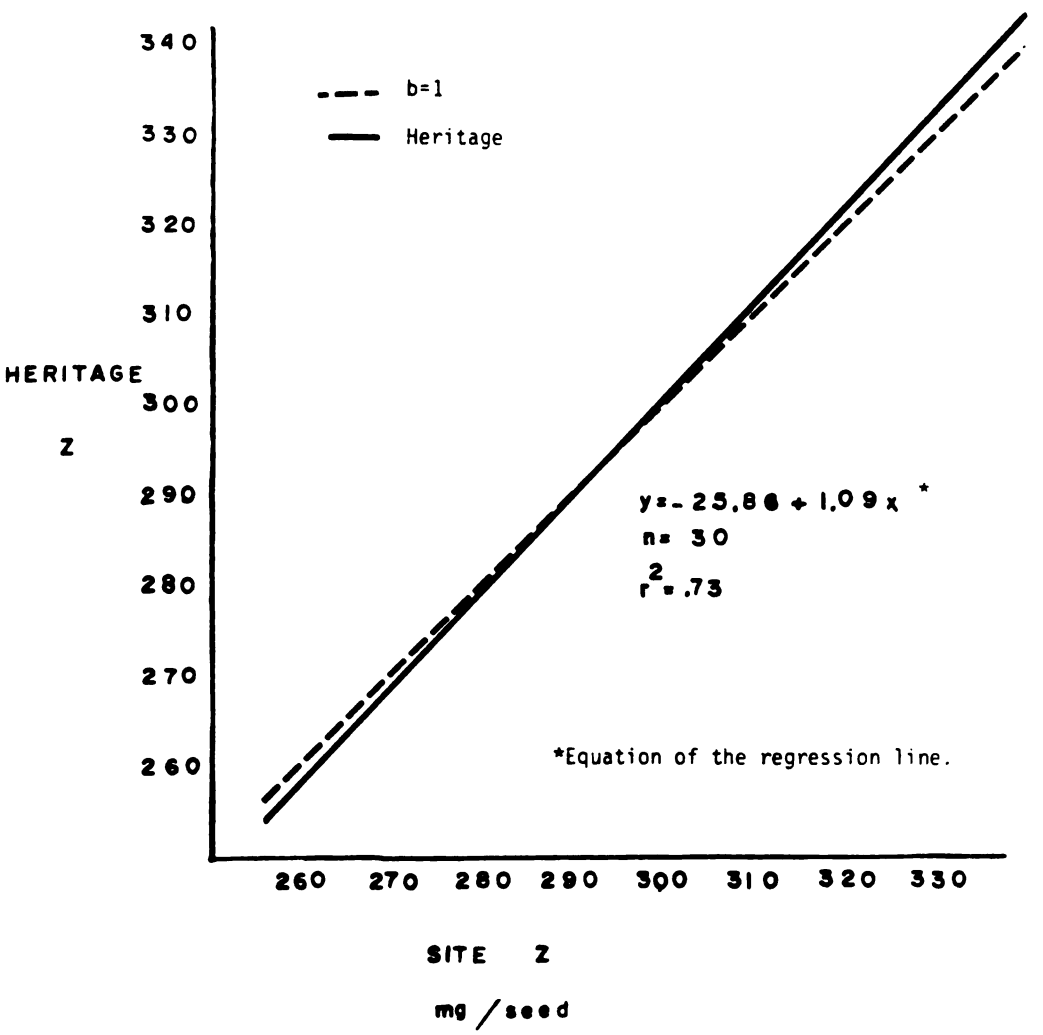


Figure 5 is the distillation of the preceeding yield component regressions. The regression of Heritage yield onto site yield for thirty year-sites amply demonstrates the superiority of Heritage with a high degree of determination. This superiority is primarily a function of the production of more seeds per unit area than competing varieties, without a severe reduction in seed weight, Z.

It should be remarked that any oat population is, to a considerable extent, a predictor of the response of any single genotype within the population. Avena sativa is a population of homozygous genotypes which will, therefore, necessarily predict its own performance. The plant breeder, however, operates narrowly within the broad parameters expressed by the species as a whole, since he deals with a sample of the population. This sample, or subpopulation, is more adapted to the specific environments for which the plant breeder engineers his varieties. Thus, one would expect a high coefficient of determination for the trait of yield. This high degree of prediction results from the homogeneous nature of the adapted population as well as from the developmental nature of yield. This latter point can best be expressed by the term "component compensation" (7). The pathway to yield, by way of the primary components, is a fluid one. Therefore, a stress on X (panicles/unit area) should reflect differential genotypic response with respect to site X and a low r^2 would imply that the environments included in the sample were highly stressed. However, yield is the product of three components and of the direct and indirect effects through the components. A stress in one is frequently buffered by superior expression in another component, and this will stabilize yield, the holistic trait. It may be concluded that Heritage represents a stable variety.

FIGURE 5. Regression Of Heritage W Onto Site W.

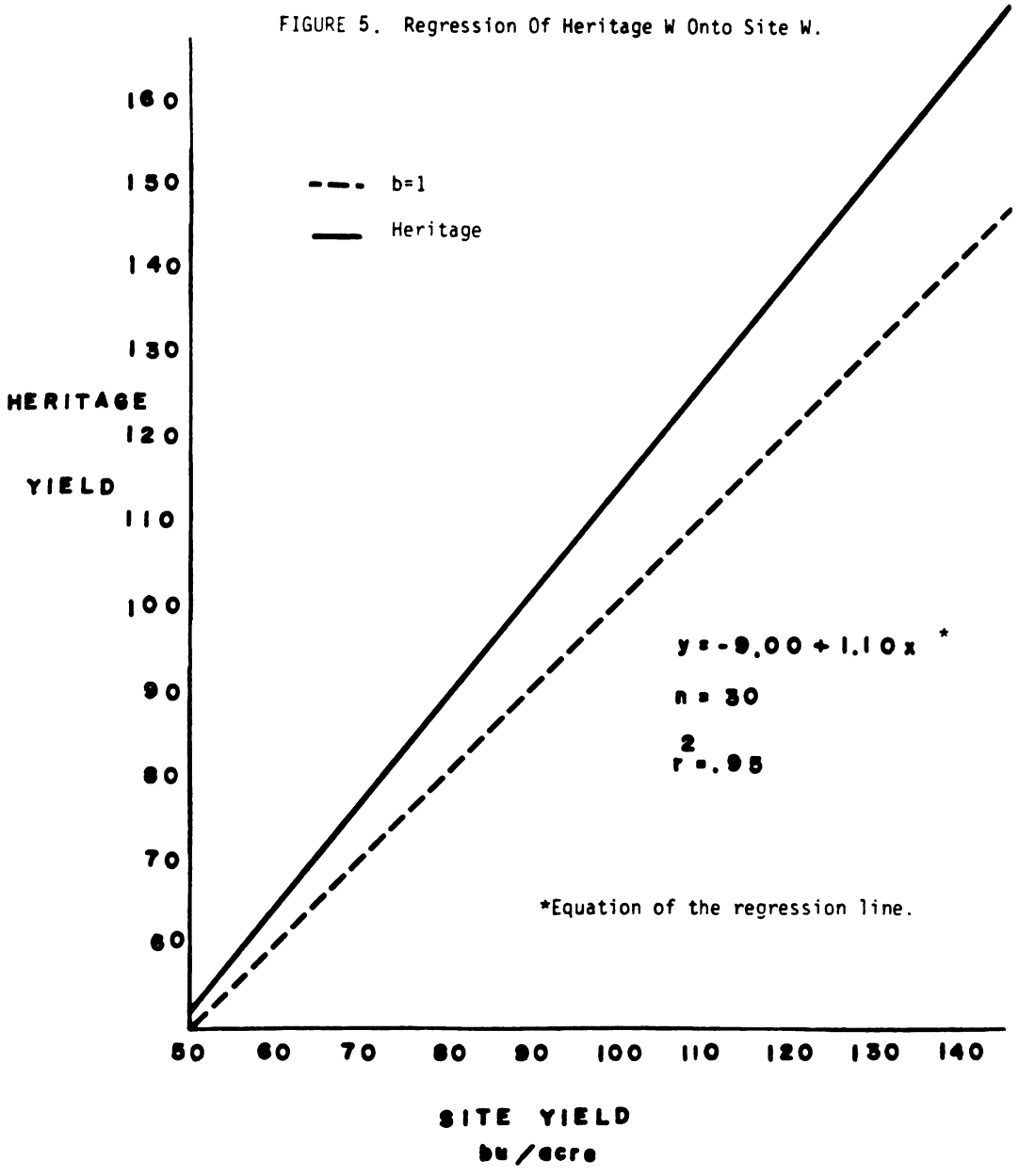
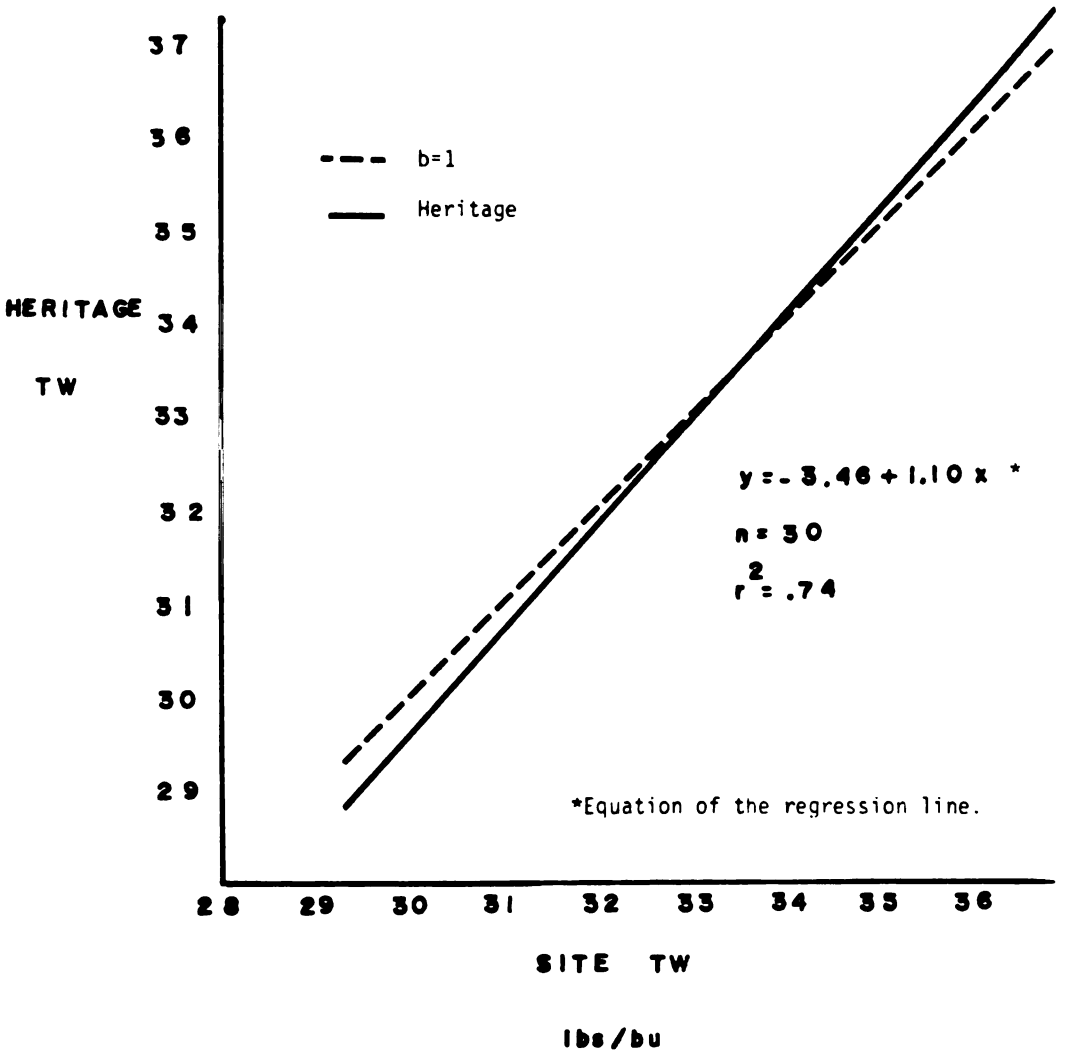


FIGURE 6. Regression Of Heritage TW Onto Site TW.



