REACTION OF PEDIGREE SELECTIONS, INBRED LINES, AND HYBRIDS TO THE BLACK-STEM DISEASE OF ALFALFA, AND THE INFLUENCE OF ENVIRONMENTAL FACTORS ON VARIABILITY OF INFECTION

by

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B. S., University of Wisconsin, 1943

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Agronomy

and

Department of Botany and Plant Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1943

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INTRODUCTION

Black-stem of alfalfa, caused by the fungus Ascochyta imperfecta Peck, is an important disease in Kansas. Considerable damage is caused by severe defoliation prior to removal of the first cutting of hay. Since much alfalfa seed is shipped to eastern states where black-stem is an important disease throughout the growing season, it is desirable for Kansas to produce disease resistant varieties if it is to serve this seed market more adequately. It was for these reasons that the workers of the Kansas Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry of the United States Department of Agriculture recommended that an intensive study be made of this disease. The study was begun by Peterson (6) in 1938-40, followed by Goodding (1) in 1940-41, and continued in the present study.

The ultimate goal of this research was the production of varieties of alfalfa resistant to black-stem. In order to accomplish this objective a knowledge of the reaction of selections, inbred lines, and hybrids to this disease had to be known. It was found while testing this reaction that there was much variability in the relative infection from one period to another. Therefore, the cause of this variability was sought in connection with the inheritance study. It was for this reason that the effect of environmental conditions on infection by black-stem was studied.

Black-stem attacks all the above ground parts of the alfalfa plant. Characteristic symptoms of the disease are the formation of small dark spots on the leaves and stems. In later stages these spots become sooty brown to black and enlarge, many of them coalescing. This results in severe defoliation, injury to many leaves that remain attached and severe blackening of the stems. The most severe damage occurs during cool moist weather. Field symptoms differ from those in the greenhouse in that stems are more severely attacked outdoors, while the leaves show more symptoms under artificial inoculation. Damage from black-stem has been reported in all alfalfa growing regions of the United States.

A review of the literature was not made as Peterson (6) and Goodding (1) have reviewed all available literature. The only previously unreported paper was the publication of part of Peterson's thesis by Peterson and Melchers (7).

MATERIAL AND METHODS

Excellent facilities were available for work on this problem. Greenhouse space, field plots, and equipment were furnished by the Department of Agronomy for studies on the reaction of alfalfa strains to black-stem. Laboratory space was furnished by the Department of Botany and Plant Pathology for increase of all cultures of the fungus.

Sources of Cultures of *Ascochyta imperfecta*

Eight strains of the fungus *Ascochyta imperfecta* were available. Strains 1, 2, and 3 were pure cultures isolated by Dr. D. B. Creager at Manhattan, Kansas. Strains 4 and 5 were isolated by Dr. F. R. Jones at Madison, Wisconsin, and strains 6, 7, and 8 were isolated from diseased alfalfa plants at the Kansas State College Agronomy Farm by Peterson (6) and Goodding (1). Transfers of these cultures were made at regular intervals to keep the fungus viable.

Securing Inoculum

A mixture of these eight strains was used for all inoculations except in one experiment where the age of the culture was varied to study its effect on the severity of infection. Separate pure cultures of each strain were grown in test tubes on potato-dextrose agar. Transfers were made aseptically to sterile sweet clover stems in test tubes. After growing the cultures on sweet clover stems for 10 days at room temperature a large number of dark colored pycnidia developed. These cultures were then ready for use as inoculum. One test tube of each strain was filled with sterile water and the sweet clover stems were scraped with a sterile needle causing the pycnida to discharge their pycnidiospores into the water. These stems were allowed to soak for three hours prior to inoculation. The test tubes were shaken thoroughly, and the solution was

strained with cheesecloth as it was poured into a 1000 cc flask. Distilled water was added bringing the solution up to 500 cc volume.

Inoculation Chamber

The alfalfa plants selected for inoculation were picked at random when the plants were eight to 12 inches tall. They were placed in a canvas covered chamber with a gable shaped roof. Water was run slowly down the roof and sides to keep the relative humidity in the chamber constantly at approximately 100 percent. The temperature varied from 60° to 80° F. except in the spring when it reached 90° to 95° F. occasionally.

Method of Inoculation

The inoculum was applied to each plant as a fine mist. Since a sufficient amount of moisture was applied by the mist, the plants were not sprayed with water before inoculation as was done by Goodding (1). When inoculating the plants the operator used one hand to hold and manipulated the plant and the other to manipulate the atomizer. Pressure for the spraying was obtained from an electric air compressor pump which operated automatically, starting at 15 pounds pressure and stopping at 25 pounds. This technic gave uniform distribution of inoculum. Plate I shows the humidity chamber, the electric air compressor pump and the technic of inoculating the plants.

The incubation period used was 72 hours which Goodding (1) found to be the optimum period. After three days the

EXPLANATION OF PLATE I

The humidity chamber, electric air compressor pump, and technic of inoculating potted alfalfa plants with spores of *Ascochyta imperfecta* are shown in Plate I.

Plate I

plants were removed from the humidity chamber and placed in the greenhouse. Care was taken to provide them with plenty of water following their removal from the humidity chamber so as to prevent excessive desiccation of the leaves.

Use of Check Plants

Four Ladak cuttings were used in each inoculation chamber as check plants to correct for the difference in degrees of infection from one inoculation to another. This Ladak clone was about medium in resistance to black-stem. These check plants were distributed at random throughout the chamber together with the other plants. Goodding (1) devised the plan of using check plants when he inserted two cuttings in each inoculation chamber. Four plants were used in the present work instead of two so as to obtain a more reliable average check score.

Plant Readings

Individual plant readings were made 10 days after inoculation. The readings were made by the same method used by Goodding (1). The number of leaf lesions and percentage of defoliation were scored on a 0-10 basis with 0 representing little or no disease symptoms and 10 very severe disease symptoms. The variation in size of leaf lesions, petiole lesions, and stem lesions was not very great. Thus they were scored from 0-5. The latter three readings and the former two were multiplied by 20 and 10, respectively, to put them on a basis of 100. The five figures then averaged to secure a plant score.

The four check plants in each inoculation chamber were scored similarly and averaged to secure an average check score. Each plant score was then divided by the average score of the checks to obtain a plant's rating or index number of infection. This figure was used in comparing the reaction of different plants. As the average score of the four check plants was used in securing a plant's index number the check was always considered as 100. A plant that had an index number below 100 was more resistant than the check, and a plant scoring above 100 was more susceptible. The method of calculating plant scores and index numbers is illustrated below. Thus a plant may be scored as follows:

Number of leaf lesions	6	x	10	= 60
Percentage of defoliation	7	x	10	= 70
Size of leaf lesions	4	x	20	= 80
Petiole lesions	3	x	20	= 60
Stem lesions	1	x	20	= 20
				290

290/5 = 58 the average plant score

If the average of the four check plants was 48, the index number of infection of this plant would be 121 as 58/48 = 120.8.

Statistics Used

Analysis of variance, correlation and regression were used in the statistical treatments. They were calculated as outlined by Snedecor (9). A correlation between the means and standard deviations of all the lines was calculated to see if analysis of variance could be used on these data. If the correlation was not significant an analysis of variance was a

good statistical method to apply. The correlation was 0.017 for 92 degrees of freedom, and was not significant as 0.205 was needed for significance at the five percent level. Thus it was justified to use analysis of variance.

Classes of resistance form an intergrading series, hence it was impossible to assign individual plants to a definite class. No plants were found to be immune from the disease nor were any killed by the disease. Plant indices tended to be distributed in a normal curve. A histogram of the distribution of plant indices is shown in Plate II. The class centers shown on the base line were obtained by recording the frequencies of all the plant indices in intervals of 10, that is, from 20 to 29, 30 to 39, 40 to 49, etc., and determining the class center of this interval. Since there are 10 indices per interval, five was taken as the class center. This is a slight error, but it is not important since class center values were not used in any calculations.

