LEAF PUBESCENCE IN COMMON WHEAT, TRITICUM AESTIVUM L., AND RESISTANCE TO THE CEREAL LEAF BEETLE, OULEMA MELANOPUS (L.)

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ABSTRACT

LEAF PUBESCENCE IN COMMON WHEAT, *TRITICUM AESTIVUM* L., AND RESISTANCE TO THE CEREAL LEAF BEETLE, *OULEMA MELANOPUS* (L.)

by Kåre Ringlund

The plant materials used in these studies were derived from crosses between glabrous and pubescent wheat varieties. A technique for evaluating pubescence density on photomicrographs of cleared leaf samples was developed. Pubescence of individual plants from parental and segregating populations was studied and these same plants were tested for resistance to the cereal leaf beetle using a larval feeding test. Correlation coefficients between larval weight and pubescence density were highly significant with high pubescence density associated with resistance. Since no chemical antibiosis type of resistance has been demonstrated, resistance to the cereal leaf beetle in wheat probably is due to the physical or mechanical protection offered by pubescence.

Analysis of the pubescence data of F_1, F_2, and BC progenies from crosses between glabrous and pubescent varieties show

this character to be quantitatively inherited. The gene action estimated on the square root scale is mainly additive. Partition of variance shows only additive gene action, but a partial dominance for pubescence density was found in an analysis of population means. Heritability in F_2 was approximately 50%.

A study of the pubescence throughout the ontogeny of a wheat plant revealed that pubescence density is generally greater on the lower surface of the leaves, and the first 2-3 leaves have more pubescence. The first 2-3 leaves on a wheat plant also have longer hairs than leaves developed at a later stage, but since the cereal leaf beetle usually produces only one generation a year, with the heaviest infestation in early spring, this change in type of pubescence has little practical importance.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The cereal leaf beetle, Oulema melanopus (L.), has been a pest of small grains in parts of Europe and Asia for centuries. On the American continent, it was first found in Michigan in 1960. Here the insect population increased rapidly and severe damage was done to oats, barley and wheat.

Through a joint effort of the United States Department of Agriculture and Michigan State University a program was initiated to produce resistant varieties. Early screening of the world collection of wheat varieties (6) indicated that a relationship existed between leaf pubescence and resistance to the beetle. Furthermore, initial observations indicated pubescence density varies throughout the life of the plant.

The objectives of this study were: (a) to establish whether there is a definite relationship between pubescence density and cereal leaf beetle resistance, (b) to study the inheritance of cereal leaf resistance and leaf pubescence in wheat, and (c) to conduct a study of pubescence in relation to the ontogeny of the wheat plant.

REVIEW OF LITERATURE

The cereal leaf beetle is found throughout the humid and sub humid areas of the western Paleoarctic zone (3), but the beetle is scarce and only a sporadic pest in most of this region. In the Balkans, the Ukraine, and the Transcaucasian area of the Soviet Union the pest is more severe and more constant in appearance in the small grains. From the Galien area in southwestern Michigan, where the cereal leaf beetle was first discovered on the American continent, it has spread to the east, south, and north to cover most of southern Michigan, northern Indiana, and northern Ohio (21). Wilson (21) estimates that the pest will soon spread to Pennsylvania and New York, but the spread westward has been very slow. He points out, however, that once the pest reaches the southern point of Lake Michigan, the spread to the west will be much faster, as has been the case with other insect pests.

The life cycle and behavior of the cereal leaf beetle have been discussed by several workers. The spring adults emerge with the first warm days of late winter. Mating starts 2-3 days after emergence, and the first eggs are laid after 5-6 days (3). Depending on the temperature, the eggs need 4 to 7 days to hatch. Mok Yun (13)

found that eggs failed to hatch, and newly hatched larvae died when they were exposed to temperatures below 48 F or above 95 F. Optimum temperature was estimated to be 85 F. The time required from egg to adult was 3-4 times longer at 58 F than at 90 F. The cereal leaf beetle has four larval instars and the total duration of the larval stage is 8-11 days. The fourth instar larvae crawl down into the soil to pupate and it takes 11-14 days before the summer adults emerge (3).

In 1964, the summer adults began emerging from the soil in southwestern Michigan in mid-June and continued until the second week in July. Koval and Apple (9) studied the movements of the summer adults and found twelve times as many beetles in a farm woodlot as in the open field and along a wooded fence row in a grass field area. It was found that the beetles do move around also in the fall after they first have sought shelter. The summer adults do not usually mate until they have been through a diapause (3).

Ruppel (18) reports overwintering mortality of the beetle of 50-70%. Seventy to eighty percent of the eggs are fertile and the egg mortality is greatest in early spring due to cold and predation. Larval mortality is slight, but pupal mortality has been extremely high through desiccation and heat. Only 10-30% of the larval population give rise to new adults. Loss of new adults in late summer has been slight.

The cereal leaf beetle can be controlled by spraying and Ruppel et al. (17) found that discing to a depth of two inches with or without application of dieldrin gave over 80% control of the pupae in the soil.

The adult beetle feeds and oviposits on young growth of several wild grasses, oats, barley and wheat, whereas rye and corn are less preferred as hosts (3). No feeding was observed on broad-leaves even if the insects were restricted to such plants. Wilson (20) observed cereal leaf beetle feeding on more than 20 species of the Gramineae with the small grains, quackgrass, orchardgrass, timothy, foxtail and the fescues being the most commonly infested.

Wilson and Shade (22) found post-diapause adult beetles to gain weight rapidly on barley, oats, wheat and rye, and they noticed a slower weight gain on corn. The beetles did not gain appreciably on tall fescue, Sudangrass, or orchardgrass, and they lost weight when restricted to grain sorghum, giant foxtail or pearl millet. Wilson and Shade (23) also evaluated several species of the Gramineae as a host for cereal leaf beetle larvae. Spelt, barley, reed canarygrass, wheat, oats, and rye were found to be superior hosts. Orchardgrass, timothy, bromegrass, ryegrass, and quackgrass were favorable, tall fescue was intermediate and crabgrass, dent corn, grain sorghum, and a sorghum × Sudan hybrid were unfavorable hosts. The cereal

leaf beetle larvae did not survive on Sudangrass, green foxtail, yellow foxtail, giant foxtail, pearl millet, Japanese millet or wild cane.

Cereal leaf beetle damage to Monon wheat resulting in up to 23% yield loss is reported by Gallun et al. (7). The yield loss is brought about by a reduction in kernel number per head and in kernel weight.

Painter (14, 15) defines resistance as "the relative amount of heritable qualities possessed by a plant which influence the ultimate degree of damage done by the insect." Resistance is divided into three different types according to mechanism; namely preference, antibiosis, and tolerance. Beck (1) excludes tolerance from his definition of resistance.

Gallun et al. (6) studied resistance to the cereal leaf beetle in field planted wheat, oats, and barley. They found wheat to be the least preferred for oviposition and feeding, and they suggest that leaf pubescence may be a factor for resistance to the insect in wheat.

Schillinger (personal communication) found that the resistance mechanism in pubescent wheat is a combined effect of non-preference by the adult female for feeding and oviposition, and a physical or mechanical antibiosis effect on the first instar larvae.