There is an elegance to this method of assaying performance through simple linear regression. It has been of help to the author in exploring relative genotypic performance and its efficacy is in no way limited to the six regression graphs herein presented. It seems a logical choice for contrasting the response of progeny to that of the parents, for both yield and its components, over a range of environments, where sufficient data are available. In an unpublished study, Nelson (1981) generated a series of regressions for such a purpose and found the information of considerable value.

APPENDIX 1. Correlational Matrix For Site And Heritage And Their Associated t-Statistics (r=0).

Correlation	Site r	t	Heritage r	t
r_{xy}	-.02	.08	-.15	.64
r_{xz}	-.11	.47	.13	.56
r_{xw}	.80	5.66***	.80	5.66***
r_{xtw}	.23	1.00	.07	.30
r_{yz}	-.12	.51	-.31	1.39
r_{yw}	.38	1.74*	.33	1.48
r_{ytw}	.32	1.43	-.79	5.44***
r_{zw}	.15	.80	-.01	.05
r_{ztw}	.60	3.97***	.61	4.07***
r_{wtw}	.36	2.04*	.38	2.17**

* P < .10

** P < .05

*** P < .001

CHAPTER 2--BIBLIOGRAPHY

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CHAPTER 3

OAT AND BARLEY COMPOSITE EXPERIMENTS

Introduction

The simple experiments detailed here originated during the teaching of CSS 408, Introduction to Plant Breeding. The subject of breeding systems was the venerable bulk population method. While writing the class notes the potential for bias in the system was seen: the breeding objective, which is to allow natural selection to operate on the bulk population, might not be a valid one, couched as it is in vague terminology. The source of the bias can be found in the primary components of yield. A brief review of the bulk population method will help explain the conflict.

A bulk population usually consists of a large field plot of segregating genotypes or, if homozygosity is well advanced, the bulk is a heterogeneous mixture of homozygous genotypes. In either case the plot is harvested in bulk and replanted in bulk, the cycle being repeated as many times as the breeder deems necessary to achieve his goals. These goals are several in number. The first is to allow natural selection to operate on the population to extract those genotypes which are superior in adaptation and presumably in yield; the secondary goals are the reductions in time, labor and expense which naturally result from the breeding system. The underlying assumption is that the natural selection

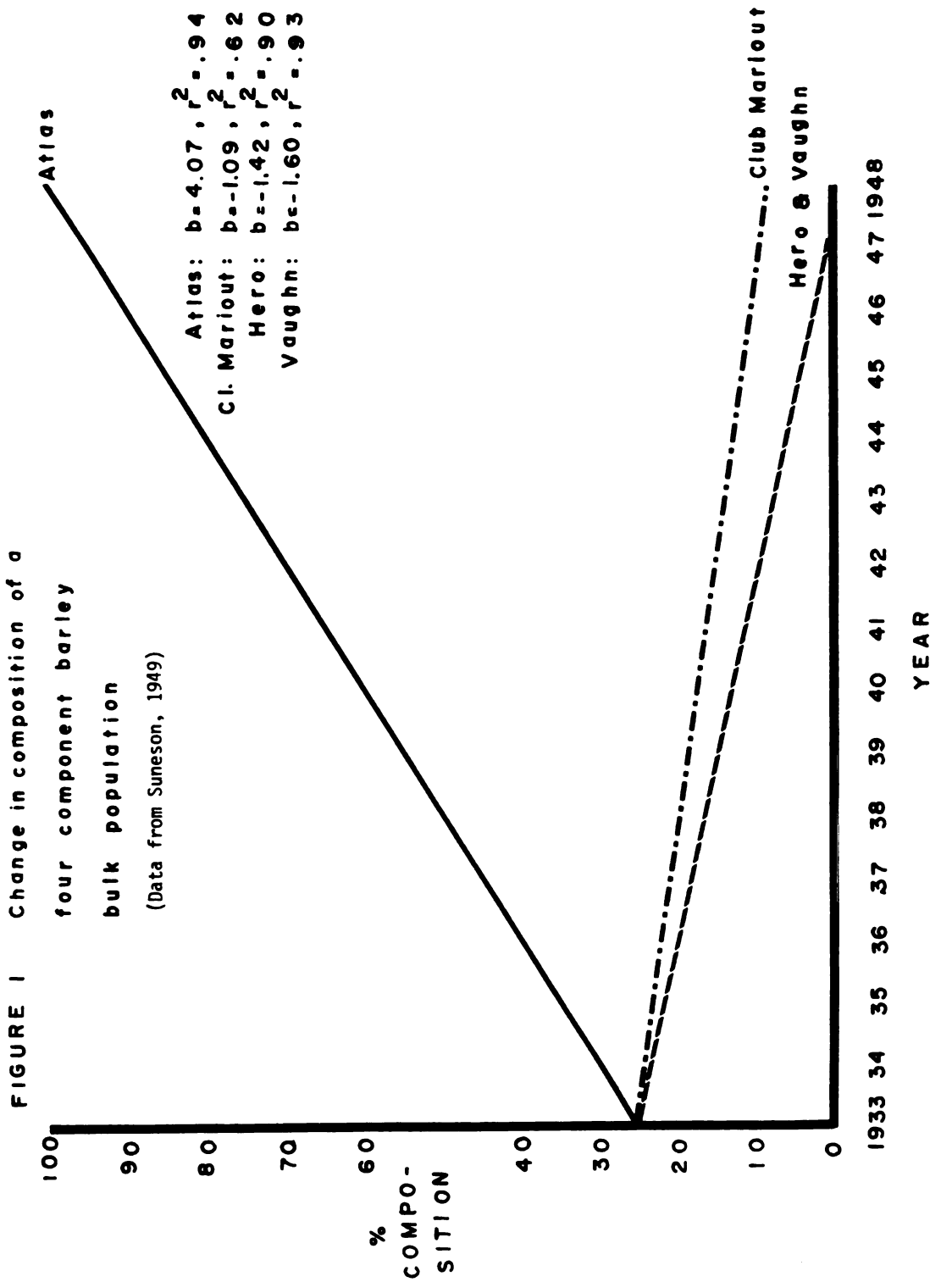

FIGURE 1 Change in composition of a four component barley bulk population

(Data from Suneson, 1949)

in the bulk increases those genotypes that have agronomic value, and that no artificial selection is practiced by the breeder upon the population. There are several potential flaws concerning both the primary goal and the assumption.

Allard (1) devotes extensive space presenting the method. Central to his discussion was the published work of Suneson, et al. (2,3,4,5). Suneson (3) began a bulk population study in 1933 in which four barley varieties were planted, each comprising 25 percent of the population. Each year the bulk plot was harvested, the frequency of each genotype was recorded and the bulk was replanted. The study was terminated in 1948 after sixteen generations. The results are displayed in Figure 1 which has been constructed using Suneson's data.

Sixteen generations changed the composition dramatically. Atlas comprised 88 percent, Club Mariout 10.5 percent, Hero 0.7 percent and Vaughn 0.4 percent. Grown in pure stands, however, the order of yield was Vaughn > Hero > Atlas > Club Mariout. Suneson was unable to explain these results. The answer seemed obvious to this author: despite a low yield in pure stands, Atlas produced more seeds per unit area than the competing varieties. I exclude from consideration seedling vigor which may have strongly influenced the outcome. In any case, even considering vigor, Atlas had to have produced more seeds for any given harvested area. Expressed as yield components and over years $XY_{Atlas} > XY_{C.M., V., H.}$, where X = # of spikes per unit area and Y = # of seeds per spike, the combined component representing seed number per unit area. The reasoning was that Atlas, as an old variety, had not been selected for high Z, or individual weed weight. Its smaller seeds would account for high XY by virture of component compensation, despite its intrinsic low yield.

Reviewing the literature more extensively it was found that seed number was proposed as the force behind changes in bulk composition. Harlan et al. (6) and Laude et al. (7) remarked on the phenomenon, but Suneson chose to explain the differential composition of his bulk by ascribing disease as the cause, despite its comparative absence and despite the fact that the changes in composition were highly linear, as detailed by the high r^2 values in Figure 1. As a consequence of this an hypothesis was proposed: The percentage composition of a variety grown in bulk with other pure lines will be a function solely of its XY component as determined by the XY estimates taken from the pure stands. Mathematically, the function would be

$$P_1 = \frac{XY}{\sum_{1}^{n} XY} P_0,$$

where P_0 is the beginning proportion of a variety, n is the number of varieties grown in the bulk and XY is the seed number per unit area per variety as determined by the pure stand. Over time the function would be $P = b\,(\Delta t)$, where b is the regression coefficient and Δt is the number of years the bulk has been harvested.

Although I have no substitute, the term natural selection seems to me a misnomer. The bulk population method of breeding supposedly operates independently of man, but as a consequence of planting and harvesting, artificial selection is taking place--the interdiction of man occurs. My point is more philosophical, or semantical, than that concerning the dynamics of the population. However, natural selection is a function of great expanses of time and it is not an isolated phenomenon

operating upon a crop whose maintenance, as such, is entirely dependent upon constant human intervention.

The ramifications of artificial selection based upon seed number were intriguing enough that two experiments were initiated. One experiment would be with barley and the other with oats. The intent was to test the hypothesis that a genotype's relative composition in a bulk population could be determined by XY component analysis of that variety in a pure stand.

Materials and Methods

The constituents of the oat and barley bulks were chosen first on the basis of easily distinguishable differences in their seed to facilitate sorting after bulk harvest. Secondly, an attempt was made to maximize the potential yield component differences in the varieties based upon the performance summaries of Michigan oat and barley experiments. Thus, two barleys were chosen from many potential genotypes because one was a naked barley and the other a red seeded variety. The former was named Hulless Vantage and the latter Red Lemma Titan. To these two six-row varieties was added Coho, a well adapted Michigan two-row variety with large seeds and high tiller number. The oat composite was made up of two varieties, Orbit and Hulless Terra. As it was impossible to distinguish red oats from yellow and white when mixed together, a two component bulk would have to suffice. Orbit was chosen for its large seed weight and Terra was the other choice since it was a naked oat and was adapted, if not directly to Michigan, then at least to Indiana where it was developed. Terra was provided by Dr. David Smith and the two

barley varieties, Red Lemma Titan and Vantage, were obtained through the courtesy of Dr. Eugene Hockett.

Composite bulks were made of the oat and barley varieties such that each variety was equally represented by seed number. The oat bulks and pure lines were packaged 35 grams per seed envelope with five replications for Terra and four each for Orbit and the composite for a total of thirteen plots. The barley bulks and pure lines were packaged 30 grams per envelope in four replications, totaling sixteen plots. Each envelope was planted with a four row belt plot planter. Row spacings were 11 inches and the plots were twelve feet in length. The plots were subsequently trimmed to eight feet in length. The barley experiment was planted in Tuscola County April 23, 1980, with 420 lbs. 8-32-16 fertilizer. The oat experiment was planted May 5, 1980, in Ingham County with similar fertilization. The sixteen barley plots were sprayed prophylactically in late May with Benlate to protect against mildew.

All barley plots were mechanically harvested July 25. Only the center two rows were taken to eliminate border effect. The oat plots were harvested by hand August 3. Again, only the center two rows were harvested and the plot harvester was not used due to extreme lodging in the nursery. The grain was dried for five days, cleaned and final yields were recorded for both the pure lines and the bulks. Multiple samples for each pure line from each replication were weighed and the seeds in each sample were counted to arrive at an estimate of Z, individual seed weight. The replicated Z values were then divided into the respective plot yields to calculate an estimate of XY, or seed number per plot.

The oat and barley bulks were sampled three times per replication and the seeds were divided into their respective classes. These genotypic seed classes were weighed and the seed number was calculated. Thus, the proportions of each genotype in the harvested bulk were estimated. The results are tabulated in Table 1. Analysis of variance was calculated and can be found in Tables 2 and 3. Chi square values adjudging differences between the expected and the observed values of XY--based upon pure line component evaluations--are listed in Table 5.

Results and Discussion

Analysis of variance for the oat pure lines was not significant at P = .05 for XY, but was highly significant for W (yield) (Table 2). Differences for both XY ans W were highly significant for barley pure lines (Table 3). Chi square estimates for differences between the observed and estimated values for XY in the bulks, calculated using analyses of the pure lines, were significant at levels far less than P = .001 (Table 5). Based upon the contingency analysis from one year's data on two bulk populations I would reject without reservation the hypothesis that changes in bulk composition can be predicted from cultivar performance as a pure line.