EXPERIMENTAL RESULTS

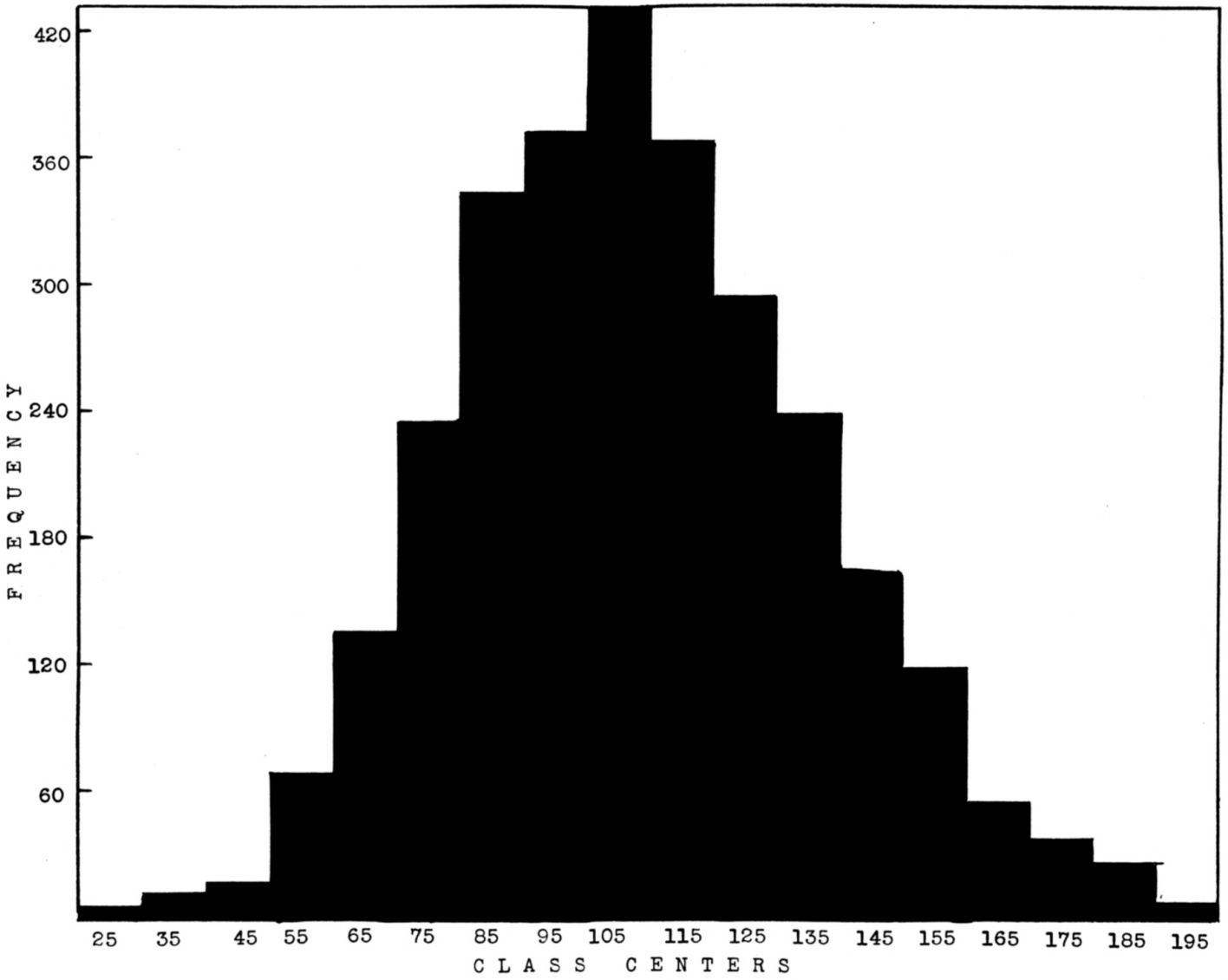
Effect of Age of Culture on Black-Stem Infection

A controlled experiment was set up to determine if the age of the culture used had any effect on the amount of disease produced under artificial inoculation. Strain 3 of Ascochyta imperfecta was the only culture used in this experiment. The age of the culture was determined from the data of the transfer of mycelium and spores from potato dextrose agar to sterile

EXPLANATION OF PLATE II

Plate II is a histogram of the plant indices of all of the plants tested. The plant indices were divided into intervals of 10, and each class is indicated by the class center or mid-point of each class.

PLATE II


420
360
300
240
180
120
60
FREQUENCY
25 35 45 55 65 75 85 95 105 115 125 135 145 155 165 175 185 195
CLASS CENTERS

sweet clover stems until the date of inoculation. Transfers were made every five days for two months. Thus cultures of 12 different ages were available. Only four were used as the number of clonal plants to be inoculated was limited. The ages of culture selected were 20, 35, 50, and 60 days. A preliminary experiment showed that spores were produced within 10 days following the transfer. It was the general practice to use cultures from 10 to 30 days of age in the regular inoculation work. Therefore, the four ages were selected over a wider range of age than was normally used.

Four strains of alfalfa besides a Ladak check were used in this experiment. Strain B was an open-pollinated Kansas Common plant. Strain F was an open-pollinated Ladak plant. Strain C4 was a one generation inbred Kansas Common plant, and Mc was a plant of unknown origin, but had the characteristics of Turkestan. Eight cuttings of each strain were available, thus making it possible to have two plants from each clone for each age of culture. The plants were selected at random for each treatment, inoculated and placed in the inoculation chamber. When the plants were removed from the chamber each plant was assigned a number at random, the original was replaced by the numbered stake. A key copy was made as the random numbers were assigned, and this copy was filed away until all readings had been made. This tended to eliminate bias as neither treatment nor strain was known as each plant was read.

Table 1 shows the plant indices of the strains of alfalfa

Table 1. The effect of the age of culture on the plant indices of four strains of alfalfa.

Variety	Strains	Age of culture								Average
		20 days		35 days		50 days		60 days		
Kansas Common	B	98	78	98	94	92	94	96	80	91
Ladak	F	88	98	110	108	76	96	64	104	93
Kansas Common	C4	108	92	130	96	132	94	124	144	115
Turkistan	Mc	92	90	118	132	118	124	84	132	111
Average		93		111		103		104		

for the four ages of culture. The means of each strain and each treatment are given.

Table 2 shows the analysis of variance on the effect of the age of culture on the degree of infection. The difference due to the age of culture was not significant. The four strains of alfalfa differed significantly as the F value of 4.99 for the estimated variability between strains was significant at the five percent level. The interaction of strains by the age of culture was not significant. These results indicated that the ages of culture used had no effect on the amount of disease produced. The strains reacted similarly to each age of culture as the interaction of strains by ages was not significant. The variance (mean square) for the interaction was about equal to the variance for within a treatment combination. Thus it was possible to pool these variance as both of them are estimates of the (same) variation of strains and ages, but obtained in different ways. By so doing a more reliable estimate of the true variation was secured and the number of degrees of freedom for this estimate of variance was increased. Table 2 also shows the analysis of variance on the effect of the age of the culture on the degree of infection when the variances for interaction and within a treatment combination are pooled as the error term. In this case, pooling did not change the analysis. The variation due to strains nearly reached the one percent level as the F value was 4.63, and 4.68 is necessary to be highly significant. Practically, it would be possible to say that the probability of a variation this large would not be

Table 2. Analysis of variance on the effect of the age of culture on the degree of infection.

Source of variation	Degrees of freedom	Sums of squares	Mean square	F value		Levels of significance			
						5% points		1% points	
						1/	2/	1/	2/
Between strains	3	3,597	1,199	4.63*	4.99*	2.99	3.24	4.68	5.29
Between ages	3	1,279	426	1.64	1.78	2.99	3.24		
Error	25	6,472	259						
Interaction (S x A)	9	2,159	240						
Within variation	16	4,313	270		1.12		2.98		
Total	31	11,348							

* Significant
1/ With 25 degrees of freedom
2/ With 16 degrees of freedom

due to chance more than one time out of 100. In all of the following analyses only one analysis will be shown. If the variances due to interaction and within a treatment combination are about equal they will be pooled and used as the error term. The minimum significant difference between the strain means is 16.5 for the five percent level, and 22.3 for the one percent level. The strain means are shown in the last column of Table 1. Strains B and F are significantly more resistant than strains C4 and Mc.

Effect of Age of Top Growth on Black-Stem Infection

A second controlled experiment was set up to determine the effect of the age of the top growth on the amount of disease produced. Six clonal lines were used in this experiment. The strains were L check, an open-pollinated Ladak plant; B, an open-pollinated Kansas Common plant which was also used in the age of culture experiment; B4 and B5, sister one generation inbreds from Kansas Common plant B, and C3 and C5, sister one generation inbreds from a Kansas Common plant. The number of plants per clone varied from two to 14. These plants were selected and placed in a group on March 18. Three weeks later half of the plants of each clone were selected at random, and the tops were removed. The plants were about 16 to 20 inches tall, and blooming at that time. After two weeks when the growth on the plants that were clipped back was about eight to 10 inches high both groups were inoculated. On removal from

the inoculation chamber random numbers were assigned each plant, a key was made of the treatment and strain, and the label stakes replaced by the random numbered stakes, thereby eliminating personal bias from readings made one week later.