Everson et al. (5) report on a screening of 14,444 lines of wheat for resistance to the cereal leaf beetle. In two years' tests

2.2% or 323 lines were found to have no or only a trace of feeding damage. Of these lines, 147 were from Russia, 103 lines were of Chinese origin and the major part of the rest were from southwestern Asia, Asia minor and southeastern Europe. The authors suggest that Asia minor is the main gene center for resistance to the cereal leaf beetle in wheat and point out Spain, Portugal, and Ethiopia as possible other centers of resistant germ plasm.

A close relationship between hairiness and resistance to jassid, Empoasea facialis, in cotton was found by Parnell et al. (16). Hairiness was evaluated by a photographic technique, and a numerical value was assigned to each plant according to hair length and density. It was found that high densities without adequate length of the hairs were ineffective in promoting resistance. Insect infestation and density of leaf hairs longer than 0.3 mm were highly correlated on a logarithmic scale. Hairs on the stem and the petiole were found to be of very little importance in preventing insect damage.

Esau (4) discusses the different types of epidermal cells in Gramineae, and Lawrence (10) gives a general classification of plant hairs. His classification, however, is aimed at distinguishing between different species, and is not very useful in describing differences between plant types of the same species.

Love and Craig (11) studied node pubescence and glume pubescence in wheat and found that both these characters are controlled by one gene.

MATERIALS AND METHODS

The plant materials used in these studies were derived from crosses between five pubescent Russian wheat varieties, CI 8286, 8287, 8487, 8514, and 9321, that showed strong resistance to the cereal leaf beetle, and four American varieties, Genesee, Avon, Talbot, and SW 1513. Individual F_1 plants from a diallel cross between the five Russian varieties, and F_1 plants from a set of crosses between these five varieties and the four American varieties were tested for cereal leaf beetle resistance together with the parents, using the larval test developed by Schillinger (19). F_2 populations derived from the crosses CI 8286 × CI 9321, CI 8286 × Genesee, and CI 9321 × Genesee together with parents and the four backcross populations CI 8286^2 × Genesee, CI 8286 × Genesee^2, CI 9321^2 × Genesee, and CI 9321 × Genesee^2 were tested with a modified larval test on an individual plant basis. Three early first instar larvae were put on each plant. Since only larvae 1-6 hours old were used, they were very uniform in size and the determination of initial weight could be eliminated.

The larvae were put on the plants at the second leaf stage. A sample for evaluation of pubescence was taken from the middle of

the first leaf just before starting the larval test. The leaf samples, approximately 1 cm long, were cleared in a solution of phenol and chloral hydrate in a 50-50 mixture (2). The samples were stored in 85% lactic acid and for evaluation of pubescence density they were mounted in lactic acid on a slide and covered with a coverslip. A picture was taken using 35 mm film under 80× magnification, thus covering a leaf area of 10.8 mm^2. The pictures were always taken adjacent to the midrib. The number of hairs per picture was counted on a strip print.

In a study of the change in pubescence density throughout the life history of the plant the three varieties CI 8286, 8287, and 9321 were used. The plants were grown in a growth chamber under constant light at 70 F. Samples were taken from the base, the middle, and the tip of each leaf at three different stages of development, and pubescence density determined as described above for both the upper and the lower surface. Seven different leaves were sampled. Once a leaf had been removed, the plant was discarded. This was done to eliminate any change in growth due to the sampling. For plants with four to six leaves, it was difficult to determine whether a leaf belonged to the main stem or to a secondary tiller. Usually a wheat plant has only five or six leaves and data collected for leaves four through six may be double sampling of the same leaf. The first three leaves and the flag leaf (leaf number seven) were easy to determine.

Statistical procedures

Data from the larval tests and the pubescence density determinations were punched on IBM cards, and means, variances, covariances, correlation and regression coefficients, and analyses of variance were computed on the M. S. U. computer.

The F_1 data from the diallel cross were analyzed using formulas for genetic and environmental variances developed by Grafius (8). He showed that if the frequency of AA in a set of homozygous parents is equal to u and the frequency of aa = v, the variance for F_1 progeny from different females and different males in a diallel cross is $uv(d + (v - u)h)^2$ where d is the effect of AA and h is the effect of Aa measured from the midparent. The sum of these two variances upon summation over $\underline{n}$ genes constitutes the additive portion of the F_2 genetic variance. The non-additive portion of the F_2 genetic variance is found from the F_1 data as the interaction between male and female parents.

A new set of formulas was developed for the case where two sets of parents with different gene frequencies for a character are crossed using one set as females and one set as male parents. By setting the gene frequencies for the female parents equal to u and v, and the gene frequencies for the male parents equal to u' and v', it can be shown that the variance for F_1 progeny from different

females is $uv(d + (v' - u')h)^2$, and the male variance is $u'v'(d + (v - u)h)^2$. The male × female interaction is $4\ uu'vv'h^2$. Upon summation over $\underline{n}$ genes the female plus the male variance constitute the additive portion of the F_2 variance, and the male × female interaction is the non-additive portion of the same F_2 variance.

Mather (12) stated that a scale adequate for analytical purposes should be such that the genes and non-heritable agents all are additive in action. However, a transformation that homogenizes the variance is not always one on which the gene action is additive.

In these studies the variance was correlated with the means both for total weight of larvae per plant and for pubescence density expressed as number of hairs per picture. In both cases a square root transformation made the variance independent of the means. No transformation was made for the data from the larval test of the F_1 plants. Percent gain as expressed by Schillinger (19) is a transformation of logarithmic nature.

Variances from the F_2, backcross, and parental populations were partitioned into additive, non-additive, and environmental variances according to Mather (12). Heritability was estimated both for pubescence density and resistance evaluated by the larval feeding test.

Population means for both these characters were analyzed by a one-way analysis of variance, and the population variance was

partitioned into linear, quadratic, and other effects. The population means $\overline{P}_1$, $\overline{BC}_1$, $\overline{F}_2$, $\overline{BC}_2$, and $\overline{P}_2$ have mean effects of -d, $\frac{1}{2}h - \frac{1}{2}d$, $\frac{1}{2}h$, $\frac{1}{2}h + \frac{1}{2}d$, and +d respectively. By numbering the populations 1 through 5 in the order the population means are written above, the linear effect from the analysis of variance is due to additive gene action and quadratic and other effects are due to non-additive components.

RESULTS AND DISCUSSION

The four American varieties, Genesee, Avon, Talbot, and SW 1513, and the five Russian varieties, CI 9321, 8286, 8287, 8487, and 8514, were evaluated both for pubescence density and for resistance by use of the larval test. The data from the larval test are presented as larval weight gain, percent survival and weight gain in percent of end weight (Table 1).