Table 2 shows that the pure line XY difference between Terra and Orbit is negligible; their differences in bulk composition are profound, with deviation from expected values equaling nearly 22 percent (Table 1). Seed number per plot, or XY, differences for barley based upon estimates derived from pure lines were significantly different, unlike the oat estimates (Table 3). The prediction equation which reflected the

Table 1. Component and yield data for oat and barley pure lines and bulks, where W = yield in grams per plot, Z = seed weight and XY = seeds per plot.

Oats

Entry	Replication	W	Z	XY
Terra	1	428	.0223	19,174
	2	410	.0219	18,737
	3	405	.0207	19,602
	4	413	.0206	20,030
	5	416	.0196	21,216
means		414	.0210	19,752
S_x		8.6	.0011	950
Orbit	1	545	.0293	18,584
	2	534	.0305	17,515
	3	578	.0287	20,134
	4	527	.0273	19,288
means		546	.0290	18,880
S_x		22.6	.0013	1,109
Composite	1	453		
	2	508		
	3	477		
	4	521		
mean		490		
S_x		30.7		

Barley

Coho	1	633	.0499	12,692
	2	647	.0488	13,264
	3	635	.0476	13,335
	4	701	.0461	15,212
means		654	.0481	15,212
S_x		31.9	.0016	1,096

Table 1, continued

Barley

Entry	Replication	W	Z	XY
Red Lemma Titan	1	565	.0400	14,125
	2	652	.0399	16,333
	3	695	.0394	17,653
	4	710	.0402	17,644
means		656	.0399	16,439
S_x		65.1	.003	1,662
Hulless Vantage	1	398	.0399	10,448
	2	398	.0381	10,627
	3	425	.0373	11,390
	4	408	.0373	10,934
means		407	.0376	10,850
S_x		12.7	.0004	412
Composite	1	456		
	2	623		
	3	672		
	4	636		
mean		597		
S_x		96.1		

Summary

Crop	Entry	Bulk Compositions % Expected	% Observed	Δ %
Oats	Terra	51.13	29.29	-21.84
	Orbit	48.87	70.71	21.84
Barley	Coho	33.30	58.63	25.33
	RLT	40.18	38.56	- 1.62
	HV	26.52	2.81	-23.73

Table 2. Mean square of XY (seeds/plot) estimates of Orbit and Hulless Terra grown as pure lines and of W (yield).

Variable XY

Source of Variation	Degrees of Freedom	Mean Square	F
Blocks	3	1,526,796	
Entries	1	822,404	3.73 n.s.
Error	3	220,532	

Variable W

Source of Variation	Degrees of Freedom	Mean Square	F
Blocks	3	219	
Entries	1	34,716	89.40 *
Error	3	318	

* $P < 0.005$

Table 3. Mean squares of XY (seeds/plot) estimates of Coho, Red Lemma Titan and Hulless Vantage grown as pure lines and of W (yield).

Variable XY

Source of Variation	Degrees of Freedom	Mean Square	F
Blocks	3	2,691,174	
Entries	2	31,237,402	36.03*
Error	5	867,067	

*P < 0.005

Variable W

Source of Variation	Degrees of Freedom	Mean Square	F
Blocks	3	2,988	
Entries	2	81,677	55.83*
Error	5	1,463	

*P < 0.005

hypothesis would place Titan as first in the bulk, with Coho and Vantage following. The observed values were dramatically different and the most startling difference was the near extinction of Hulless Vantage in a single generation.

There is an explanation for the deviations from expected values: vegetative vigor. This is, perhaps, another way of stating that those varieties best adapted to Michigan predominated in the bulk, regardless of the expression of their relative XY components. For example, Orbit constituted 71 percent of the bulk which was 22 percent more than was predicted. Orbit has long been an approved variety for Michigan, although it is a New York release. Michigan growing conditions are sufficiently good that certified seed is grown in this state for eventual sale in New York and other eastern states. For the 1980 harvest year Orbit was the second highest yielding entry in both rod row experiments grown in Kalamazoo and Tuscola counties. In two additional experiments containing advanced generation material and various parents, Orbit was the single highest yielding entry. The year was ideal for a cool season, large seeded oat of which Orbit has been a premier example for more than a decade. Terra is an Indiana variety and although it was tested for several years in the Uniform Cooperative Mid-Season Oat Performance Nursery it never found a market. Like other naked oats it is a curiosity of no great commercial value considering the current technological ease by which oats are dehulled. The mean yield of 78 bu/acre is respectable, especially in the absence of hulls, when contrasted with a mean yield of 102 bu/acre for Orbit.

Vegetative vigor is again the logical explanation for the disparity between the observed and the expected values for XY in the barley bulk

population. In this case I suspect that early tiller initiation was the critical component of vigor that determined very early the eventual relative seed number production. Coho predominated due to its adaptation to Michigan (it is a Michigan-developed variety) and to its two-row characteristic. Two-row varieties are necessarily high tiller producers since the lateral florets are sterile and as such contribute nothing to yield. The competition provided by an abundance of tillers which were initiated early would retard growth of less vigorous six-row varieties. Hence, the virtual elimination of Vantage from the composite occurred. In fact, Vantage had extremely large heads (Table 4) which would be reflected in a reduced number of tillers. On the other hand, Red Lemma Titan, aside from being one of the most phenotypically stunning barleys this author has ever seen, had relatively small heads and good tiller production. In short, this variety showed remarkable adaptation and vigor considering that it was developed in Montana. The relative order of the varieties in the bulk after one generation was, in fact, the inverse of the order of the mean weight of individual spikes.

An apologium: The hypothesis which I proposed and which generated this experiment was necessarily simplistic. It was, however, easily tested and the results are not without value. First, there is a lesson to be drawn concerning the composition of bulks or, under a different appelation, the composition of multilines. With equivalence of yield it is apparent that other factors are operating in the population which can quickly and deleteriously eliminate one of the constituent genotypes, depending upon events determined early in growth as demonstrated by Wiebe, et al. (8). The use of highly adapted varieties with adequate genetic markers to determine their survival in composites is a possible

Table 4. Estimates of spike weights for Coho, Red Lemma Titan and Hulless Vantage as determined by sampling pure line plots.

Entry	Replication	Sample	25 Spike Weight (gms)	Mean Spike Weight (gms)
Coho	1	1	29.31	
		2	30.85	
		3	27.65	1.17
	2	1	29.35	
		2	26.58	
		3	26.62	1.10
	3	1	30.55	
		2	31.64	
		3	31.05	1.24
	4	1	25.84	
		2	28.39	
		3	26.37	1.07
Red	1	1	32.00	
Lemma		2	33.15	
Titan		3	34.12	1.32
	2	1	32.84	
		2	32.35	
		3	33.26	1.31
	3	1	39.73	
		2	37.55	
		3	40.66	1.57
	4	1	34.75	
		2	36.31	
		3	38.04	1.45

Table 4, continued

Entry	Replication	Sample	25 Spike Weight (gms)	Mean Spike Weight (gms)
Hulless	1	1	56.62	
Vantage		2	51.60	
		3	52.79	2.15
	2	1	56.60	
		2	54.69	
		3	52.14	2.18
	3	1	62.68	
		2	60.98	
		3	59.04	2.44
	4	1	58.91	
		2	60.71	
		3	51.29	2.28

Summary

Entry	Mean Individual Spike Weight Over Replications and Over Samples (gms)	$S_{\bar{x}}$
Coho	1.15	0.04
Red Lemma Titan	1.41	0.06
Hulless Vantage	2.26	0.07

Table 5. Chi square test of significant differences between XY estimated and observed values in oats and barley.

Oats: Chi square = 671.6 with 3 degrees of freedom.
$P < 0.001$

Barley: Chi square = 1290 with 6 degrees of freedom.
$P < 0.001$

solution to the problem of varietal choice. Preferrable to such a system is the method of Grafius which utilized bulks at the F_5 generation in which neither adaptation nor extreme artifical selection occurred. Such bulks are composed of F_5 head hill selections which retain a modest amount of heterozygosity which would eventually constitute heterogeneity. Another point is that extreme selection does, or can, occur in bulk populations, though the selection is neither natural nor easily predicted. The final point is instructive: the data are good educational material for any course in plant breeding since the scope of the experiments is broad and the explanations require no little thought.

To bring this chapter full circle I will conclude with Suneson's original experiment (1). I attempted at various times to contact Dr. Charles Schaller, the current barley breeder at the University of California, Davis, and Coit Suneson's successor. I was unable to communicate with Dr. Schaller until well after my experiments were concluded. I did speak with Linda Prato, Dr. Schaller's technician in an attempt to find specific information concerning Suneson's four varieties. What I desired was data on the four varieties grown in the same year and location and for which both yields and seed weights were recorded. Dividing Z into W (yield) would provide an estimate of XY. Despite Prato's best efforts to find such records, they do not exist.

In early December, 1980, I had an opportunity to talk with Dr. Schaller. We discussed Suneson's work at some length and Dr. Schaller concluded that the results were mostly a function of relative varietal vigor, although no controlled experiments were even conducted to test the notion. What was most interesting, however, was Dr. Schaller's

recitation of farmer preference. Despite a clear superiority of many varieties over Atlas in yield, growers consistently and for many years continued to grow Atlas to the exclusion of newer releases. The phenomenon was deemed sufficiently important to conduct a survey of barley growers with respect to their preference for Atlas. The answer was simple: Atlas always looked better in the field. Thus are aesthetics and vigor united.

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CHAPTER 4

THE USE OF OUTLIERS IN BREEDING FOR YIELD IN BARLEY

Introduction

The first use of the primary components in explaining yield was first proposed by Balls (1) and their use in raising yields was subsequently proposed by Woodworth (2). Frankel (3) cited the strong genotype-environment interaction as an explanation for why plant breeders failed to utilize them. The environment notwithstanding, Adams (4) offered the most plausible explanation for the difficulties which a plant breeder encounters when working with yield components: the strong negative correlations between the primary components would confound attempts to increase yield by the mechanism of component compensation. In a similar vein Grafius et al. (5,6,7,8) delineated the effects of component compensation in barley which, by extension, would apply to other cereals. The application of this research in conjunction with regression analysis led to an experiment (9) which highlighted a method of isolating genotypes which, in proper crossing combinations, would produce selected homozygous progeny transgressive to both parents for yield. The selected progeny, cited as 1×4^2 (9), was Michigan line 68-105-15 which was eventually released as the variety Bowers, an extremely high yielding feed barley (10,11,12,13). The confidence engendered by the development of Bowers, coupled with an extended knowledge

of the use of yield components, provided the inspiration for this experiment.

Materials and Methods

The mathematical identity X Y Z = W, where X = spike number/unit area, Y = seed number/spike, Z = weight/seed and W = weight/unit area means, simply, that yield is determined by its primary components. Hence, any yield differences between genotypes must be reflected in their determinants, X, Y and Z. The primary problem lies in assessing differences with respect to the negative correlations between components, or component compensation. The method of Grafius (9) to ascertain the extent of these differences was through the simple expedient of linear regression.

Data from 1978 barley experiments were plotted for Z (seed weight) against Y (seed number per spike). Of eleven entries three were "outliers" (Figure 1) in the sense that they appeared to lie above and to the right of the axis of the eight remaining entries. Two of the outliers, 68-105-1 (Michigan) and M33 (Minnesota) (see Nos. 5 and 11, respectively), were each crossed to the four genotypes having the highest values for seed number per spike, Y. The constraint on increasing the number of crosses and utilizing the third outlier was that of greenhouse space needed to simultaneously advance no more than eight bulks through two generations of single seed descent (14). The crosses are detailed in Appendix 1.

The seven surviving F_1 lines were increased to F_2 and subsequently grown for two generations under single seed descent, producing F_4 seed.