Table 3 shows the plant indices of the seven strains for the two treatments for all plants in the age of tops experiment. The strain means of each treatment, for both treatment and the treatment means are given also.

Readings were made as usual, and the average of the 12 Ladak checks was used in figuring the plant indices. From the analysis of variance in Table 4 the age of the top growth had no effect on the plant score in this experiment as the F value was not significant. Likewise, the strains were found to be non-significant, and no significant difference was found in the interaction of strains by ages of top growth. However, as the variance of the interaction was much higher than the "within variation" they were not pooled. Thus the treatments had no effect on the degree of infection. The fact that strain differences did not reach the five percent level of significance probably was due to the low numbers in the different clones, the close pedigree relationship of some of these clones and the interaction of strains by ages though not significant. Clones B4 and B5 were sister inbreds from clone B, and clones C3 and C5 were sister inbreds from another Kansas Common plant. As these six strains were quite closely related it was possible to place them into three groups, group 1 containing the

Table 3. The effect of the age of top growth on the plant indices of six strains of alfalfa.

Strain	Plant indices											
	L check		B4		B5		B		C3		C5	
Treatment	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
	89	100	96	107	107	137	111	59	111	118	126	100
	111	96	111	90			85	82	93	89	118	104
	98	104	98	83					102	100		
	100	85	78	70					115	100		
	89	117	117	111					106	120		
	106	111	98	109					115	130		
			82	67					122	130		
Treatment mean	99	102	97	91	107	137	98	70	109	112	122	102
Strain mean	100		94		122		84		111		112	

Grand mean of new growth 103
Grand mean of old growth 101

Table 4. Analysis of variance on the effect of the age of the top growth on the degree of infection.

Sources of variation	Degrees of freedom	Sums of squares	Mean square	F value	Level of significance 5%
Between strains	5	4,448	890	2.57	5.05
Between ages of tops	1	85	85	0.25	230.0
Interaction (S x A)	5	1,724	345	1.86	2.46
Within variation	38	7,046	185		
Total	49	13,303			

Ladak clone, group 2 containing clones B, B4, and B5, and group 3 containing clones C3 and C5. Analysis of variance on these groups is shown in Table 5. The variation due to groups was highly significant but the age of top growth was not significant. Thus the results are identical with those secured on the six strains except the grouping by relationship showed the different groups to be significantly different.

These results corroborate those secured by Peterson (6). He found no difference in the infection on leaves varying in age from one to 23 days in an experiment in which the age of each leaf on the plants tested was known.

Effect of Temperature on Black-Stem Infection

A separate experiment was not set up to test the effect of temperature on the amount of black-stem produced, but an examination of the temperatures throughout the inoculating season and the scores of the Ladak checks showed that there was a significant correlation between the degree of infection produced and the temperatures in the inoculation chamber during the infection period. That is, as the average temperature during the three-day infection period increased, the average plant score of the four Ladak checks increased. A scatter diagram of the temperatures and plant scores for 31 infection periods is shown in Plate III. The sloping line is the regression of temperature on the scores of the Ladak check. The correlation coefficient of 0.425 between temperature and the

Table 5. Analysis of variance of the effect of the age of the top growth on the degree of infection of three unrelated groups.

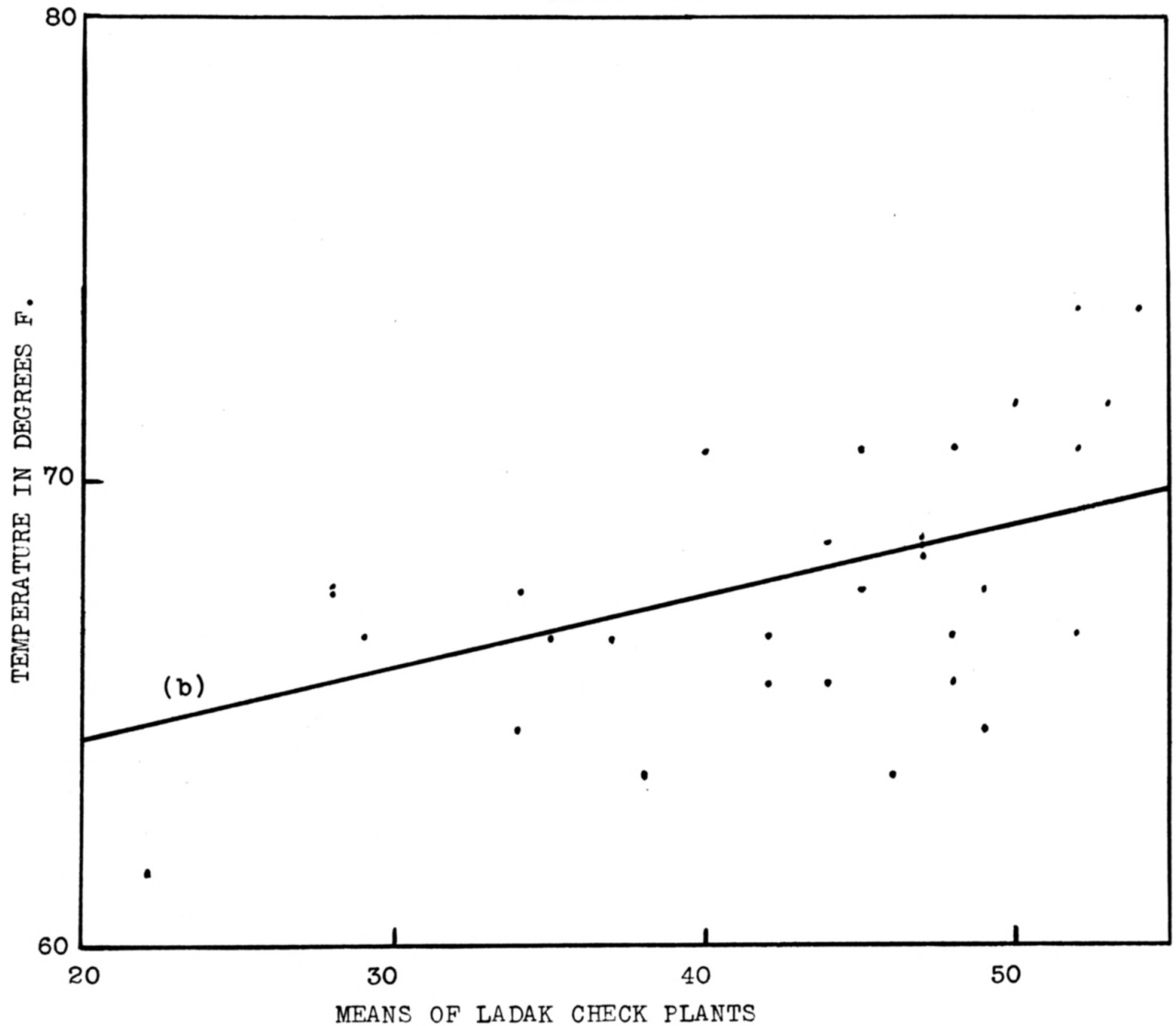
Sources of variation	Degrees of freedom	Sums of squares	Mean square	F value	Levels of significance 5%	1%
Between groups	2	2,511	1,256	5.39**	3.2	5.1
Between ages	1	85	85			
Error (Interaction plus within variation)	46	10,707	233	2.70	252.0	
Total	49	13,303				

** Highly significant

EXPLANATION OF PLATE III

Plate III is a scatter diagram of the relation of the temperature in the humidity chamber for the three-day infection period to the degree of infection of the Ladak check plants. The straight line is the regression line of temperature on the plant score.

PLATE III


80
70
60
20
30
40
50
TEMPERATURE IN DEGREES F.
MEANS OF LADAK CHECK PLANTS
(b)

the plant scores was significant at the five percent level. Temperature records were available for only 31 of the 45 inoculations made. The 11 lowest average plant scores of the Ladak checks had a mean of 35.2 while the corresponding temperature mean was 69.9° F. The mean of the 11 highest average Ladak checks scores was 50.4 and the corresponding temperature mean was 72.8° F. Peterson (6) found that about 22° C. or 71.6° F. was optimum for growth of the mycelium and spores of Ascochyta imperfecta on agar while fruiting took place at all temperatures from 9° to 30° C. Thus the highest plant scores occurred at approximately the same temperature as found best under artificial culture.