Table 1. -- Pubescence density and resistance to the cereal leaf beetle in four American and five Russian wheat varieties

	$\sqrt{\text{No. hairs per 10.8 mm}^2}$	Larval wt. gain in mg/72 hrs.	Percent survival	Percent gain
Genesee	1.0	1.65	83	61.9
Avon	0.5	1.80	92	60.2
Talbot	2.1	1.10	92	44.2
SW 1513	3.2	2.23	75	65.5
CI 9321	7.3	0.46	17	15.8
CI 8286	7.6	1.04	42	38.6
CI 8287	7.9	1.25	58	52.2
CI 8487	7.6	0.50	50	20.2
CI 8514	7.9	0.35	41	18.8

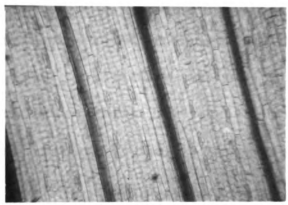
There were statistically significant differences between varieties for all four characters, and with one exception, these were due to differences between the American and Russian varieties. Only for pubescence density was there a significant difference between varieties within groups. This effect was mainly due to the two varieties SW 1513 and Talbot having more hairs than Genesee and Avon.

The F_1 plants from the two groups of parents were intermediate between the respective parents both with respect to larval feeding and pubescence density (Table 2). Figure 1 shows photomicrographs of leaf samples of two of the parents and their F_1 progeny.

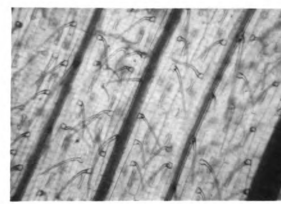
Table 2. -- Pubescence density per 10.8 mm^2 and larval feeding on four American and five Russian wheat varieties and their 20 F_1 progenies

	$\sqrt{\text{No. Hairs}}$	Larval wt. gain (72 hrs.)	Percent survival	Percent gain
Mean of 4 American var.	1.70	1.69	86	58.0
Mean of 20 F_1 progenies	5.69	1.38	77	50.7
Mean of 5 Russian var.	7.65	0.72	42	29.1
Mean of midparents	4.68	1.21	64	43.6

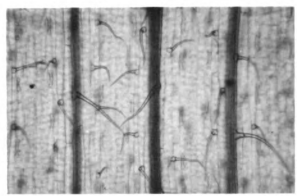
Reciprocal crosses were made for all twenty possible combinations between the two sets of parents. No reciprocal



Genesee, 1 hair



CI 9321, 54 hairs



F_1, 23 hairs

Figure 1. -- Photomicrographs of leaf samples from Genesee, CI 9321, and their F_1 progeny showing pubescence density in a 10.8 mm^2 area, 350X

differences were found, and hence the reciprocals were pooled and for simplicity the four American varieties will be referred to as female parents and the Russian varieties as the male.

A factorial analysis of the data for the F_1 progenies showed significant differences between progenies from different female parents for the two characters survival and pubescence density (Table 3).

Table 3. -- Mean survival and pubescence density on F_1 progenies of four female parents with low pubescence and five pubescent Russian lines, CI 9321, 8286, 8287, 8487 and 8514

Female parent	Percent survival of 5 F_1 progenies	$\sqrt{\text{No. hairs/10.8 mm}^2 \text{ area}}$ of 5 F_1 progenies
Genesee	83	5.24
Avon	78	5.48
Talbot	85	5.70
SW 1513	62	6.33

Progenies from SW 1513 had the highest pubescence density, and the cereal leaf beetle larvae had a lower survival on progenies from this variety than on progenies from the other three varieties.

An analysis of variance of the number of hairs (on a square root scale) and the expected components of the mean squares of the above F_1 progenies are shown in Table 4.

Table 4. -- Analysis of variance for square root of number of hairs on F_1 progenies of the four American varieties (female) and five Russian varieties (male), and the expected components of the mean squares

Source of variance	Degrees of freedom	Mean square	Expected composition in mean squares
Replication	3	0.782*	$\sigma^2 + 20\sigma^2_r$
Female parents	3	4.370**	$\sigma^2 + 20\sigma^2_f + 4\sigma^2_{mxf}$
Male parents	4	0.434	$\sigma^2 + 16\sigma^2_m + 4\sigma^2_{mxf}$
F × M	12	0.199	$\sigma^2 + 4\sigma^2_{mxf}$
Error	57	0.238	σ^2
Total	79	0.420	$\sigma^2 + \sigma^2_r + \sigma^2_f + \sigma^2_m + \sigma^2_{mxf}$

Estimates of the components, their relation to the formulas for genetic variances presented earlier, and an estimate of heritability for pubescence density are presented in Table 5.

The gene frequencies u, v, u' and v' refer to the frequencies of those genes that are different in the two sets of parents. If the five male parents have the same genes for pubescence density, u' = 1, v' = 0, and σ^2_m must be zero regardless of the size of the other parameters. The significant σ^2_f showed that some genes for pubescence exist among the female parents used in this set of crosses.

Table 5. -- Components of variance and heritability estimated from F_1 data for pubescence density expressed as number of hairs on a square root scale

Component	Corresponding formulae	Estimate
σ^2_f	$uv\sum(d + (v'-u')h)^2$	0.2066
σ^2_m	$u'v'\sum(d + (v-u)h)^2$	0.0122
σ^2_{mxf}	$4uu'vv'\sum h^2$	0
h^2 = heritability = .2188/.420 =		52%

The cereal leaf beetle larvae that were restricted to resistant parental and F_1 plants both had a lower survival rate and lower weight gain per larvae (Tables 1 and 2). In the modified larval test used for the F_2 and backcross populations, differences in initial larval weights were eliminated. The data from these larval tests are presented as total weight of all surviving larvae and include both differences in larval gain and differences in survival.

The relationship between total weight and pubescence density expressed as number of hairs is shown in Figure 2, a and c. The correlation coefficient between the two characters for 413 plants of Genesee, CI 9321, and their F_2 and backcross populations (Figure 2a) was -.620 which is highly significant. When both total weight and number of hairs were transformed to square roots of the original observations, the simple correlation coefficient was -.652 (Figure 2b).

Figure 2. -- Relationship between pubescence density and larval feeding presented on the original scale, a and c, and on a square root scale, b and d. Data presented in a and b were collected on Genesee, CI 9321 and the F_2 and backcross populations derived from these two varieties. c and d represent Genesee, CI 8286 and their F_2 and backcross populations. Some points represent more than one observation but all observations were used to calculate the regression lines.