FIGURE 1a Regression of Z onto Y for Possible Parents

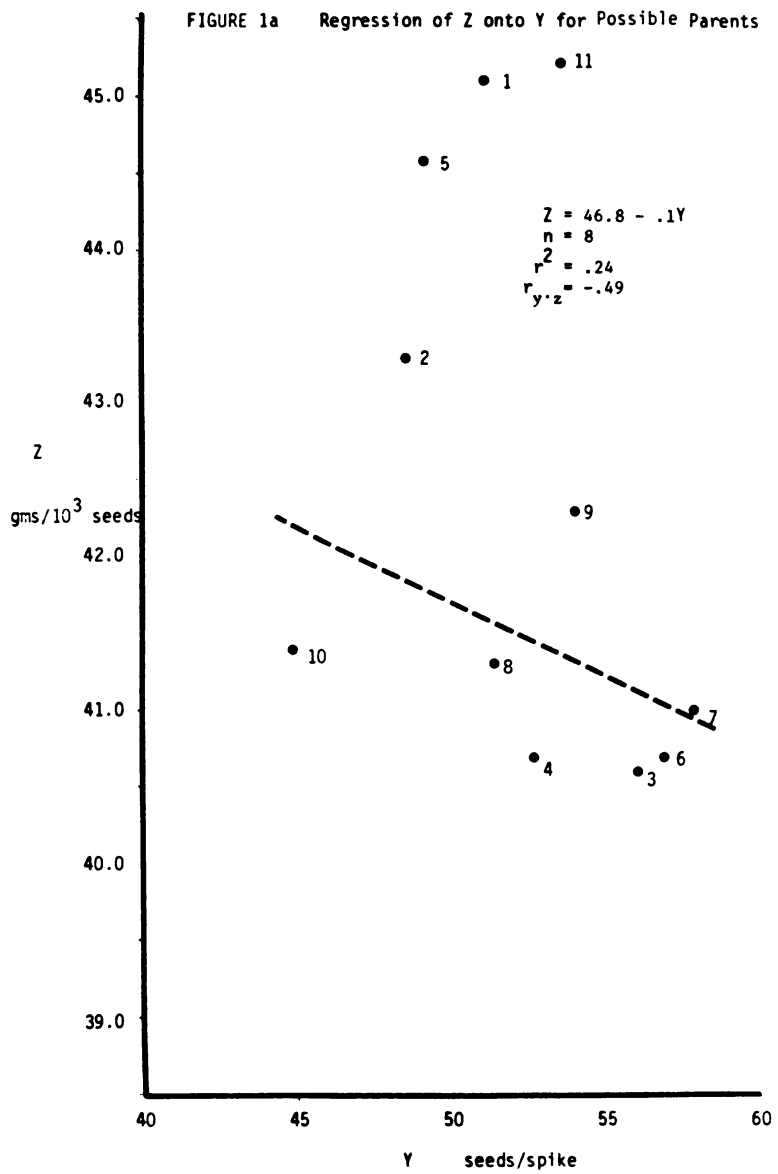
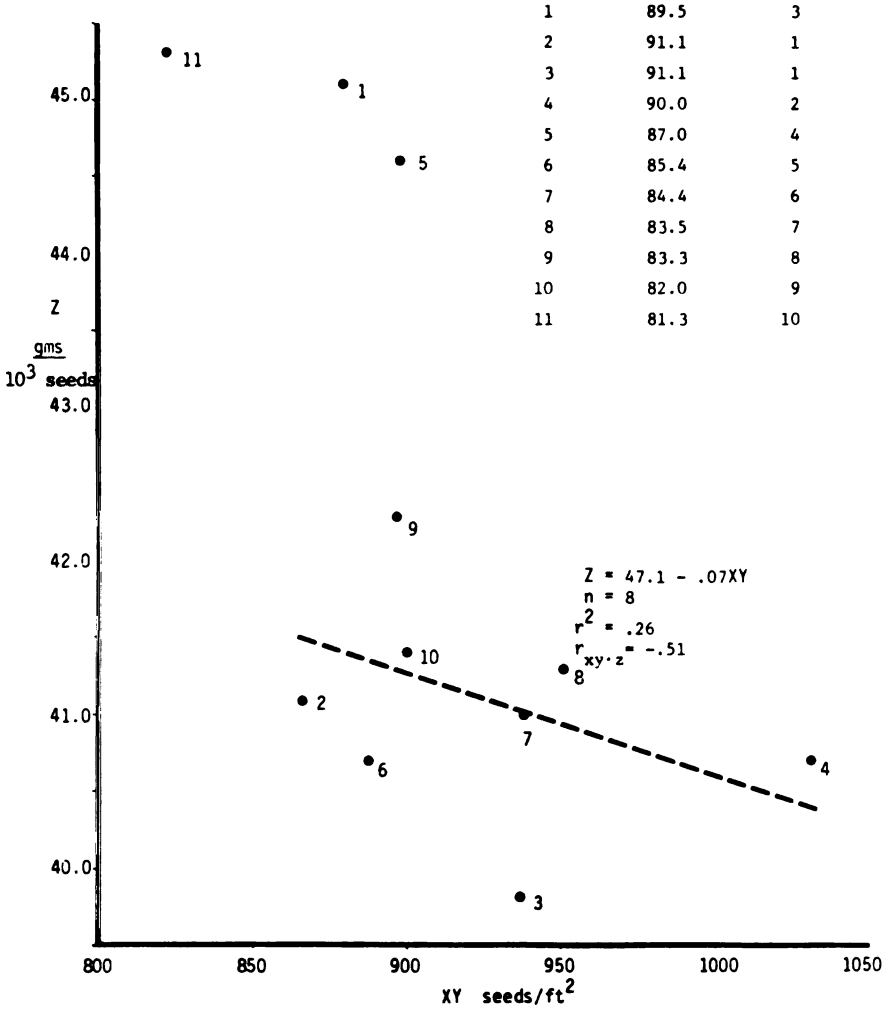


FIGURE 1b Regression of Z onto XY for Possible Parents and Their Respective Yields.

ENTRY	YIELD (bu/A)	RANK
1	89.5	3
2	91.1	1
3	91.1	1
4	90.0	2
5	87.0	4
6	85.4	5
7	84.4	6
8	83.5	7
9	83.3	8
10	82.0	9
11	81.3	10



Approximately 1,000 seeds of each bulk were planted in single plots at East Lansing in the spring of 1979 for seed increase. The plots consisted of four rows on eleven inch centers, eight feet in length. They were fertilized with 400 pounds/acre 8-32-16 and the center two rows were harvested using a plot harvester (15).

F_5 seed of each bulk, all six parents and seven checks were planted in Tuscola County April 24, 1980, with 420 pounds/acre 8-32-16. Plots were four rows, eight feet in length with eleven inch row spacings. The twenty total entries were planted as a 4 x 5 rectangular lattice with four replications. The soil type was a Parkhill Clay Loam.

The experiment was harvested July 25 and yield component data were taken on all entries. Only the center two rows were harvested for yield. The grain was dried in a greenhouse for four days and subsequently cleaned before data were recorded. Table 2 summarizes the results and Table 3 contains the analyses of variance associated with the traits of interest.

Results and Discussion

The expectation upon which these crosses were based was that the mean yields of all seven bulk populations would exceed all of the respective parents. Table 2 and Figure 2a display the remarkable results. There are four points of importance concerning these results which need to be stated. First, of the seven bulks, five outyielded all the parents. Secondly, the two bulks which did not outyield all parents had, nevertheless, very respectable yields. More importantly, however, these two bulks (B79-105 and B79-106) were associated with

TABLE 1a.

EXPERIMENT 8095 BULKS

TABLE OF UNADJUSTED MEANS

(SORTED ON VARIABLE 12 IN DESCENDING ORDER)

ENTRY NAME		VARIABLE 11 X TILLERS/SQ FT	32 Y SEEDS/SPIKE	14 Z GMS/SEED	12 W GMS
B79-1 03	•	16.3231	78.4028	.0404	824.0000
B79-1 07	•	17.3524	69.1132	.0421	806.0000
B79-1 04	•	17.7331	73.2593	.0391	804.0000
B79-1 01	•	15.4906	80.5646	.0400	795.0000
B79-1 02	•	16.9532	76.4986	.0388	793.3333
68-10 5-10	♦	15.8290	74.8937	.0413	775.6667
68-10 5-17		16.5736	71.0444	.0409	767.0000
68-10 5-15	♦	17.1405	76.2069	.0364	758.0000
M33	♦	16.5523	70.0785	.0409	756.6667
B79-1 06	•	15.6758	75.3451	.0395	746.0000
M37		16.0250	72.9563	.0395	739.0000
68-10 3-1	♦	15.5912	74.0778	.0391	722.6667
B79-1 05	•	15.8577	67.9743	.0415	714.3333
BONAN ZA		16.3335	74.2792	.0368	712.3333
68-10 5-1	♦	16.0242	64.0389	.0435	711.0000
68-10 4-7	♦	16.5197	65.0861	.0409	704.6667
M36		14.7641	73.0479	.0402	691.3333
COHO		28.9779	25.1604	.0495	571.6667
LARKE R		15.3931	67.2757	.0400	658.3333
M34		14.3475	66.6535	.0423	646.6667
AVERAGE OF PROCEDING 20 MEANS		16.7729	70.0003	.0406	739.9833
LSD (P2.05)		2.9649	10.0791	.0018	100.6874
(P=.01)		3.7244	13.1029	.0024	130.8936
COEFFICIENT OF VARIATION		10.4597	8.8174	2.7667	8.3324

• Bulks

♦ Parents

TABLE 1b.

EXPERIMENT EGGS PULKS

T A B L E O F U N A D J U S T E D M E A N S

(SORTED ON VARIABLE 13 IN DESCENDING ORDER)

ENTRY NAME	VARIABLE 13 TW(LB/BU)	33 XY SEEDS/PLOT	30 BU/ACRE	11 20 HD WT
B79-1 03	50.3370	20416.2222	103.0000	50.6000
B79-1 07	50.2430	19219.6667	101.0000	46.5000
B79-1 04	49.8670	20519.5556	100.5000	46.0000
B79-1 01	49.8200	19885.3333	99.3750	51.5000
B79-1 02	49.1150	20450.7778	99.1667	47.4667
68-10 5-10	50.3940	18792.3333	96.9583	49.5000
68-10 5-17	49.9610	18760.4444	95.8750	46.5667
68-10 5-15	50.1490	20793.8889	94.7500	44.4667
M33	50.6190	18498.2222	94.5833	45.8333
B79-1 06	49.6320	19866.1111	93.2500	47.5667
M37	50.9950	18719.0000	92.3750	46.0667
68-10 3-1	51.0420	18504.2222	90.3333	46.3667
B79-1 05	50.6660	17215.0000	89.2917	45.1000
30MAN 7A	48.8330	19395.6667	89.0417	43.6333
68-10 5-1	50.5720	16349.3333	88.8750	44.5333
68-10 4-7	50.3840	17205.1111	88.0833	42.6667
M36	50.2900	17220.7778	86.4167	46.9667
COMO	53.2040	13547.2222	83.9583	23.1333
LARKE R	50.2900	16434.4444	82.2917	43.0333
M34	51.0420	15303.3333	80.8333	45.0667
AVERAGE OF PROCEDING 20 MEANS	50.3722	18304.8333	92.4979	45.1283
LSD (P2.05)	.5679	2482.9343	12.5859	6.1599
(P=.01)	.9602	3227.8146	16.3617	8.0078
COEFFICIENT OF VARIATION	.9119	8.3064	8.3324	8.3587

TABLE 1c.