The temperature in the inoculation chamber during the infection period was not the only critical one as the plants were subject to different temperatures in the week following inoculation. The effect of the temperature after removal from the inoculation chamber was noted in late spring when the infection was so slight it was decided that accurate readings could not be made on the inoculated plants. The temperature in the inoculating chamber during the infection period was somewhat higher than previously, but the running water tended to keep it down in comparison to the high greenhouse temperatures. Peterson (6) found that in the early fall when the temperatures were somewhat higher, a longer infection period was necessary than in the winter. The effect of a long infection period was demonstrated accidentally when one replication of a technic

experiment on root reserves was left in the inoculation chamber for one week. The tops of all the plants were killed completely, and some of the plants failed to recover.

As stated previously no attempt was made to test the effect of extremely high or low temperatures on the degree of black-stem infection. Thus the results available are within the range of good infection as these plants were inoculated under proper conditions for infection to see the differences in resistance of several strains of alfalfa. It is believed that temperatures above 78° F. with correspondingly high greenhouse temperatures would cause lower amounts of infection. If this is the case the correlation would be curvilinear instead of simple when tested over a wider range of temperature.

Effect of Root Reserves on Black-Stem Infection

A third experiment was set up to determine the effect of root reserves on black-stem infection. Fifty-one plants from seven clones were selected, and the tops were cut back to one inch on March 26. These plants were then divided into three groups, and labeled high reserves, low reserves, and control. The plants in the low reserve group were cut back on April 5, 15, and 26. Those in the high reserve group were cut back on April 26, and those in the control group were not cut.

The practice used in securing plants of high and low root reserves is a standard one among alfalfa workers concerned with organic food reserves. Graber and co-workers (2),

Nelson (5), Grandfield (3), Hildebrand and Harrison (4), and others have reported that early and frequent cuttings result in a lower carbohydrate and nitrogen content of the roots. Under field conditions Grandfield (3) found the minimum carbohydrate and nitrogen content was reached about 20 days after cutting. Maximum accumulation occurred at about the full bloom stage. Under greenhouse conditions where temperature, moisture, and soil fertility are kept near the optimum, the plants grow much faster than in the field and thus reach their minimum carbohydrate and nitrogen content sooner. Therefore, the low reserve plants were clipped at intervals of 10 days instead of 20. Hildebrand and Harrison (4) found that weekly, biweekly, or monthly clippings of potted plants to one inch caused a great reduction in the consequent top growth and root growth. In this experiment it was assumed that clipping of the tops when four to six inches high would reduce the reserves considerably. The low reserve plants were clipped three times after about 10-day intervals while the high reserve plants were clipped once in the full bloom stage. No root samples were taken to see how successful the treatments had been, but it was noted that after the last clipping the low reserve plants recovered much more slowly than previously. These plants were easily distinguished from the high reserve plants for two to three weeks after clipping because of the tardy recovery; two plants were so severely treated that they died.

On May 20 all plants were inoculated together with six Ladak checks. On removal from the inoculation chamber random numbers were assigned each plant and a key made. Table 6 shows the plant indices of the seven strains for each treatment. The analysis of variance shown in Table 7 indicates that the differences between strains and the effect of treatments were highly significant. The interaction of strains and treatments was not significant in this case; therefore, it was pooled with the "within variation" and used as the error term. This indicates that all strains reacted in the same way to the clipping treatments. Thus from these results one may conclude that plants with low root reserves tend to be less susceptible than those with high root reserves.

There was not much difference between the control and the high reserve group. Very likely there was little if any difference in the reserves of these two groups. Graber and co-workers (2) reported that under field conditions the dry matter was higher in the root samples from uncut plots than those cut at full bloom. On a percentage of dry weight basis there was little difference between the uncut plots and the plots cut in full bloom. However, there was a great difference between these two treatments as compared with the plots cut in the succulent stage in regard to percent of dry matter, percent of total available carbohydrates, percent of total nitrogen, and percent of total sugars. In the present experiment the low reserve group would correspond to the plots cut

Table 6. The effect of root reserves on the plant indices of seven strains of alfalfa.

	Plant indices of strains by treatments																			
	Mc			J			B8			C4			B2			D			F		
	H*	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L
	128	128	94	72	77	102	108	79	72	160	117	85	117	94	98	145	142	140	94	96	75
	121	136	104	89	126	89	77			94		111	109	83	72	143	158	164			
	136	125	106														162				
	128	136	94																		
	100	136	117																		
	142		109																		
Mean	126	132	104	80	102	96	92	79	72	127	117	98	113	88	85	144	154	152	94	96	75

* H is high reserve group.
C is control group.
L is low reserve group.

Table 7. Analysis of variance of the effect of root reserves on black-stem infection.

Sources of variation	Degrees of freedom	Sums of squares	Mean square	F value	Levels of significance 5%	1%
Between strains	6	20,239	3,373	13.5**	2.34	3.30
Between treatments	2	2,683	1,342	5.4**	3.24	5.20
Error (Interaction plus within variation)	39	9,712	249			
Total	47	32,634				

** Highly significant

in the succulent stage, the high reserve group to the full bloom stage and the control group to the uncut plots. Thus probably the only difference between the control and the high reserve groups would be in the age of the top growth, and in one of the control experiments it was found that the age of the top growth did not have any effect on infection.

It should be noted that the difference between the high reserve group and the low reserves, though significant, is not large. Caution should be used before a universal statement is made as this experiment was performed near the end of the inoculating season, and there was not time to repeat it.

The strain means by treatments are shown in the lower line of Table 6. In all cases the strain means were lower for the low reserve group than the control group. There were two strain means that were lower in the high reserve group than in the low reserve. The magnitude of these reversals was not large, however. Thus the plants with low food reserves tended to be more resistant to black-stem than those with high reserves. These results parallel those of some of the rusts of cereals, leaf spot of celery and crown gall of beet. Raines (8) found the vegetative vigor of these hosts and the severity of the disease to be in a direct relation.

Reaction of 30 Pedigree Lines

Ten plants from each of 30 bacterial wilt resistant selections from Kansas Common were potted in the field and

brought into the greenhouse in November. These plants were young seedlings from seed planted on August 30. When the plants became eight to 10 inches high they were selected at random on the basis of height only and inoculated for black-stem. Readings were made in the usual manner, and the scores and indices were calculated. In this case a Turkistan clone, Mc, was used as the check instead of the Ladak. Considerable variability within lines was found. These lines were from open-pollinated plants. Much variability was expected because of the well-known heterozygosity in alfalfa. It was thought that two inoculations would give a more reliable estimate of a plant's reaction to black-stem than one so the plants were cut back after the readings had been made on the first inoculation. Many of the plants were blossoming at that time. Four weeks later these plants were inoculated again and readings made.

A highly significant difference existed between inoculations and the lines were significantly different. Kansas Common line 1-102-5 had the lowest average score of 82 for seven plants as shown in Table 8. The second inoculation exceeded the first inoculation in 23 of the 30 lines tested. In the other seven lines the reverse was true. It is difficult to explain adequately the significant variation between inoculations as all plants were of the same age and received the same treatment. Some of the variation may be due to the error in readings and some to the difference in recovery of the plants

Table 8. Means and rank of 30 Kansas Common selections for two inoculations of black-stem.

Pedigree line	Number plants tested	Mean of first inoculation	Mean of second inoculation	Mean of both inoculations	Rank
1-102-5	7	75	89	82	1
1-103-1	9	64	105	84	2
1-1014-2	8	88	84	86	3
1-102-6	8	75	105	90	4
1-1014-1	8	80	104	92	5
1-1014-3	8	84	102	93	6
1-105-2	4	85	106	96	7
1-101-1	9	89	107	98	8
1-103-2	8	84	114	99	9
1-102-2	8	89	110	100	10
1-101-2	6	99	101	100	10
1-106-1	7	96	108	102	12
1-1011-1	8	104	99	102	12
1-104-1	8	96	111	104	14
1-1017-1	9	92	118	105	15
1-1013-1	10	94	117	106	16
1-106-2	7	102	111	106	16
1-109-1	9	108	103	106	16
1-1014-4	5	98	116	107	19
1-1018-1	10	113	102	108	20
1-102-1	7	103	113	108	20
1-109-2	9	103	115	109	22
1-1011-2	7	107	112	109	22
1-102-3	6	94	127	111	24
1-1012-1	9	116	109	112	25
1-105-1	10	115	112	114	26
1-102-4	7	115	112	114	26
K.C. Wilt Res.	10	108	122	115	28
1-1012-2	8	107	130	118	29
681 K.C.	7	124	131	128	30
Unweighted mean		97	110	103	

after cutting as some plants recovered faster than others. The root reserves may have been higher in the plants when inoculated the second time than the first as the majority of the plants were blooming or about to bloom when cut back after the first inoculation. Therefore, differences in root reserves would be expected. Also the greenhouse temperatures were higher during the early part of the season than later. Other factors which are not known at present may have contributed to this difference.