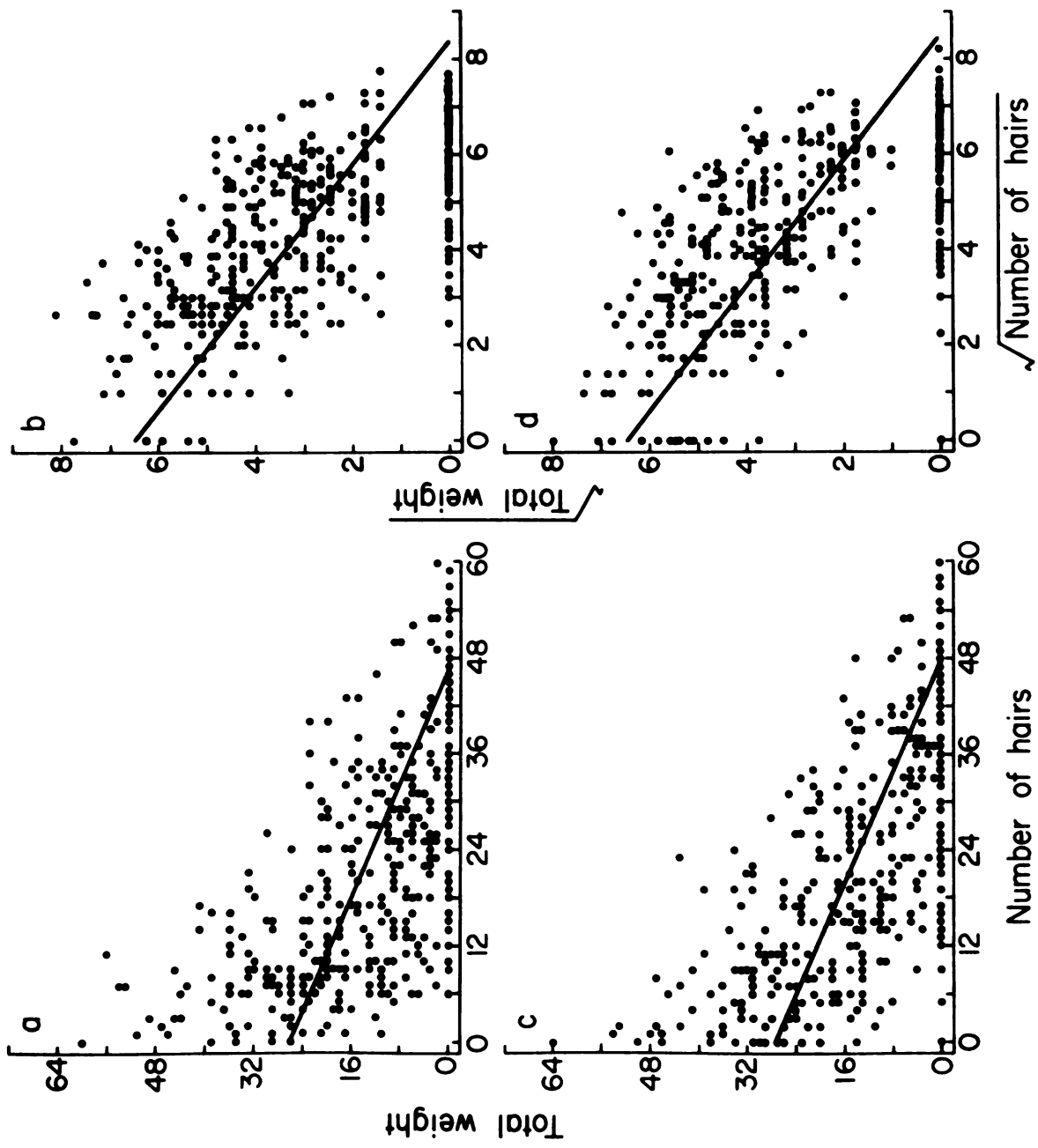

a
b
c
d
Total weight
√Total weight
Number of hairs
√Number of hairs
0
12
24
36
48
60
0
16
32
48
64
0
2
4
6
8

Figure 2

The correlation coefficients for 378 plants of Genesee, CI 8286, and their F_2 and backcross populations were -.671 and -.670 for untransformed and transformed data respectively (Figure 2c and d).

In order to show the effect of heritable and non heritable differences in larval feeding and pubescence density simple correlation coefficients between these two characters were computed for the parents, F_2 and backcrosses in each of the two crosses discussed above. These simple correlation coefficients are presented in Table 6 for each of the populations, and the plotted diagrams for segregating populations are presented in Figures 3 and 4.

Table 6. -- Simple correlation coefficients between larval feeding and pubescence density in square root transformations

Population	r	Population	r
Genesee	-.036	Genesee	-.054
BC_1	-.297**	BC_1	-.434**
F_2	-.529**	F_2	-.423**
BC_2	-.206	BC_2	-.297*
CI 9321	-.112	CI 8286	-.348*

Figure 3. -- Relationship between pubescence density and larval feeding on a square root scale. Some points represent more than one observation, but the regression lines are computed from all observations.