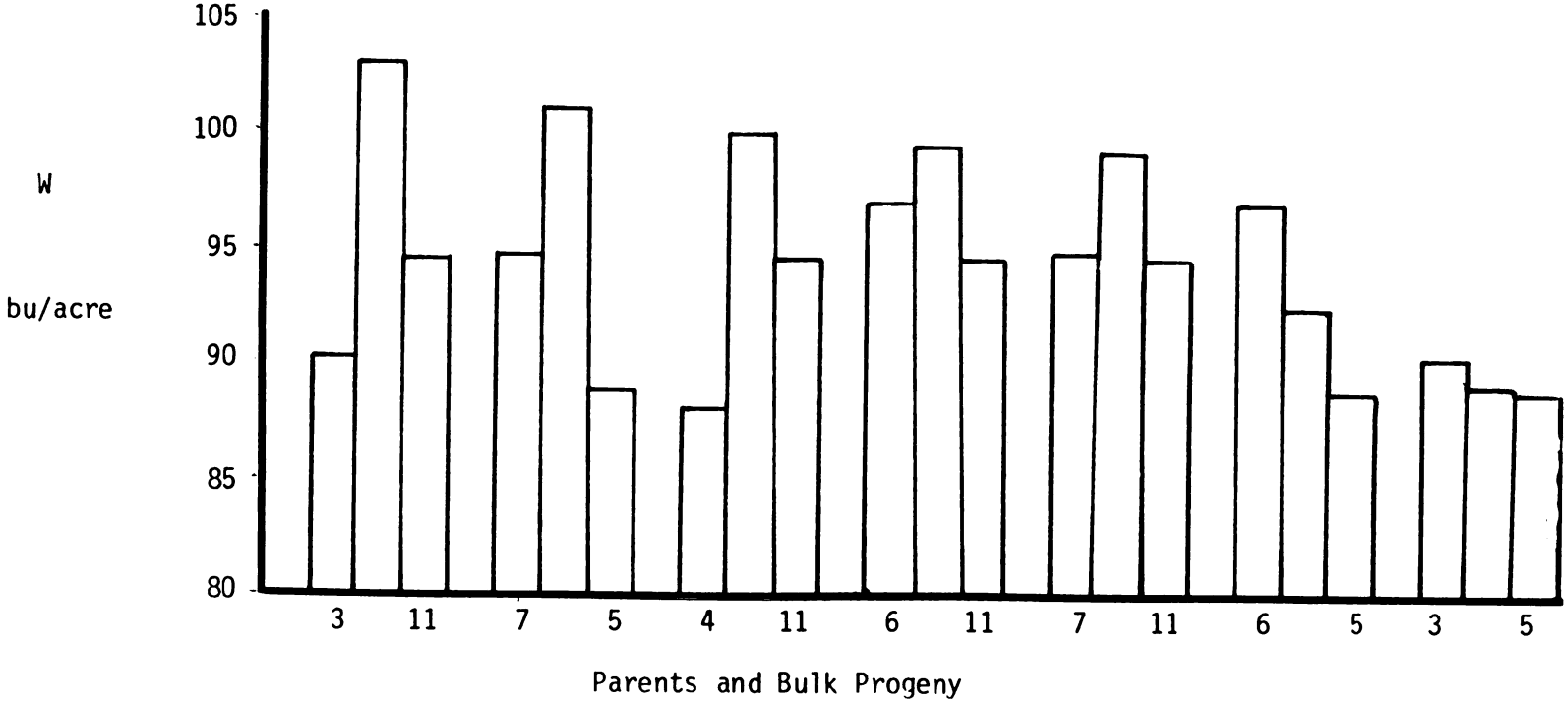
EXPERIMENT 8095 BULKS

TABLE OF UNADJUSTED MEANS

(SORTED ON VARIABLE 12 IN DESCENDING ORDER)

ENTRY NAME	VARIABLE 6 HEIGHT (INCHES)	VARIABLE 10 LODGING
B79-1 03	40.0000	20.0000
B79-1 07	42.3333	48.3333
B79-1 04	40.0000	35.0000
B79-1 01	41.0000	25.0000
B79-1 02	39.6667	38.3333
68-10 5-10	39.0000	28.3333
68-10 5-17	37.6667	21.6667
68-10 5-15	41.0000	20.0000
M33	40.6667	20.0000
B79-1 06	38.3333	68.3333
M37	38.3333	15.0000
68-10 3-1	37.3333	21.6667
B79-1 05	42.6667	45.6667
JONAN ZA	45.3333	36.6667
68-10 5-1	41.0000	30.0000
68-10 4-7	40.3333	33.3333
M36	41.6667	16.6667
COHO	39.0000	5.6667
LARKE R	38.0000	28.3333
M34	40.0000	10.0000
AVERAGE OF PROCEDING 20 MEANS	40.1667	28.5000
LSD (P=.05)	3.2477	27.2867
(P=.01)	4.2220	35.4726
COEFFICIENT OF VARIATION	4.9513	58.6302

FIGURE 2a Parental and Bulk Progeny Performance for Yield.



high degrees of lodging (68 percent and 47 percent, respectively). It is not inconceivable that this lodging contributed to a yield reduction which, when adjusted for the loss by machine harvesting (15), might have ranked these bulks above the parents, as well. Thirdly, it must be borne in mind that in the development of these bulks, that no selection for yield had been applied. Fourthly, these bulks are collections of homozygous genotypes which assuredly are normally distributed with respect to the polygenic trait of yield. It is logical, therefore, to assume that each bulk contains pure lines which are transgressive not just to their parents, but with respect to the high mean yield of the bulks themselves. The practical consequences of this are obvious.

With the exception of the late Dr. John E. Grafius, there has been no corpus of theory which conveniently explains these results since they are predicated upon the primary components of yield. The difficulty of explanations using yield components is perhaps best summarized by the following analogy. Yield components are like eating Jello with fingers: no sooner does one get a grip in two dimensions, the Jello squirts off into a third dimension. It was precisely this plasticity or compensation or collection of negative correlations which this experiment was designed to circumvent. Yield cannot be explained by one or two components, but must be presented and dealt with holistically. Figures 2b through 2e demonstrate this point.

The seven bulks are represented in histograms nested between each of their parents for the traits X, Y Z and the combined trait YZ. The individual graphs of X, Y and Z do not explain the yield performance of the top five bulks in a consistent fashion. The combined trait, XY (Figure 2e), however, does. Of the five superior bulks, all were

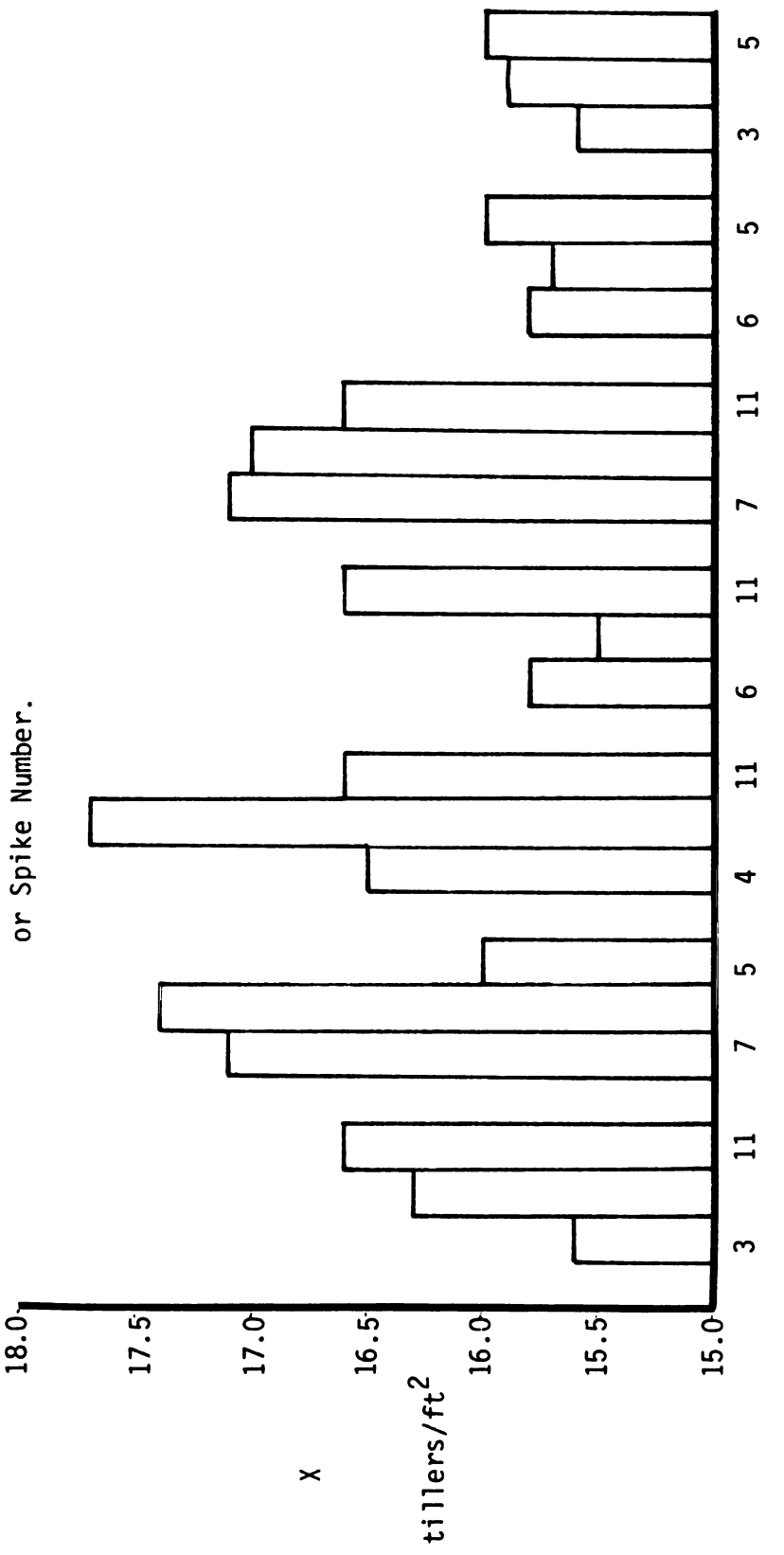
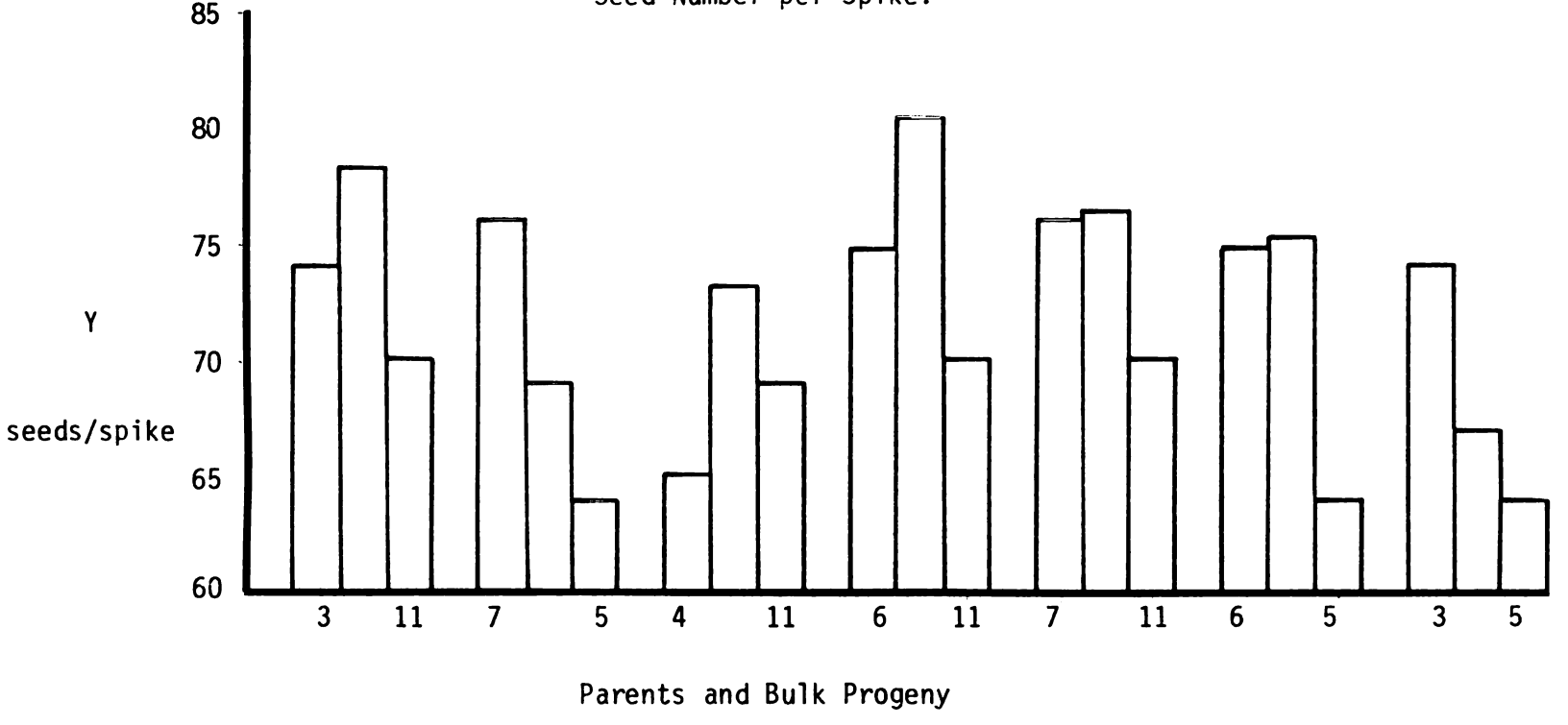


FIGURE 2b Parental and Bulk Progeny Performance for Tiller or Spike Number.