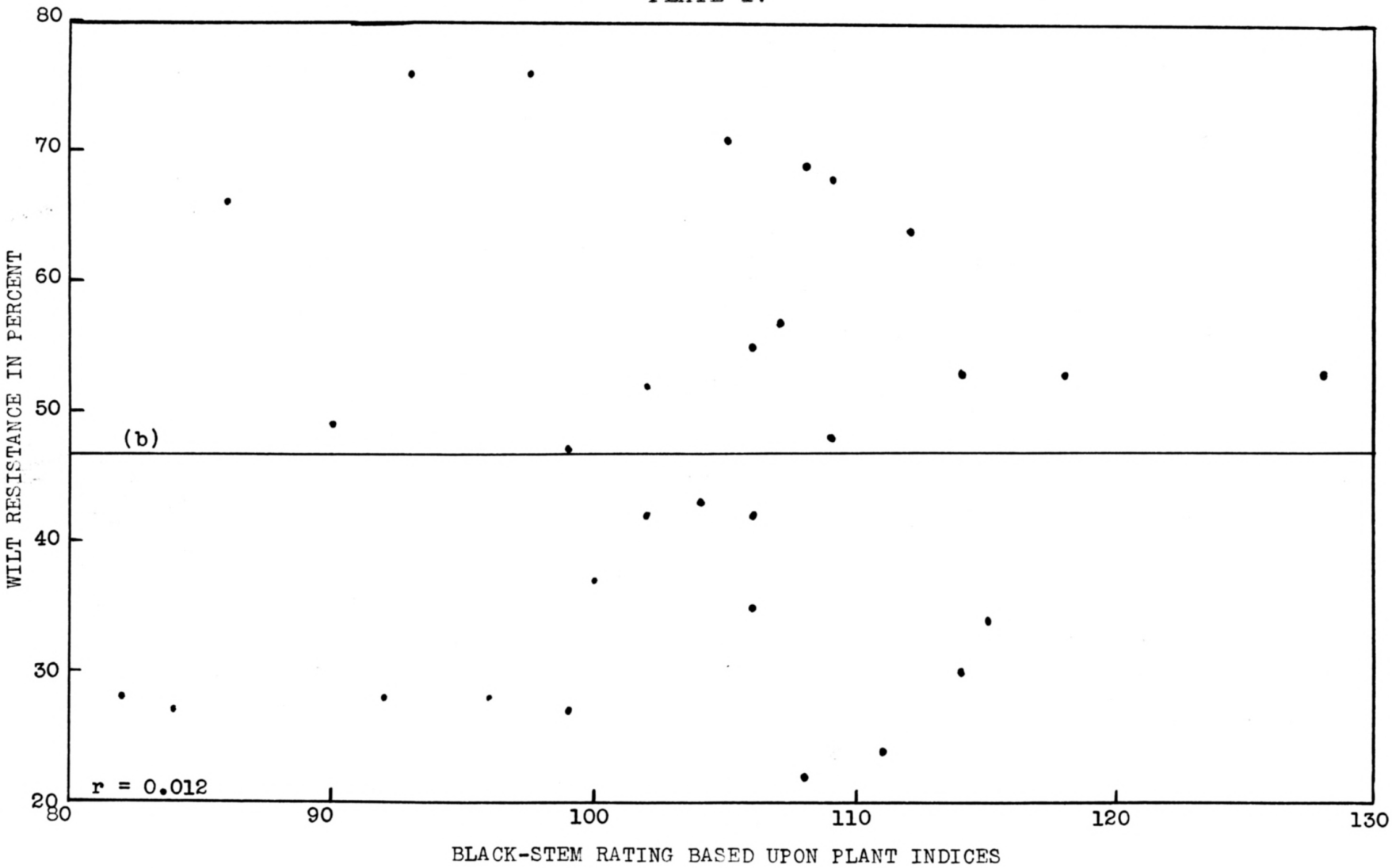
Although the analysis of variance showed that significant differences were present between lines there was so much variation within lines that the highest ranking mean differed only from those lower than 22 in rank as found when the "t" test was used. Thus it is readily seen that most of the lines were statistically similar.

The correlation of resistance to black-stem with the resistance to bacterial wilt was investigated in these 30 lines. The value for r was found to be 0.012, which was not significant. The reaction of these lines to bacterial wilt was secured from tests in the wilt nursery at Manhattan, Kansas, in 1941. Plate IV shows a scatter diagram and regression line of the reaction of 30 Kansas Common selections to black-stem and to bacterial wilt. The regression of the wilt rating on the black-stem rating was 0.008. Thus there was no change in the relation of the reaction of the lines to black-stem or bacterial wilt as they became more resistant or susceptible to either disease. In other words, lines resistant or susceptible

EXPLANATION OF PLATE IV

Plate IV is a scatter diagram of the reaction of 30 Kansas Common selections to black-stem and bacterial wilt. The slope (b) of the regression line is 0.008 indicating little or no regression.

PLATE IV


80
70
60
50
(b)
40
30
r = 0.012
20
80
90
100
110
120
130
WILT RESISTANCE IN PERCENT
BLACK-STEM RATING BASED UPON PLANT INDICES

to black-stem may be either resistant or susceptible to bacterial wilt.

Reaction of Inbred Lines

Goodding (1) tested the selfed progeny from 18 open-pollinated plants selected for inheritance studies. Inbreds from seven varieties and species were used. In every case the progeny gave a reaction that was similar to the parent. That is, if two plants from a variety were found to be resistant and susceptible respectively, the indices of the selfed progeny of the first would be statistically lower than the latter. Much variation existed within lines as this material was only inbred for one generation. Selfed progenies from 20 to 30 plants of each line were tested. The average of the indices of these selfed plants in a line was thought to be a good indication of a plant's transmitting ability. Analysis of variance showed that there was a highly significant difference between strains tested. This indicated that the plants transmitted differently and that selection of resistant lines would be possible. There also was a highly significant difference in the reaction of the varieties and species tested.

Six to eight plants each from one generation inbred lines were grown in the greenhouse during the summer of 1941. Usually half of them had been selected for high resistance and half for susceptibility. During the period of six months while these plants were growing in the greenhouse many did not bloom.

Some of them were self sterile or had a very low percentage of selfed seed. Others produced so few seeds that no plants remained after losses due to damping off and transplanting. Thus second generation inbreds were obtained from only nine of the 18 original (P_1) plants.

Table 9 shows the means of infection of nine lines of the first and second generation of inbreeding. The correlation between the two generations was 0.716 which is significant at the five percent level according to Fisher's table of correlation coefficients given by Snedecor (9). This corroborates the results secured by Goodding (1) who found that the selfed progenies of 18 lines reacted in a manner similar to their open-pollinated parent. The variation was lower in the S_2 generation than in the S_1. This was true in all but one strain tested, and would be expected since homozygosity increases as inbreeding proceeds. Table 9 shows the standard deviations of the S_1 and S_2 generations of each line. The average standard deviation for the S_1 generation was 30.02 as compared with 20.72 in the S_2 generation. This decrease of 9.3 was 31 percent of the variability of the S_1 generation. Thus inbreeding has caused a large reduction in the heterozygosity present. All of the variation present was not due to the heterozygous nature of a line as some occurred from the reading method and inoculation technic. This was shown by the variability among the four clonal check plants used with every inoculation. Only on rare occasions would the scores of these clones be alike. In every

Table 9. Infection means and standard deviations of nine lines for S_1 and S_2 generations.