a. BC_1 $Genesee^2 \times$ CI 9321

b. BC_1 $Genesee^2 \times$ CI 8286

c. F_2 Genesee $\times$ CI 9321

d. F_2 Genesee $\times$ CI 8286

Figure 3

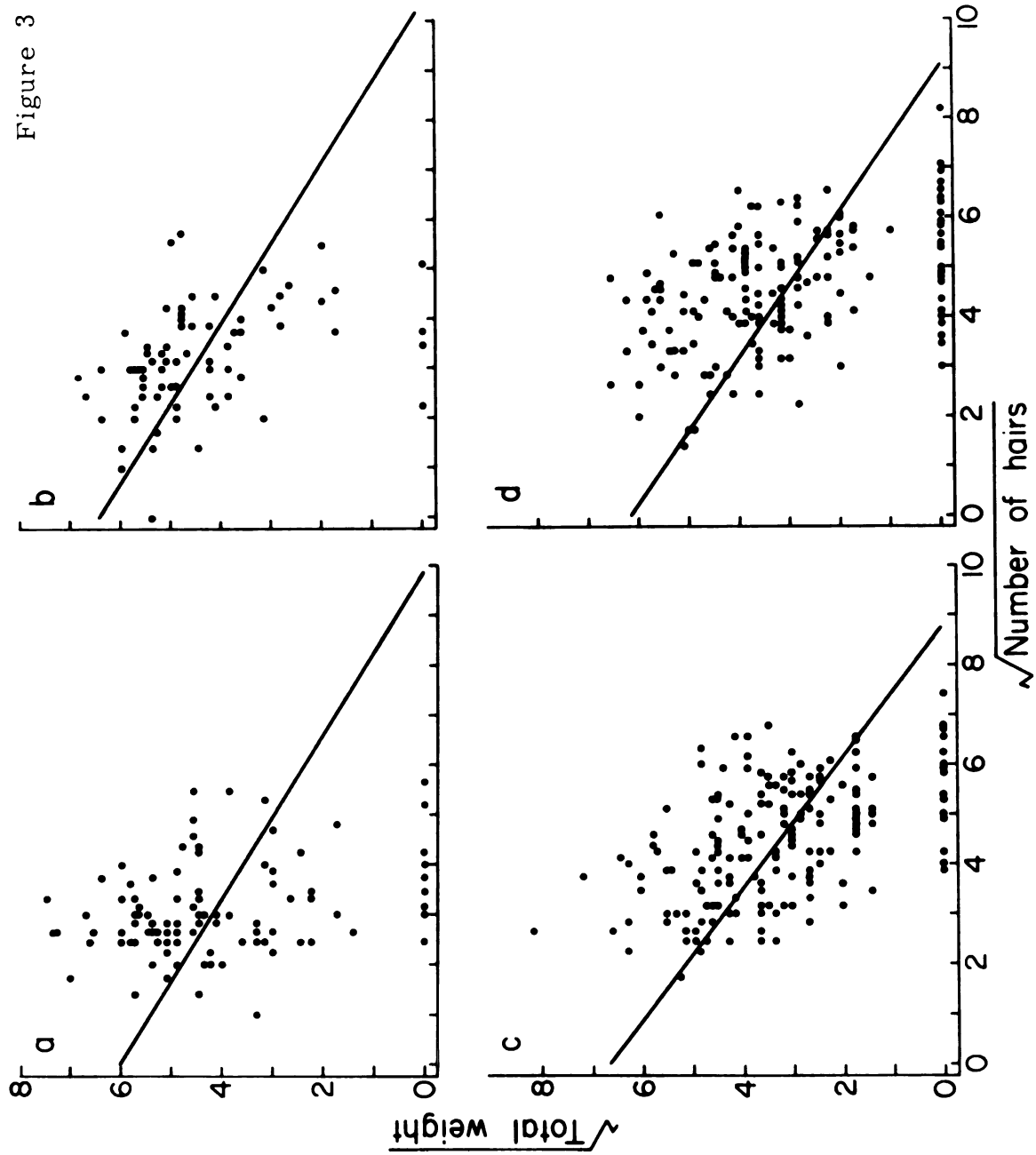


Figure 4. -- Relationship between pubescence density and larval feeding on a square root scale. Some points represent more than one observation, but the regression lines are computed from all the observations.

a. BC_2 Genesee × CI 9321[2]

b. BC_2 Genesee × CI 8286[2]

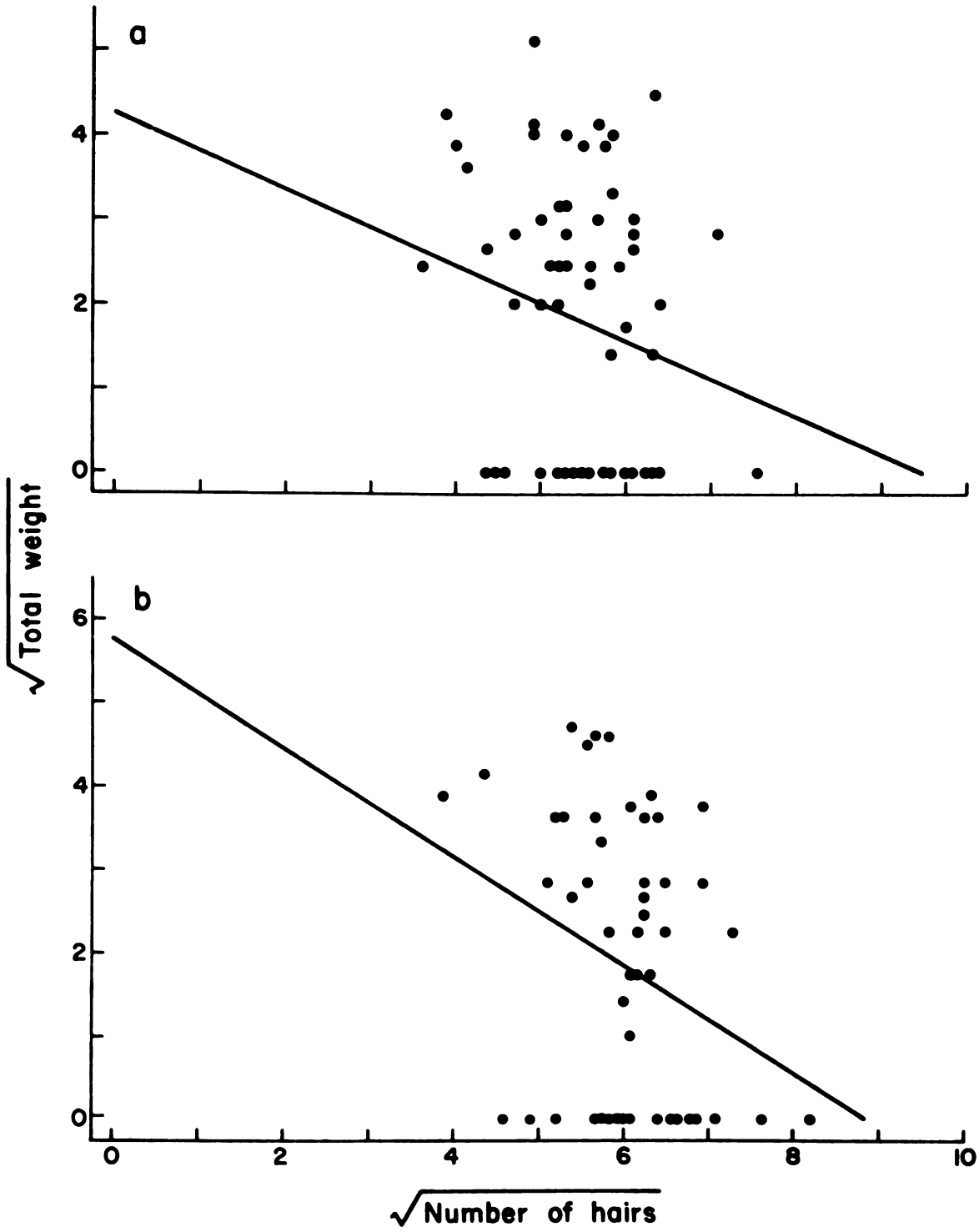


Figure 4

Genesee has a very low pubescence density, and for this variety there is no correlation between the two characters. A highly significant correlation between larval feeding and pubescence density exists for both the backcross populations to Genesee and the F_2 populations. The correlation coefficient for the backcross populations to the resistant parents is significant at the 5% level for one set of data and non-significant for the other. It can be seen from Figure 4 that all plants in these populations have from medium to high pubescence density and even the least pubescent plants have a fairly high degree of resistance. The random variations in the pubescence determinations and in the larval test, therefore, tend to cover up the correlation between the two characters for these populations.

The significant correlation for the variety CI 8286 indicates that non-heritable variation in pubescence density can play a role in resistance to the cereal leaf beetle.

The question that at once arises is whether the correlation between pubescence density and larval feeding is due to the mechanical effects of pubescence alone or to chemical antibiosis controlled by genes closely linked with one or more of the genes for pubescence. A study of Figures 2 and 3 shows that there are no pubescent plants which are susceptible. However, it should be noted that in these same figures there are some plants with very little pubescence that show resistance on the larval feeding test. Caution

should be exercised in the interpretation of these data since the young larvae are very delicate and readily damaged during the transfer from the testing chamber to the test pots. Therefore, the absence of highly pubescent susceptible plants is strong evidence that resistance is due to the mechanical effects of pubescence alone and not to other linked factors.

Tables 7 through 10 show frequency distribution for F_2, backcross, and parental populations from Genesee × CI 9321 and Genesee × CI 8286. It is evident from these tables that pubescence density and resistance expressed as larval weights are quantitatively inherited. Data are presented on total weight and on pubescence density both on the original scale and in the square root transformation. For both characters there was a skewness towards low values on the original scale, and the square root transformation therefore made the distributions more normal. However, zero values were not changed and for total weight there was a noticeable deviation from normality even on the transformed scale.