FIGURE 2c Parental and Bulk Progeny Performance for Seed Number per Spike.



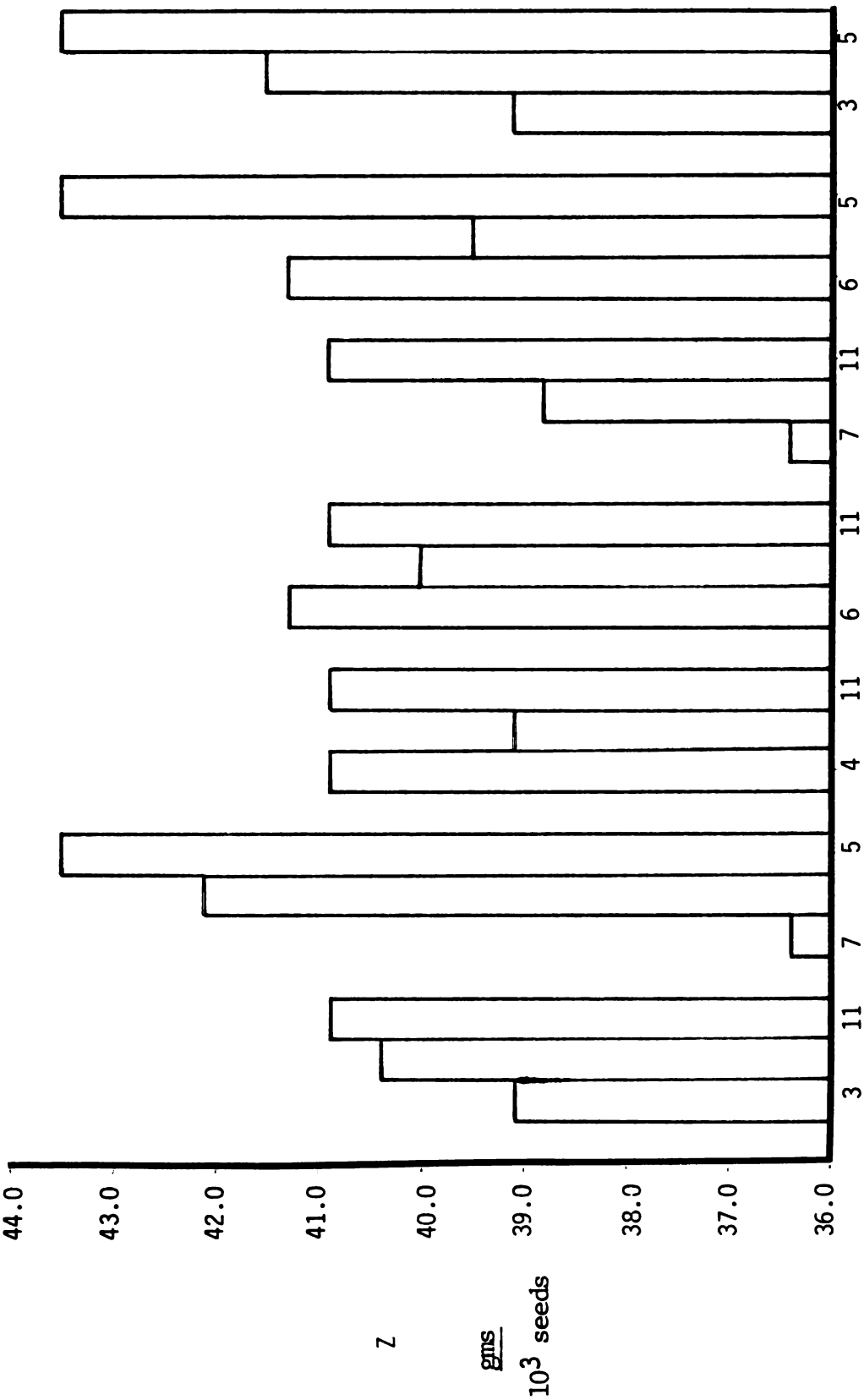
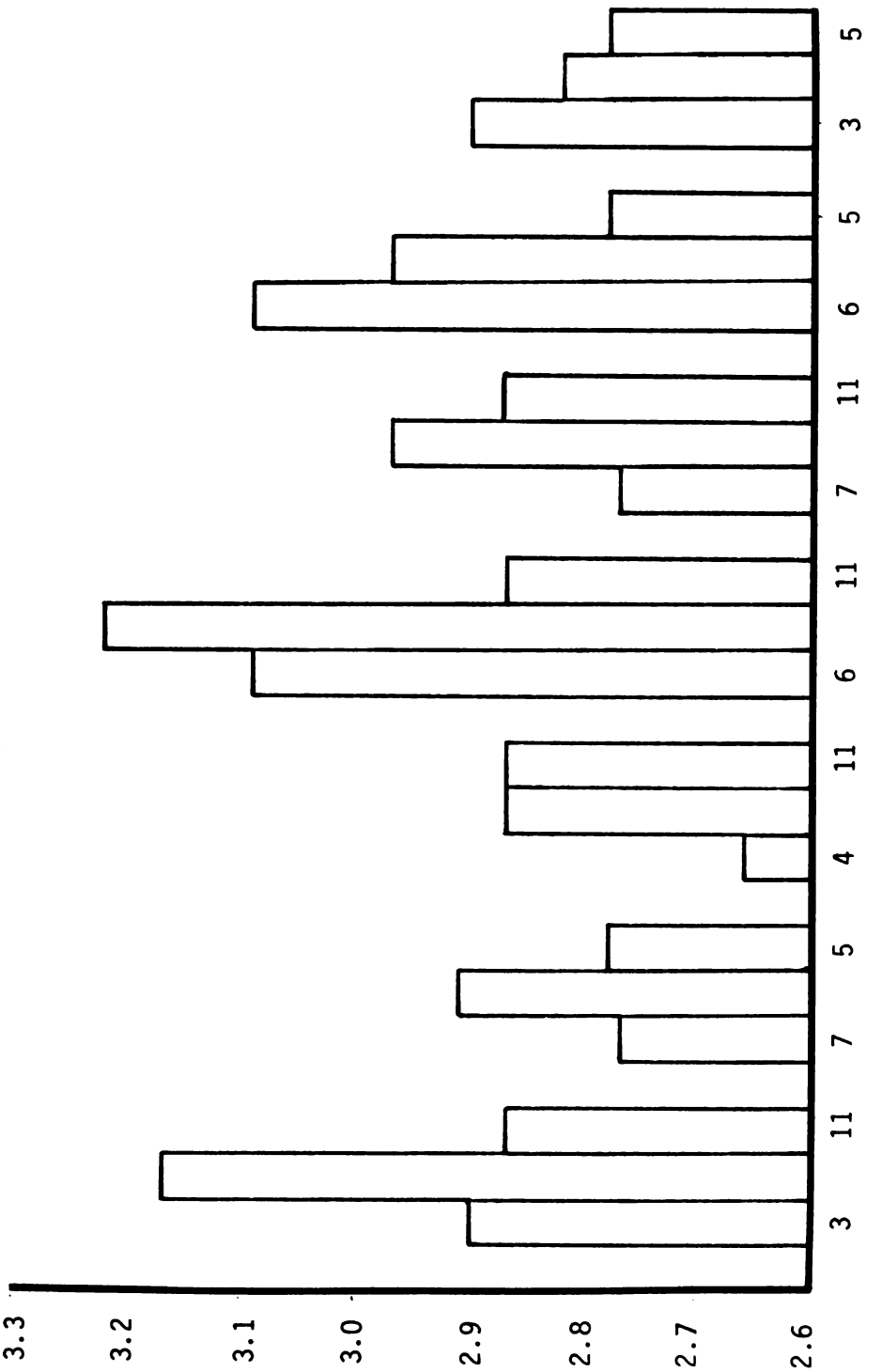


FIGURE 2d Parental and Bulk Progeny Performance for Seed Weight.

FIGURE 2e Parental and Bulk Progeny Performance for the Combined Trait, YZ.



superior to each of their parents with the exception of B79-104 which was superior to one parent and equal to the other. This is not surprising since the regression upon which the crosses were based was that of Z onto Y. Net superiority in the progeny with respect to both Y and Z should necessarily result, provided that the regression related to heritable traits with a genotype-environment interaction which was reasonably consistent among all six parents. In other words, the regressions of yield components upon one another should strongly reflect true genotypic, and not merely phenotypic, performance. The exclusion of the trait X is not without reason since its expression in the bulks with respect to the parents is seemingly random (Figure 2b), which should be a consequence of the manner in which the crosses were constructed. Malting barley requires a high percentage of plump seed to command a premium in the market. For that reason, Z was a yield component of major importance in the breeding goals of the Michigan barley project.

A causal analysis of Figures 2a through 2d and Figure 3 would suggest several points concerning the genetics of these crosses. Taken separately, the expression of the traits X, Y and Z are randomly distributed among the bulks with respect to their parents. Some are mid-parental, some are superior and some are inferior. Thus, additivity cannot be excluded as a mode of genetic action (16). Neither may epistasis (non-additivity) be excluded since five of the bulks out-yielded all parents. Only dominance and its interactions can safely be excluded from consideration since these bulks were approximately 96 percent homozygous. The author is reluctant to draw further conclusions for several reasons. This experiment was not constructed as a genetic study to extract the various components of variance and the application

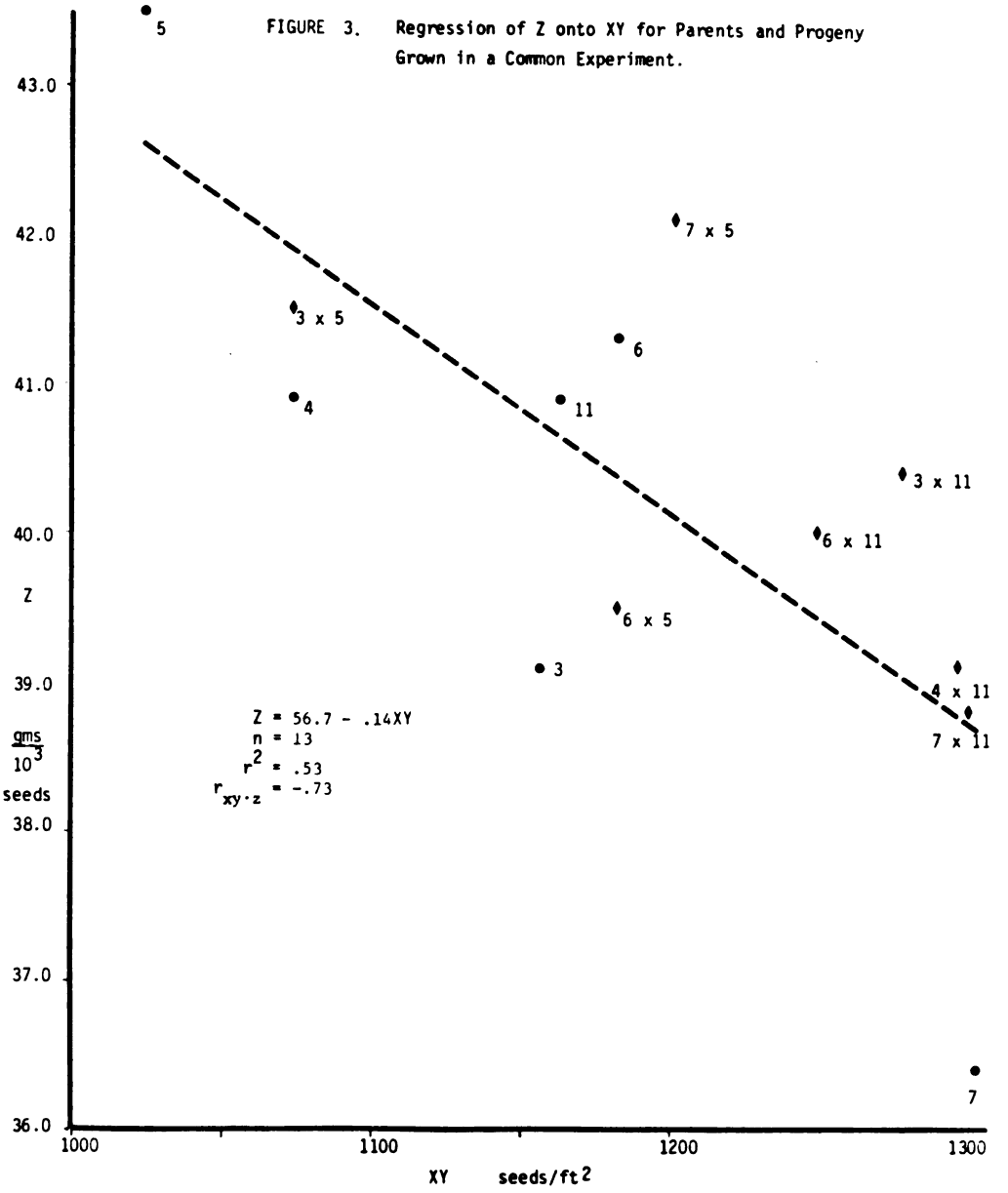


FIGURE 3. Regression of Z onto XY for Parents and Progeny Grown in a Common Experiment.

of the quantitative genetical lexicon to this study is largely inappropriate. With exactitude, one cannot even describe the yield performance of the five superior bulks as being transgressive since this term, as originally conceived, applies only to pure lines (17).