Variety	Line	S_1 generation		S_2 generation	
		Mean	Standard deviation	Mean	Standard deviation
Ladak	G	101.8	24.9	81.8	14.5
Kansas Common	B	104.6	37.9	97.7	22.3
Semipalatinsk	J	108.7	30.4	92.1	18.3
Kansas Common	C	114.7	29.0	109.4	17.8
Ladak	H	119.9	32.0	124.3	22.0
Turkistan	N	120.5	32.9	133.2	16.7
Kansas Common Selection	L	124.5	20.1	105.1	27.4
Kansas Common Selection	K	126.2	30.4	116.7	28.0
Hairy Peruvian	D	134.9	32.6	117.0	19.5
Average		117.3	30.0	108.6	20.7

case, however, the variation between the check plants was small in comparison to the variation within a strain.

A significant correlation of 0.583 was found between the means of the S_1's tested in 1940 by Goodding and the means of the few plants selected for further breeding as tested in 1941. Goodding tested from 20 to 30 S_1 plants from each of 18 lines. Four plants were selected from each of the extremes of a line and thus about an equal number of resistant and susceptible plants were present. This showed that the plants reacted similarly under different operators.

Reaction of Hybrids

Several crosses were made using the alcohol emasculation method of Tysdal and Garl (10) both with the open-pollinated plants and the inbreds.

Ten crosses of open-pollinated plants are shown in Table 10. All crosses of open-pollinated plants produced both resistant and susceptible progeny where more than two or three plants were present in the cross. Statistically the 51 F_1's from the 10 crosses of P_1's were similar. This may have been due to the small number of plants in each cross. There was, however, a significant difference between the pooled F_2 lines shown in table 11. Analyses of variance calculated on F_2 populations from reciprocal crosses did not show significant differences; hence, it was possible to pool all of the F_2's from a cross. This provided larger numbers to work with, and therefore, a more reliable estimate of the reaction of a cross

Table 10. Means of infection and number of plants in the F_2 generation of the crosses of open-pollinated plants.

Cross No.	Varieties crossed	Number in F_2	Mean
1*	Hairy Peruvian x Medicago falcata	18	96
2	Kansas Common x Semipalatinsk	10	131
3	Hairy Peruvian x Kansas Common	104	112
4	Medicago falcata x Hairy Peruvian	10	102
5	Kansas Common x Hairy Peruvian	14	85
6	Ladak x Hairy Peruvian	49	104
7	Semipalatinsk x Ladak	126	93
8	Ladak x Hairy Peruvian	24	122
9	Ladak x Hairy Peruvian	66	113
10	Semipalatinsk x Kansas Common	10	102

* Includes reciprocal cross

Table 11. Analysis of variance on pooled F_2 lines from crosses of open-pollinated plants.

Sources of variation	Degrees of freedom	Sums of squares	Mean square	F value	Levels of significance 5%	1%
Between lines	10	55,158	5,516	11.6**	1.85	2.37
Within lines	420	199,650	475			
Total	430	254,808				

** Highly significant

than was secured from the F_1 generation. Since the F_2 populations were statistically different this indicated that the P_1's transmitted differently. It is thought that the true means of the F_1's of each cross were not secured by averaging the indices of the one to seven plants present in each cross except in one case where 26 plants were available. This was further substantiated by the fact that the correlation between the means of the pooled F_2 lines and the F_1 lines was not significant. Undoubtedly, the larger numbers in the F_2 generation gave a more accurate estimate of a cross than the mean of the F_1's.

Of several crosses among inbred lines and back crosses made in the summer of 1941, 11 had more than 11 progeny. Table 12 shows the crosses that were made. Analysis of variance showed that highly significant differences existed between crosses. This was not true the previous year among crosses of open-pollinated plants. The reason may have been the larger numbers present in the crosses of S_1's and back crosses, the more homozygous nature of the parents, and the closer selection of parents than in the previous year. The F_2 population resulting from these crosses were not tested.

Effect of Inbreeding and Selection in Inbred Lines

Plate V is a graph of the distribution of plant indices of the S_1 and S_2 generations of resistant Kansas Common plant, B. The mean of the S_1 generation was 102 with a standard deviation of 37.9. The average score of the original P_1 plant

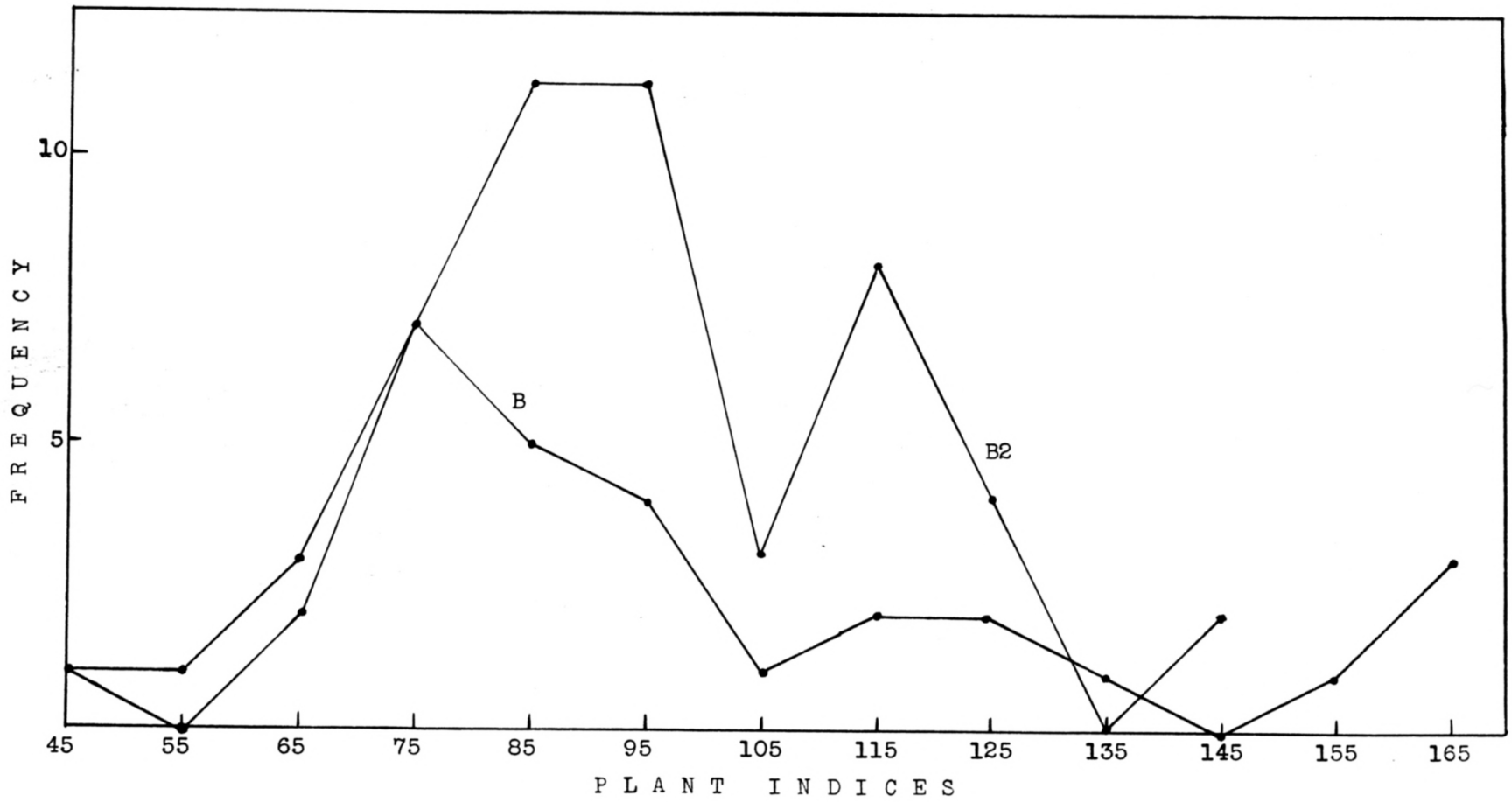
Table 12. Means of infection for crosses of inbreds and back crosses.

Parent varieties	Lines crossed	Number plants in cross	Mean of infection
(Semipalatinsk x Ladak) x Ladak	7- x F	32	80.7
Kansas Common	C3 x C4	36	86.7
Kansas Common	B2 x C3	11	89.6
Kansas Common	B8 x C5	14	94.2
Kansas Common x Hairy Peruvian	C3 x D2	11	96.8
Kansas Common	B8 x C4	15	96.9
Kansas Common	B2 x B8	40	99.4
Kansas Common	C4 x C5	31	103.0
(Ladak x Hairy Peruvian) x Hairy Peruvian	6- x D	31	105.8
(Hairy Peruvian x Kansas Common) x Kansas Common	3- x B	11	111.3
Hairy Peruvian x (Kansas Common x Hairy Peruvian)	D x 5-	12	112.8

EXPLANATION OF PLATE V

Plate V is a graph of the plant indices for the S_1 and S_2 generations from the Kansas Common variety. The line B represents the S_1 generation and line B2 the S_2 generation. There was no significant difference between the progeny of B and B2.