The gene frequencies (u and v) in an unselected population from a cross between two homozygous parents are .5 and the genetic variance in F_2 is $\frac{1}{2}\sum d^2 + \frac{1}{4}\sum h^2$ or $\frac{1}{2}D + \frac{1}{4}H$ (12), where D is the additive and H the non-additive genetic variance after summation over $\underline{n}$ genes. These components and the F_2 heritability of pubescence and

Table 7. -- Frequency distributions of total weight of surviving larvae for F_2, backcross, and parental populations from the cross Genesee × CI 9321

	Total weight in .1 mg																							Total
Upper class limit			4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68					
Lower class limit			0	5	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65					
Genesee					1		1	4	3	6	3	3	1	3	1		1							27
BC_1			13	6	12	4	15	12	8	9	12		3	1	1	3								99
F_2			44	27	27	24	21	18	5	7	4	3	2		1				1					184
BC_2			32	14	6	7	4		1															64
CI 9321			32	3	3			1																39

	Square root of total weight																							Total
Upper class limit	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0	6.4	6.8	7.2	7.6	8.0	8.4			
Lower class limit	0	.5	.9	1.3	1.7	2.1	2.5	2.9	3.3	3.7	4.1	4.5	4.9	5.3	5.7	6.1	6.5	6.9	7.3	7.7	8.1			
Genesee									1		1	1	6	6	3	3	3	2		1				27
BC_1	10			1	2	5	1	8	5	3	6	15	12	9	14	1	3	1	3					99
F_2	21			6	17	10	17	16	25	10	11	23	9	7	5	4	1	1			1			184
BC_2	25			2	5	8	6	5	2	6	3	1	1											64
CI 9321	27			3	2	1	2	1	2			1												39

Table 8. -- Frequency distributions of pubescence density for F_2, backcross, and parental populations from the cross Genesee × CI 9321

	Number of hairs per 10.8 mm^2 leaf surface																				Total
Upper class limit			4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64			
Lower class limit			0	5	9	13	17	21	25	29	33	37	41	45	49	53	57	61			
Genesee			27																		27
BC_1			10	41	24	10	5	4	2	3											99
F_2			1	19	24	23	23	20	22	17	18	7	5	4		1					184
BC_2						3	5	6	17	8	10	11	2		1		1				64
CI 9321										4	2	3	5	3	12	6	4				39
	Square root of number of hairs																				
Upper class limit	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0	6.4	6.8	7.2	7.6	8.0	Total
Lower class limit	0	.5	.9	1.3	1.7	2.1	2.5	2.9	3.3	3.7	4.1	4.5	4.9	5.3	5.7	6.1	6.5	6.9	7.3	7.7	
Genesee	10		10	3	4																27
BC_1			1	2	7	19	22	14	12	8	5	3	2	3	1						99
F_2					1	9	10	17	13	17	20	18	23	19	20	7	9		1		184
BC_2									1	2	3	5	13	13	12	13		1	1		64
CI 9321														2	4	5	4	14	7	3	39

Table 9. -- Frequency distributions of total weight of surviving larvae for F_2, backcross, and parental populations from the cross Genesee × CI 8286

	Total weight in .1 mg																					Total
Upper class limit			4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64				
Lower class limit			0	5	9	13	17	21	25	29	33	37	41	45	49	53	57	61				
Genesee			1		1	4	4	3	4	4	4	4	2	4	1	2		1				39
BC_1			8	3	3	7	8	12	12	11	9		2	2								77
F_2			48	22	17	31	13	12	8	8	7	2	2									170
BC_2			26	12	1	9	2	3														53
CI 8286			35	3		1																39
	Square root of total weight																					
Upper class limit	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0	6.4	6.8	7.2	7.6	8.0		Total
Lower class limit	0	.5	.9	1.3	1.7	2.1	2.5	2.9	3.3	3.7	4.1	4.5	4.9	5.3	5.7	6.1	6.5	6.9	7.3	7.7		
Genesee	1								2	3	1	6	4	4	4	4	4	3	2	1		39
BC_1	4				4		3	3	3	4	7	9	13	13	10	2	1	1				77
F_2	34		1	1	12	10	12	14	16	18	8	12	9	10	8	2	2					170
BC_2	21		1	1	3	5	7		6	4	1	3										53
CI 8286	26		1	1	7	2	1			1												39

Table 10. -- Frequency distributions of pubescence density for F_2, backcross, and parental populations from the cross Genesee × CI 8286

	Number of hairs per 1.8 mm^2 leaf surface																					Total
Upper class limit			4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68			
Lower class limit			0	5	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65			
Genesee			33	6																		39
BC_1			10	21	20	11	8	2	2	2	1											77
F_2			4	10	18	21	24	24	19	20	13	8	5	2	1				1			170
BC_2						1	1	2	4	7	10	12	8	4	1	1	1		1			53
CI 8286							1			2		7	6	8	8	4	3					39

	Square root of number of hairs																					Total
Upper class limit	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0	6.4	6.8	7.2	7.6	8.0	8.4	
Lower class limit	0	.5	.9	1.3	1.7	2.1	2.5	2.9	3.3	3.7	4.1	4.5	4.9	5.3	5.7	6.1	6.5	6.9	7.3	7.7	8.1	
Genesee	16		4	8	5	6																39
BC_1	1		1	3	5	12	9	13	7	11	5	5	2	2	1							77
F_2				1	3	4	6	8	13	18	20	23	20	19	18	10	4	2			1	170
BC_2										1	1	1	4	5	13	15	6	4	2		1	53
CI 8286												1		1	1	8	8	13	6	1		39

resistance estimated by the larval feeding test were estimated from population variances shown in Tables 11 and 12.

Table 11. -- Means and variances of larval feeding and pubescence density for populations derived from crosses between Genesee and CI 9321

Population	$\sqrt{\text{No. hairs/10.8 mm}^2}$		$\sqrt{\text{Total weight}}$	
	Mean	Variance	Mean	Variance
Genesee	0.85	0.48	5.55	0.90
BC_1	3.08	0.85	4.15	3.57
F_2	4.50	1.44	3.24	2.89
BC_2	5.44	0.55	1.82	2.62
CI 9321	6.80	0.40	0.78	1.72
D		1.52		0
H		0		7.16
Heritability		53%		0

The non-additive component of the variance for resistance, expressed as square root of total weight, in Table 11 disagreed with the other estimates of gene action in this study. The high value for H was caused by an extremely high variance for the first backcross population. The deviation from normality in the frequency distribution for

this character, which was especially noticeable for this set of data (Table 7), strongly biased the estimates obtained from variances.

Table 12. -- Means and variances of larval feeding and pubescence density for populations derived from crosses between Genesee and CI 8286

Population	$\sqrt{\text{No. of hairs/10.8 mm}^2}$		$\sqrt{\text{Total weight}}$	
	Mean	Variance	Mean	Variance
Genesee	0.97	0.79	5.41	2.13
BC_1	3.17	1.14	4.47	2.37
F_2	4.60	1.37	3.04	3.57
BC_2	6.03	0.59	1.84	2.86
CI 8286	6.71	0.40	0.67	1.06
D		1.30		1.91
H		0		0.06
Heritability		48%		54%

The estimated genetic variance for square root of total weight in the set of data presented in Table 12 and for pubescence density in both sets of data contained only an additive component. The heritabilities for number of hairs and total weight on the square root scale were approximately 50%.