What might be concluded is that this system of identifying yield component outliers and the genetic variability they impart to crosses is the first potential technique to aid cereal breeders in parental selection where yield is the trait of greatest interest. It might also be proposed that when outliers are found they represent a second population, distinct from the first, or main, population, despite similarities in yield. The system might also be extended to other species as well since it offers a plausible method for predicting specific combining ability in corn and other hybrid crops. This experiment has been planted spring, 1981, to improve its resolution and to determine its repeatability.

Appendix1. Notation for Genotypes of Possible Parents, Parents and Bulk Progeny.

Notation	Genotypic Designation
1	Michigan 68-104-14
2	" 68-106-9
3	" 68-103-1
4	" 68-104-7
5	" 68-105-1*
6	" 68-105-10
7	" 68-105-15 (Bowers)
8	" 68-104-21
9	" 68-104-14
10	Larker
11	Minnesota M33 *
6 x 11	B79-101
7 x 11	B79-102
3 x 11	B79-103
4 x 11	B79-104
3 x 5	B79-105
6 x 5	B79-106
7 x 5	B79-107

*Outliers

APPENDIX 2a.

EXPERIMENT 8095 BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS X TILLERS/SQ FT

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	.88551526	2	.44275763	
ENTRIES				
UNADJUSTED	509.55205568	19	26.81852925	8.71326
ERROR				
RANDOMISED COMPLETE BLOCK	116.96010058	38	3.07789738	
TOTAL	627.39767153	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	2.8649
P= .01	LSD=	3.7244

COEFFICIENT OF VARIATION

10.4597 PER CENT

APPENDIX 2b.

EXPERIMENT BOOS BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS Y SEEDS/SPIKE

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	4.15404039	2	2.07702020	
ENTRIES				
UNADJUSTED	6395.19764319	19	336.58934964	8.83535
ERROR				
RANDOMISED COMPLETE BLOCK	1447.63903549	38	38.09576409	
TOTAL	7846.99071908	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	10.0791
P= .01	LSD=	13.1029

COEFFICIENT OF VARIATION

8.8174 PER CENT

APPENDIX 2c.

EXPERIMENT 8095 BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS Z SMS/SEED

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	.00000115	2	.0000005R	
ENTRIES				
UNADJUSTED	.00041153	19	.00002166	17.13130
ERROR				
RANDOMISED COMPLETE BLOCK	.00004804	38	.0000012E	
TOTAL	.00046073	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05 LSD= .0018
P= .01 LSD= .0024

COEFFICIENT OF VARIATION

2.7667 PER CENT

APPENDIX 2d.

EXPERIMENT 8085 BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS W GMS

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	2169.67333356	2	1084.81666678	
ENTRIES				
UNADJUSTED	151553.65000021	19	7976.50789475	2.09817
ERROR				
RANDOMISED COMPLETE BLOCK	144465.69999981	38	3801.72894736	
TOTAL	298188.98333359	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	100.6878
P= .01	LSD=	130.8936

COEFFICIENT OF VARIATION

8.3324 PER CENT

APPENDIX 2e.

EXPERIMENT 8095 BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS TW(LB/BU)

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	.08548832	2	.04274416	
ENTRIES				
UNADJUSTED	44.39924327	19	2.33680228	13.97091
ERROR				
RANDOMISED COMPLETE BLOCK	6.35595568	38	.16726199	
TOTAL	50.84068727	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	.6679
P= .01	LSD=	.8682

COEFFICIENT OF VARIATION

.8119 PER CENT

APPENDIX 2f.

EXPERIMENT BOBS BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS XY SEEDS/PLOT

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	868482.47402203	2	434241.21704102	
ENTRIES				
UNADJUSTED	20470713A.11193A4A	19	1077A059.90062833	4.66034
ERROR				
RANDOMISED COMPLETE BLOCK	87850722.23254395	38	2311861.1113A272	
TOTAL	293426342.77856445	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	2082.9343
P= .01	LSD=	3227.8146

COEFFICIENT OF VARIATION

8.3064 PER CENT

APPENDIX 2g.

EXPERIMENT 8095 BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS BU/ACRE

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	33.90052084	2	16.95026042	
ENTRIES				
UNADJUSTED	2368.02578125	19	124.63293586	2.09813
ERROR				
RANDOMISED COMPLETE BLOCK	2257.27656250	38	59.40201480	
TOTAL	4659.20286459	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	12.5859
P= .01	LSD=	16.3617

COEFFICIENT OF VARIATION

8.3324 PER CENT

APPENDIX 2h.

EXPERIMENT 2005 BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS 20 HD WT

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	3.51033334	2	1.75516667	
ENTRIES				
UNADJUSTED	1827.96850001	19	96.20886842	6.76145
ERROR				
RANDOMISED COMPLETE BLOCK	540.70299998	38	14.22902632	
TOTAL	2372.18183334	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	6.1599
P= .01	LSD=	8.0078

COEFFICIENT OF VARIATION

8.3587 PER CENT

APPENDIX 2i.

EXPERIMENT ROSS BULKS

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS HEIGHT (INCHES)

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	11.03333333	2	5.51666667	
ENTRIES				
UNADJUSTED	213.00000000	19	11.21052632	2.83433
ERROR				
RANDOMISED COMPLETE BLOCK	150.30000000	38	3.95526316	
TOTAL	374.33333333	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	3.2477
P= .01	LSD=	4.2220

COEFFICIENT OF VARIATION

4.9513 PER CENT

APPENDIX 2j.

EXPERIMENT POOR RULES

ANALYSIS OF VARIANCE TABLE
FOR RANDOMISED BLOCK DESIGN

DEPENDENT VARIABLE IS LODGING

SOURCE OF VARIANCE	SUM OF SQUARES	DEGS. OF FREEDOM	MEAN SQUARES	F STATISTIC
REPLICATIONS	490.00000000	2	245.00000000	
ENTRIES				
UNADJUSTED	12015.00000000	19	632.36842105	2.26494
ERROR				
RANDOMISED COMPLETE BLOCK	10610.00000000	38	279.21052632	
TOTAL	23115.00000000	59		

LEAST SIGNIFICANT DIFFERENCES (APPROXIMATE)

P= .05	LSD=	27.2867
P= .01	LSD=	35.4726

COEFFICIENT OF VARIATION

58.6302 PER CENT

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CHAPTER 5

CHEMICAL INDUCTION OF GENOME ELIMINATION IN SOMATIC TISSUE OF A WIDE HYBRID WITH BARLEY

Introduction

Barley (Hordeum vulgare) has, as its primary agronomic weakness, low winter survival in cold-stressed environments (1). Furthermore, the prospects for improving winter hardiness through the exploitation of the remaining intraspecific variability are exceedingly poor as stated by Grafius (1) and as judged from other reports in the literature (2,3,4,5,6). Winter hardiness is by no means the only polygenic character for which barley might be improved. Other goals include further improvement for salt tolerance and resistance to other stresses such as puddled soils. Mendelian traits such as disease resistance are also of interest.

The barley wide hybridization program at Michigan State University was initiated by the late Dr. John E. Grafius to find genetical solutions to these problems by incorporating alien genetic variability in the barley genome. The problem has been a particularly difficult one in view of the many man-years expended to the purpose at this institution. The author has been intimately associated with this effort for four years and he has experienced the many frustrations that have been the consequence. On the other hand, progress has been made, not so much in terms of the tangible improvement of barley, but more in terms

of finding solutions to the many barriers to successful genetic transfer from wild species.

The research herein reported is narrow in focus and deals exclusively with the wide hybrid between H. vulgare and H. jubatum. What will be reported are the results of the author's previously reported efforts to effect somatic recombination by three means (7). Additionally, this research will detail the use of three chemicals intended to induce genome or chromosome elimination in the wide hybrid with the intention of recovering haploid H. vulgare ($2n = x = 7$) which, upon the doubling of the chromosome complement would restore fertility and allow the introgression of wild germplasm (if present) into the cultivated population of barley. The necessity for this system has been previously reported by the author (7).

Literature Review

The value of barley (Hordeum vulgare) as a genetical research organism is not necessarily correlated with its agronomic value. Its diploid state ($2n = 2x = 14$) provides for easier genetic studies than those in wheat (Triticum aestivum) which is an allo-hexaploid ($2n - 6x = 42$). Wheat has distinct advantages over barley, however, and these advantages stem from its polyploid nature. There are three distinct genomes in wheat, each derived from a different species. A deficiency for an agronomic trait in one genome may be compensated by genetic expression for that trait in another genome. Harlan (12) has termed this "buffering" and it is not found in barley. Buffering has advantages other than genomic complementation for specific genetical traits: the existence

of multiple genomes is what allows the manipulation of one genome, yet insures adequate physiological response expressed by the undisturbed genomes.

Polyploidy in wheat and its physiological stability when disturbed is a valuable condition utilized by cytogeneticists and plant breeders. The greater economic importance of wheat has also fostered more intensive efforts toward its improvement. The gene transfer systems of Riley (8), Sharma (9), Knott (10) and Sears (11), et al., are achievements as yet not accomplished in barley. Barley suffers a paucity of closely related wild relatives which might serve as a convenient source for additional variability. Regions of extensive homoeology between the barley genome and those of other species are rare (12).

Despite these difficulties there have been several reports of successful introgressions of wild germplasm into barley. Hamilton et al. (13) and Schooler et al. (14) transferred disease resistance through wide crosses and Schooler (15) recovered male sterile lines by similar methods. These few reports represent the extent of successful gene transfers. No success has been reported for other traits such as insect resistance, resistance to other diseases, enhanced salt tolerance, or, most importantly, for improved winter hardiness.

Success in the author's wide hybridization program is contingent upon the chemical induction of haploid cells in somatic tissue of barley and this has been variously reported (16,17,18). It has also been achieved in tissue cultures of a medicago hybrid (20).

Materials and Methods

Chemical Induction of Chromosome Elimination

I. Chloramphenical (CAP)

Chloramphenicol is an antibacterial and antirickettsial drug derived from cultures of the soil bacterium Streptomyces benezuelae (16). Aside from its pharmacological properties, CAP is known to be a chlorophyl inhibitor and, in at least one case, it has been shown to induce chromosome elimination in barley root tips (17). Clones from the perennial hybrid Hordeum vulgare x H. jubatum (2n = 3x = 21), known as VJJ', were treated as follows:

		Chemicals and Concentration		
Date	Number of VJJ' Clones	CAP	DMSO	Duration of Treatment
4/9/80	12	0.3g/1	2%	2.0 hours
"	"	"	"	3.0 hours
"	"	"	"	4.5 hours

Healthy clones of VJJ' were subdivided into sections of 1.0-1.5 cm. in crown diameter. Fifty percent of the root tissue was removed and the clones were then placed in culture tubes into which the solutions were poured until the level was 2 cm. above the crown. The treatments were conducted in the greenhouse under supplemental florescent lighting and the clones were removed according to the time schedule listed above. The plants were thoroughly washed, trimmed of 50 percent of their leaf matter and potted in 4 inch pots with fresh soil. All pots were labeled and the plants were watered daily and fertilized once a week for the duration of the study.