PLATE V


10
5
FREQUENCY
B
B2
45
55
65
75
85
95
105
115
125
135
145
155
165
PLANT INDICES

B was 100, which is the result of approximately 10 inoculations as several cuttings were available. Thus this plant transmitted to its offspring approximately as it reacted to black-stem. The plant, B2, a selection out of the selfed progeny of B, scored an average of 93. Selfed progeny of B2 had a mean of 95 with a standard deviation of 21.4. Thus B2 transmitted similarly to B. The progenies of B and B2 do not differ from each other statistically. In this case the selection of a plant towards the resistant side of the mean caused the second selfed generation to be slightly more resistant, though not enough to be statistically significant.

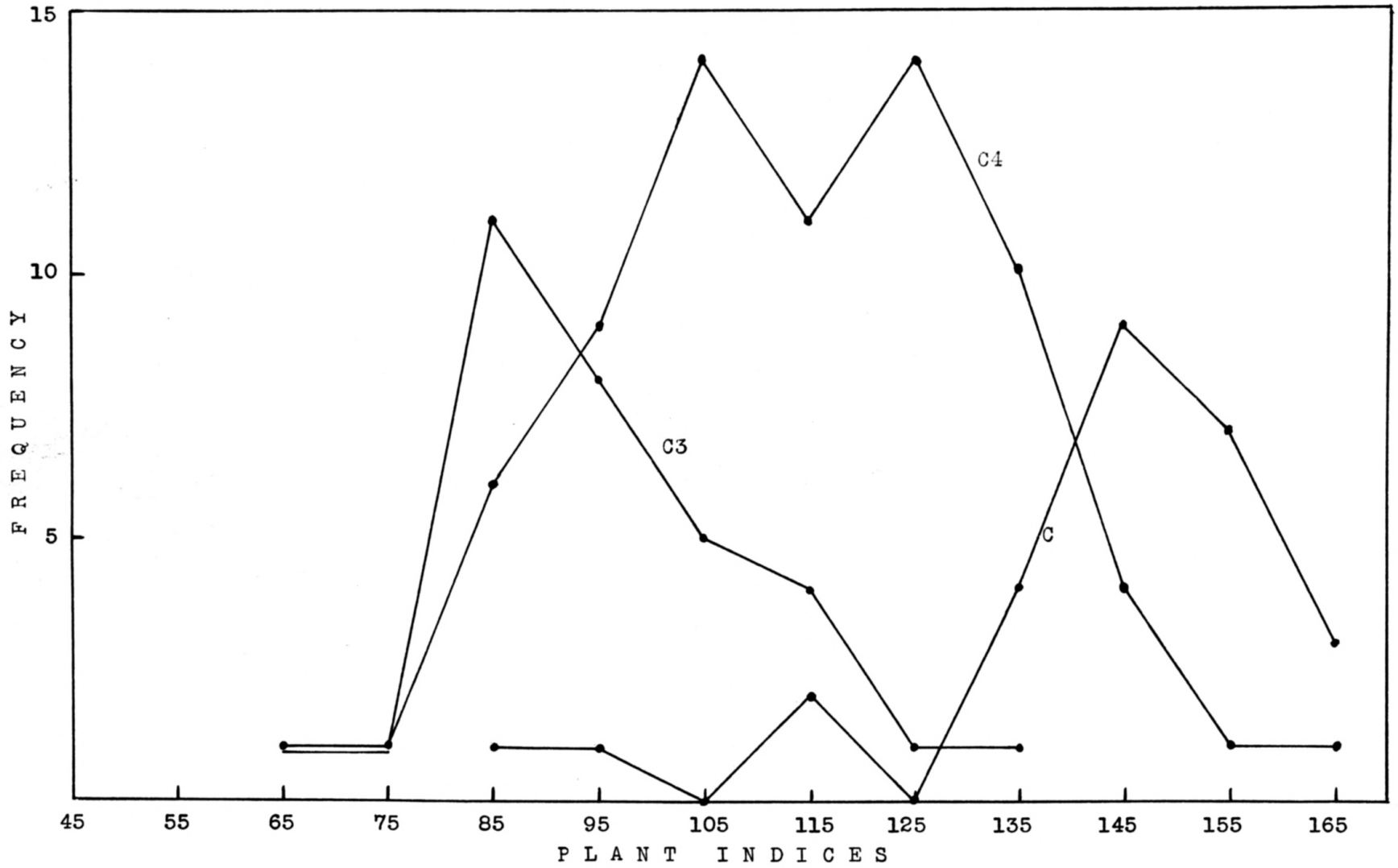
Plate VI shows the distribution of plant indices of the S_1 generation and S_2 generation of a susceptible Kansas Common plant, C. It is readily seen from the graph that this plant produced mostly susceptible offspring as only two plants scored less than 100. The mean of the 27 selfed progeny was 147 with a standard deviation of 29.0. Plant C3 had an average score of 110, and the average of its 32 selfed progeny was 96.1 with a standard deviation of 14.1. Plant C4, a sister of C3 averaged 116, and its 72 selfed progeny averaged 115 with a standard deviation of 19.6. Analysis of variance showed that all three progenies differed from each other significantly. Thus selection had caused the resistance of this line to rise considerably above the original level in the P_1 generation.

Plate VII shows the distribution of plant indices for a selection from the Hairy Peruvian variety for the S_1 and S_2

EXPLANATION OF PLATE VI

Plate VI is a graph of the plant indices for the S_1 and S_2 generations from the Kansas Common variety. Line C represents the S_1 generation and lines C3 and C4 the S_2 generation. Significant differences existed between the three progenies. Thus inbreeding followed by selection has raised the level of resistance.

PLATE VI


15
10
5
FREQUENCY
C4
C3
C
45
55
65
75
85
95
105
115
125
135
145
155
165
PLANT INDICES

generations. This plant was like the susceptible Kansas Common plant. Its selfed progeny averaged 140, having a standard deviation of 32.6. D2, a selection from the S_1 progeny scored 117 and the mean of its progeny was 117. Analysis of variance showed that the progeny of D and D2 differed significantly. Thus the selection of a more resistant type out of the selfed progeny of D was successful.