Analyses of variance for the population means are presented in Tables 13 and 14. The populations were numbered one through five in the order of Genesee, BC_1, F_2, BC_2, and CI 8286 or 9321. The effect of population on the means was then broken down into linear effect, quadratic effect, and other effects.

Table 13. -- Analyses of variance for population means of larval feeding and pubescence density for populations derived from crosses between Genesee and CI 9321

Source of variance	$\sqrt{\text{Total wt.}}$		$\sqrt{\text{No. hairs}}$	
	D. F.	M. S.	D. F.	M. S.
Between populations	4	167.08**	4	240.81**
Linear	1	662.48**	1	927.65**
Quadratic	1	0.12	1	25.65**
Other	2	2.86	2	4.96**
Within populations	422	2.82	422	0.98

Table 14. -- Analyses of variance for population means of larval feeding and pubescence density for populations derived from crosses between Genesee and CI 8286

Source of variance	$\sqrt{\text{Total wt.}}$		$\sqrt{\text{No. hairs}}$	
	D. F.	M. S.	D. F.	M. S.
Between populations	4	166.18**	4	232.60**
Linear	1	661.88**	1	894.30**
Quadratic	1	0.02	1	33.17**
Other	2	1.42	2	1.47
Within populations	373	2.82	373	1.06

For the square root of total weight the analyses of the means showed only additive gene action. The analyses for pubescence density in the square root transformation also showed a significant non-additive gene action. The deviation from linearity, however, even if it is statistically significant, has only a minor effect on the magnitude of the means (Figure 5).

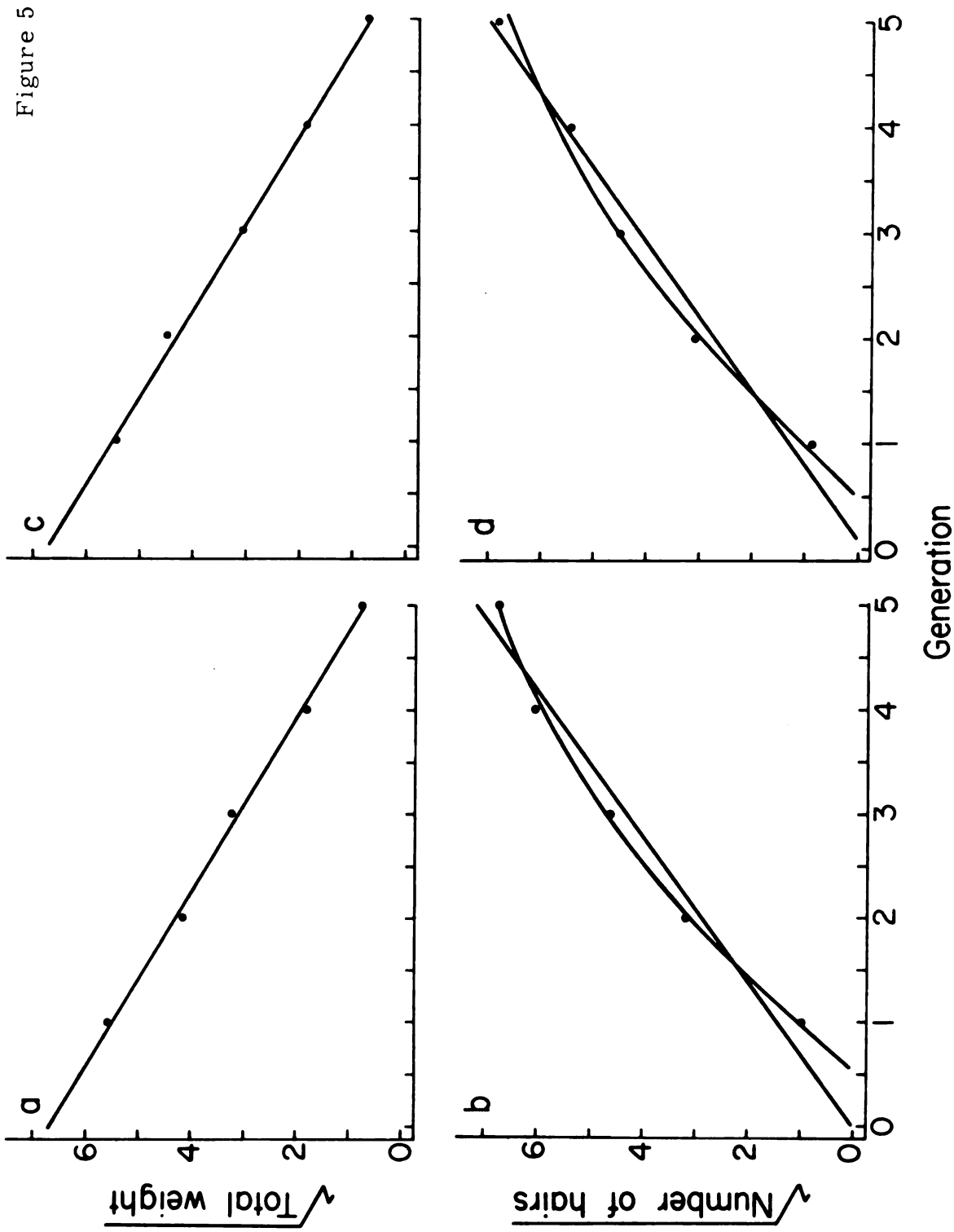
Non-additivity can be due to intra and inter allelic interaction. Epistatic gene action interferes with estimates from partition of variances whereas dominance does not. Mather's (12) scaling test for additiveness allows for dominance but not for epistasis, and hence can be used to distinguish between the two types of non-additive gene action. One possible test for additiveness is to set C equal to $4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2$. This C-value has an expected mean of zero and an expected variance, V_C, of $16V_{\overline{F}_2} + 4V_{\overline{F}_1} + V_{\overline{P}_1} + V_{\overline{P}_2}$. C-values and their standard errors calculated on pubescence density for the two sets of populations derived from Genesee × CI 9321 and Genesee × 8286 were found to approach zero being $-.61 \pm .626$ and $.02 \pm .632$ respectively. The non-additive gene action estimated from the population means was, therefore, due to partial dominance. Theoretically, this should not interfere with the method of variance partitioning and in this study the analysis of population means was more accurate in estimating gene action than was partition of variance. Since it is believed that pubescence is the sole factor for resistance, pubescence

Figure 5. -- Population means for pubescence density, b and d, and larval feeding, a and c, on a square root scale for parental, F_2, and backcross populations from Genesee × CI 8286 (a and b) and Genesee × CI 9321 (c and d)

Generation

1 P_1, Genesee
2 BC_1
3 F_2
4 BC_2
5 P_2, CI 8286 or CI 9321

Figure 5



density and larval weight are two independent measurements of the same gene system. The difference in gene action obtained from the analyses of means for the two characters, therefore, is due to scaling.

Analysis of F_1 and F_2 data from a diallel cross involving five pubescent Russian varieties showed no differences between parents or progenies. These varieties must, therefore, have the same genes for pubescence density and resistance.