II. Para-fluorophenylalanine (PFP)

P-fluorophenylalanine is an antibacterial agent (16) which has been shown to induce chromosome elimination in root tips of a Ribes hybrid (18) and in the wide hybrid Festuca pratensis x Lolium multiflorum (2n = 4x = 28) (7). Clones of VJJ' were treated as follows:

		Chemicals and Concentration		
Date	Number of VJJ' Clones	CAP	DMSO	Duration of Treatment
4/9/80	12	0.3g/1	2%	2.0 hours
"	"	"	"	3.0 hours
"	"	"	"	4.5 hours

The mode of treatment was identical to that of CAP.

III. Griseofulvin

Griseofulvin is an antifungal and antibacterial chemical derived from cultures of Penicillium griseofulvum (16). It has been shown to induce abnormal chromosome numbers in plant cell cultures (20). Use of this chemical is confounded by its virtual insolubility in water. On 5/9/80 a solution of 0.3g/1 griseofulvin plus 2 percent DMSO was made. A white flocculent persisted under stirring, so the solution was placed in a cold room (griseofulvin is unstable at room temperature) for three days of continual stirring. On 5/12/80 the solution was filtered to remove the still-considerable flocculent. The treatment was identical to that of CAP, except that 43 VJJ' clones were treated and the duration of the treatment was 24 hours, due to a low concentration of griseofulvin in solution.

IV. 2 Percent DMSO (Control)

Twenty-five clones of VJJ' were treated similarly to the CAP treatments with only 2 percent DMSO. The date of the treatment was 7/20/80 and the duration was 4.5 hours. All other untreated clones were also regarded as controls.

V. Solubility of Griseofulvin in a 2 Percent DMF Solution

A report in the literature by Nutti-Ronchi indicated that as much as .3 grams of griseofulvin is soluble in one liter H_2O (20). This report ran counter to the author's experience. This experiment was devised to help quantify the treatments.

Communication with Sigma Chemical Company of St. Louis, Missouri, informed the author that at least 50 mg. griseofulvin is soluble in 1 ml. dimenthylformamide. The author was informed that a precipitate is not at all unlikely when attempting to dissolve griseofulvin in H_2O. The solubility of griseofulvin in a 2 percent DMF solution was then undertaken.

Twenty ml. of DMF was placed in a clean, dry 50 ml. beaker. 0.3000 g of griseofulvin was added and magnetically stirred for 15 minutes. All the griseofulvin went into solution. This solution was added to 980 ml. of triple-distilled H_2O and magnetically stirred for ten minutes. A precipitate, white in color, formed over the period of ten minutes.

A piece of Whatman #1 filter paper was weighed and placed in a Buchner funnel attached to a vaccum apparatus. The solution was filtered through the filter paper; the paper was then removed and placed in an oven at 60^{o} C until dry. The paper was then reweighed. The

procedure was repeated three times and the results were as follows: Grisefulvin not in solution = .2022 g ± .0037 g. Therefore, approximately 90 + mg. of griseofulvin is soluble in a 2 percent DMF solution. However, it should be remarked that the filtered solution had a fine, white precipitate in it the following day after sitting for 24 hours at room temperature.

VI. Griseofulvin Treatment of Six Diploid Cultivars of Barley

Approximately three hundred seeds of each of the varieties--Bowers, Munn, Coho, Manker, Larker and Morex--were immersed in tap H_2O, supplemented with 5 percent sodium hypochlorite. The seeds were in 250 ml. Erlenmeyer flasks and were drained and rinsed three times after 24 hours. The sodium hypochlorite was added to suppress fungal diseases during seed treatment.

A saturated solution of griseofulvin (approximately .09g/liter in 2 percent DMF and 2 percent DMSO was filtered and added to the six flasks, 125 ml. per flask. The treatment time was 24 hours, after which the solutions were drained and the seeds were rinsed three times in tap water.

The flasks were removed to the greenhouse and remained there for another 24 hours to better identify those seeds which had germinated. The criterion was radicle emergence.

Each variety was planted in 20 pots, split in four groups of five pots each and these groups were randomized on the greenhouse benches. Bowers, Munn and Morex were planted three seeds to the pot and the other varieties were planted more densely, due to a reduced radicle emergence. The plants were watered daily and fertilized weekly until complete maturity.

VII. DMF Toxicity Study in Barley and a Wide Hybrid with Barley

Ten clones of VJJ' and twenty-five barley seedlings of approximately six inches in length were placed in a 2 percent solution of DMF for five hours in a greenhouse under florescent lighting. The clones and seedlings were immersed approximately one inch above their crowns and, when removed from the DMF solution, they were thoroughly washed, had 50 percent of their top growth removed and were then repotted. They were watered daily and fertilized once a week. There was no mortality resulting from the treatments and the twenty-five barley plants were thrown away at maturity.

Results and Discussion

Table 1 summarizes the results of the three treatments and the controls. The haploids arising after the griseofulvin treatments merited statistical analysis. Therefore a contingency test was conducted testing a null hypothesis that there were no differences between the pooled controls and the griseofulvin treated material with respect to the occurrence of haploid sectors. Table 2 shows the results of the analysis. All haploid lines were confirmed cytologically through root top squashes for $2n = x = 7$.

The extreme significance ($P << .001$) of the griseofulvin treatment renders superfluous a discussion of the CAP and PFP treatments. Griseofulvin has been variously researched in the past decade (20,21,22, 23,24,25,26,27,28) and its mode of action seems to be through inhibition of microtubule protein synthesis. The site or sites at which it operates are different from those of colchicine, although griseofulvin

Table 1. Frequency of Haploid Sectors Arising from VJJ' Clones Treated with CAP, PFP, Griseofulvin and DMSO.

Treatments	Number of Surviving Clones	Duration of Study	Number of Haploid Sectors	Date of Sectoring
CAP	35	5.5 mo.	1	9/80
PFP	36	5.5 mo.	0	----
Griseofulvin	38	4.5 mo.	4	7/80
			7	8/80
			3	9/80
		Total for Griseofulvin:	14	
Control I (2% DMSO)	25	3.0 mo.	1	9/80
Control II (All other untreated plants)	242	6.0 mo.	1	8/80
			5	9/80
		Total for both controls:	7	

Table 2. Contingency Analysis of Griseofulvin versus Controls for Haploid Sector Induction in the Hybrid VJJ'.

H_0: The incidence of haploid sectors was independent of griseofulvin treatment.

H_A: The incidence of haploid sectors was associated with griseofulvin treatment.

	Control	Griseofulvin	Total
Haploids	7	14	21
No haploids	260	24	284
Total	267	38	305

Chi-square (with Yate's correction for continuity) = 55.54

df = 1

Chi-square $_{.001,\ 1}$ = 10.83

Therefore, reject H_0 at the 0.001 level of P.

mimics colchicine to a certain extent through an early induction of polyploidy (20,28). This polyploidy of cultured plant cells is accompanied by aneuploidy and, eventually, a range of chromosome numbers, some of which are representative of haploidy. This latter phenomenon accounted for the use of griseofulvin in this study.

What is not accounted for in any of the literature which has come to the author's attention is a plausible, yet exact, explanation for the extreme delay in the griseofulvin effect. It was fully two months before it manifested itself in haploid induction and the phenomenon continued until October, 1980, when the experiment was terminated, some four and one-half months later. Furthermore, only haploid barley sectored from the vegetative tissue of the triploid hybrid. No aneuploidy was observed either cytologically or morphologically in the sectors. Since there is an innate predisposition of the hybrid to sector spontaneously (7,29,30)--although at a low frequency--it might be argued that the aneuploidy and polyploidy reported in the scant literature concerning the effects of griseofulvin on plant system (20, 28) does, in fact, occur in the hybrid VJJ'. The difference lies in the possibility that the region containing the crown meristem of the hybrid exerts a strong selection pressure upon karyotypically abnormal cells, such that only three conditions have a reasonable probability of surviving: haploid H. vulgare ($2n = x = 7$), haploid H. jubatum ($2n = 2x = 14$) and the normal hybrid tissue, VJJ' ($2n = 3x = 21$). This might account for the delayed effects of the drug.

The confidence engendered by the increased frequency of haploidy resulted in the treatment of many of those hybrids which were themselves treated earlier with mitomycin-c and gamma irradiation to accomplish

genetic transfer from the H. jubatum genomes to that of H. vulgare. These griseofulvin treatments were initiated in late July, 1980. The treatments were identical to those of CAP, PFP and the original griseofulvin treatments. In October haploid sectors were seen. For the first time since this hybrid was made, sectors bearing a pure H. jubatum morphology resulted. Four such plants arose, but contrary to expectation, they were reconstituted segmental allotetraploids (2n = 4x = 28). Therefore, in addition to a strict reduction in chromosome number (e.g., H. vulgare haploid sectors), the elimination of the H. vulgare genome was followed by a spontaneous doubling of the chromosome number in the H. jubatum sectors.

A large and controlled study of six diploid barley cultivars treated factorially with differing concentrations of griseofulvin with 2 percent DMF (dimethyl formamide) added had completely negative results with respect to haploid induction. The only noticeable effects were the partial induction of uniculm in the barley cultivar Bowers and a generalized low frequency of sterile florets. DMF was determined prior to the study to have no effect at the 2 percent concentration on any of the plants with which the author was working. DMF is the only solvent of griseofulvin which is not toxic (Nelson, unpublished). It was used to increase the concentration of griseofulvin in plant tissue. The solubility of griseofulvin in a 2 percent DMF solution was still only 0.2022 g/l (Nelson, unpublished). It might therefore be concluded that the concentrations at which griseofulvin is effective are extremely small, since DMF was not the solvent used in the original experiment. The results of this experiment would imply that some degree of genomic

instability is, therefore, necessary for griseofulvin to induce chromosome elimination in somatic tissue.

What is disconcerting is that of all the haploids produced by the author, either spontaneously or by induction, in the preceding four years, none was phenotypically distinguishable from another. The transfer of a polygenic trait was therefore unlikely. For those clones which were untreated for somatic recombination this is not so surprising since there is no homoeology between the respective genomes of H. vulgare and H. jubatum (31). This would render unlikely the probability of somatic pairing and consequent genetic exchange. However, the irradiated plants and the haploid plants which they produced should have evinced some morphological differences. The irradiations were at 500 R which was near the LD^{50} for clones of this hybrid.

Isozyme analysis is a possible recourse to assay heterogeneity in the haploid population. Limited material and severe time constraints have hampered this study, although there is some evidence (32) for differences between lines for malic dehydrogenase (MDH) and its associated five bands. In the absence of gross morphological differences between haploid lines the author is reluctant to ascribe isozyme heterogeneity in the haploids to genes found only in H. jubatum and not in H. vulgare. This is due to a potential source of bias incorporated in the VJJ' hybrid itself. It has been mistakenly reported (30) that Coho barley was the female parent of the hybrid. This is not so and the true parentage is reported by the creator of the hybrid, Dr. Robert Steidl (29). The original F_1VJJ' seed was produced on two spikes of a male sterile (nuclear) winter barley. The male sterile character can be maintained only in the heterozygous condition to produce seed over generations.

Consequently, there is considerable potential for heterogeneity through outcrossing in the H. vulgare genome residing in the hybrid VJJ' and, possibly, heterogeneity for MDH. Nevertheless, the author believes that the haploid stocks should be assayed again and with greater specificity for heterogeneity in isozymes with respect to each other, to the hybrid, and to the pure H. jubatum recovered through griseofulvin treatment. Additionally, the hybrid should undergo another cycle of irradiation as the investment is low and the possible benefits are great. H. jubatum is exceedingly winterhardy where H. vulgare, winter habit, is not. H. jubatum also expresses extreme salt tolerance and is vigorous in puddled soils.

The wide hybridization effort at Michigan State University has encountered obstacles which were beyond comprehension when the program was initiated. Years of hard work and a fair amount of ingenuity have allowed us to develop novel means of transferring genetic variability from wild to domestic barley. With another four years of concentrated effort utilizing the recombinant and haploid systems detailed herein, economically valuable improvements in barley will be recovered. The VJJ' hybrid is a perennial of immense value and it should not be allowed to disappear in consideration of its potential in barley improvement.

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