It remains to be tested whether pubescent varieties from different countries also have the same genes for this character. Differences were found, however, between progenies from different susceptible varieties (Table 3). This means that one or more of the susceptible varieties has a gene or genes for pubescence.

Whenever it is feasible, adapted varieties with genes for pubescence should be used in the initial crosses in a program to produce cereal leaf beetle resistant varieties. Effective resistance, however, has to be incorporated into American varieties from exotic sources. Effective selection in early generations is, therefore, essential for rapid progress in breeding resistant varieties. The photographic method developed in this study for evaluating pubescence density provides the plant breeder with a simple method for selection of resistant plants in a segregating population at the seedling stage.

Developmental study

A total of 378 leaf samples were analyzed for pubescence in a complete 5-factorial experiment involving three varieties, seven leaves ranging from the older basal leaves to the flag leaf, three stages of leaf development, and three sites on each of the two leaf surfaces.

The data on pubescence distribution on leaves are summarized in Tables 15 and 16.

Statistically significant differences in pubescence density were found between varieties, leaf number (leaves being numbered in order of initiation), and surface. The following two-factor interactions were significant: variety × leaf number, leaf number × site (site being the location on individual leaf), variety × surface, and leaf number × surface.

Table 15. -- Number of hairs per 10.8 mm^2 leaf area on leaf one through seven on three wheat varieties. Each statistic is the average of 18 observations, three each on upper and lower leaf surfaces at three different stages of leaf development

Variety	Leaf number							
	1 basal	2	3	4	5	6	7 flag	Mean
CI 9321	59	66	48	32	31	32	42	44
CI 8286	61	64	48	36	37	35	35	45
CI 8287	60	60	58	40	33	40	58	50
Mean	60	63	51	36	34	36	45	46

CI 8287 had a higher mean density than the other two varieties, and the main effect of leaf number was that the first and the second leaf had highest pubescence density, leaf numbers 3 and 7 were intermediate and leaf numbers 4 through 6 had the lowest pubescence density. The interaction between varieties and leaf number was due to CI 8287 being more pubescent on leaf numbers 3 and 7 whereas approximately the same densities were found on all three varieties for the other leaves.

The decrease in pubescence from the basal leaf to the flag leaf is not expected to create a problem for the cereal leaf beetle resistance in a field planted to one resistant variety since the female beetle oviposits few eggs on pubescent leaves of the seedling plant. Only a few larvae hatching from these eggs survive so there will be a very small population of summer adults. Migration of summer adults from other fields is not likely to cause severe problems as the summer adults tend to migrate toward woodlots and areas where they can hibernate (9).

This change in leaf pubescence from the seedling stage to the mature plant is, however, important for the plant breeder if resistant and susceptible materials are interplanted. In this case there will be enough summer adults produced on the susceptible (glabrous) plants to attack the resistant plants in the least pubescent adult stage. Evaluation of plants for resistance to the cereal leaf beetle should,

therefore, be done at the seedling stage. The significant interaction for pubescence density between leaf number and location on the leaf (site) is shown in Table 16.

Table 16. -- Number of hairs per 10.8 mm^2 leaf area at the base, the middle and the tip of the leaf for leaf numbers one through seven. Each statistic is the average of 18 observations, three varieties on two leaf surfaces at three different stages of leaf development.

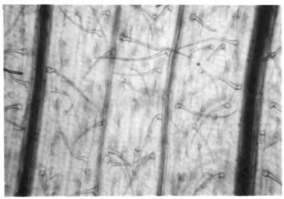
Site	Leaf number							
	1	2	3	4	5	6	7	Mean
Base	62	58	43	31	32	43	64	48
Middle	60	64	51	36	34	35	52	47
Tip	58	69	59	42	35	29	19	45

There is no main effect of site, and the interaction is due to a decrease in pubescence density from the base to the tip for leaf number 1, 6 and 7, and an increase in pubescence towards the tip for leaf numbers 2, 3, 4 and 5.

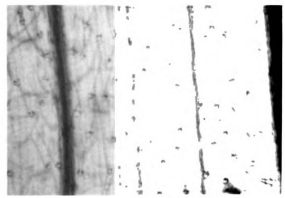
For the study of the relationship between pubescence and resistance and for the genetic study of pubescence density, the evaluation was always done on the first leaf and the sample was taken at the middle site. The difference in pubescence density between leaves and the significant interaction between site and leaf number show the importance of this sampling technique.

On the average, there were more hairs on the lower than on the upper surface; on the flagleaf, however, this was reversed. Since the cereal leaf beetle usually feeds on the upper leaf surface, these differences in pubescence are of minor importance for the resistance.

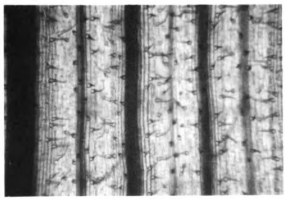
There were noticeable differences in the type of pubescence both between different leaves and between the upper and lower surface (Figure 6). The young leaves had hairs on the lower surface which were straight and usually pointed in the same direction whereas hairs on the upper surface were bent and did not have a simple pattern of orientation. The hairs on the flag leaves were shorter, and for CI 9321 and 8286 there were no hairs on the lower surface at the base of the flagleaf. These changes in type of pubescence are probably more important for the resistance to the cereal leaf beetle than are the changes in pubescence density from the basal leaf to the flagleaf.



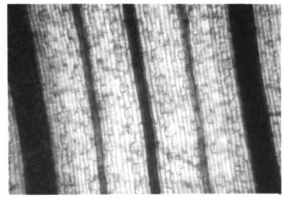
Upper surface
of basal leaf



Lower surface
of basal leaf



Upper surface
of flagleaf



Lower surface
of flagleaf

Figure 6. -- Differences in type of pubescence within a plant, 350×

SUMMARY

A technique for evaluating pubescence density on photomicrographs of cleared leaf samples was developed. Pubescence of individual F_1 and segregating F_2 plants was studied and these plants tested for resistance to the cereal leaf beetle using a larval feeding test. Correlation coefficients between larval weight gain and pubescence density were highly significant with high pubescence density associated with resistance. Since no chemical antibiosis type of resistance has been demonstrated, one can conclude that resistance to the cereal leaf beetle in wheat is actually due to the mechanical or physical protection offered by pubescence.

The original data for both larval weight and pubescence density were transformed to square roots to make variances independent of the means. The square root transformation also makes the frequency distributions for segregating populations more normal.

Analyses of the pubescence data of F_1 and F_2 progenies from crosses between glabrous and pubescent varieties showed this character was quantitatively inherited. The gene action is mainly additive on the transformed scale and heritability in F_2 was estimated

to be approximately 50%. Analyses of population means show partial dominance for high pubescence density on a square root scale.

Pubescence density is generally higher on the lower surface of the leaves, and the first 2-3 leaves have more pubescence than the others. The first 2-3 leaves on a wheat plant also have longer hairs than leaves developed at a later stage, but since the cereal leaf beetle usually produces only one generation per year with the heaviest infestation in early spring, this change in type of pubescence has little practical importance for the resistance to the beetle.

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