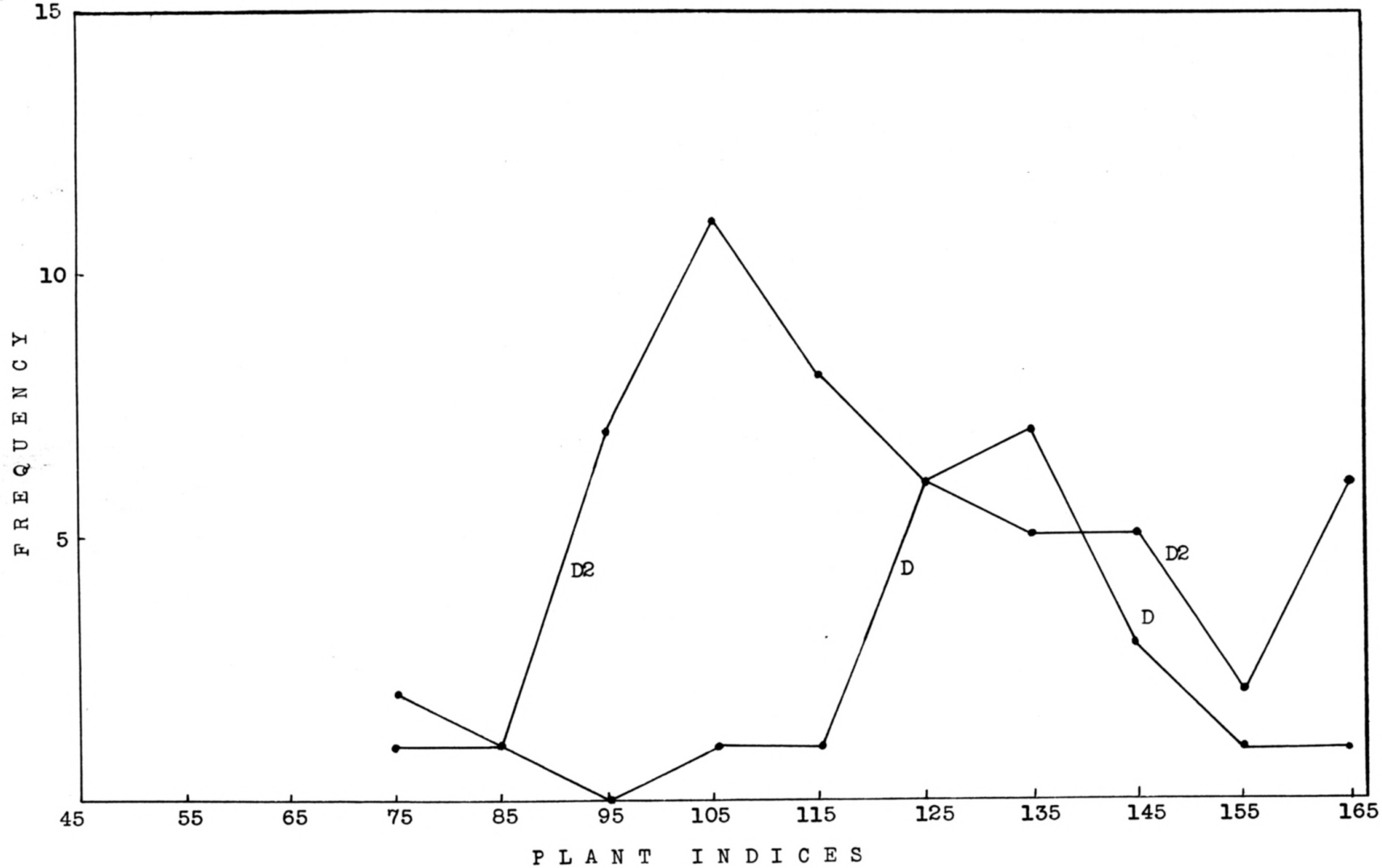
The plant indices for the S_1 and for the unselected S_2 generations from a Ladak plant H were studied. The S_2 generation was an unselected population as a number of S_1's were selfed and the seed mixed, thus producing the S_2 generation. The mean of the S_1 generation was 121 with a standard deviation of 32. The mean of the S_2 generation was 131 with a standard deviation of 23.1. The difference between the two means was not significant. Thus in this case there was no selection and the S_2 generation was not quite as resistant as the S_1 although the difference was not statistically significant.

These four examples show the value of selection in an inbreeding program on heterozygous material. In all cases the selection of resistant plants in the S_1 generation tended to produce more resistant plants in the S_2 generation than in the S_1, while in the case of an unselected population there was no change in resistance in the S_2 generation as compared with the S_1.

EXPLANATION OF PLATE VII

Plate VII is a graph of the plant indices for the S_1 and S_2 generations from the Hairy Peruvian variety. Line D represents the S_1 generation and line D2 the S_2 generation. Significant differences existed between the progeny of D and D2.

PLATE VII


15
10
5
FREQUENCY
D2
D
D2
D
45
55
65
75
85
95
105
115
125
135
145
155
165
PLANT INDICES

Effect of Selection in Hybrid Lines

Plate VIII is a graph of the distribution of plant indices of three F_2 lines, 3, 7, and 9 derived from crossing open-pollinated plants. Line 3 was the result of crossing a susceptible Hairy Peruvian by a resistant Kansas Common. Line 7 was the cross of a resistant Semipalatinsk by a resistant Ladak. Line 9 was the cross of two susceptible plants, Ladak by Hairy Peruvian. The means of the F_1's of the three crosses did not differ significantly, but the F_2's were significantly different. Line 7, which was the result of crossing two resistant plants, was significantly more resistant than lines 3 or 9. There was no difference between lines 3 and 9. Thus the cross involving two resistant plants produced more resistant offspring than either of the other two crosses, one of which had one susceptible parent and the other both susceptible parents.

These results corroborate the results secured in 1941 when crosses of inbreds and back crosses were made which carried a high degree of resistance if the parents were resistant, and susceptible if the parents were susceptible.

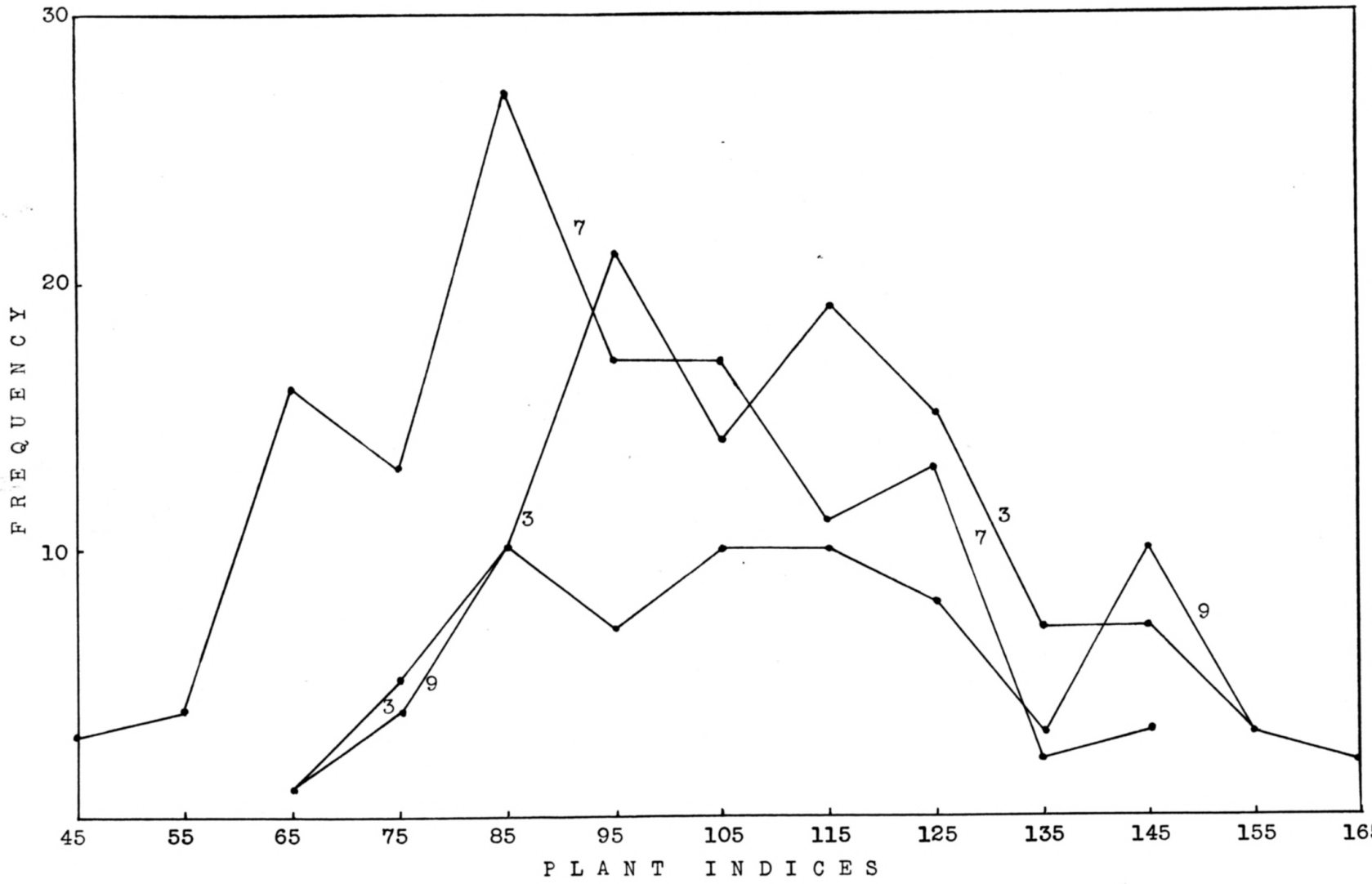
Selection of Highly Resistant Plants

One plant from one of the Kansas Common wilt resistant selections proved to be highly resistant to black-stem. On three successive inoculations it scored 43, 41, and 56 as compared with an average of 82 for the most resistant line in-

EXPLANATION OF PLATE VIII

Plate VIII is a graph of the plant indices for the F_2 generation of three crosses, 3, 7, and 9. Although the F_1^2 generation plants were alike there were significant differences between line 7 and the other two lines, 3 and 9, in the F_2 generation.

PLATE VIII


30
20
10
FREQUENCY
7
3
9
3
7
3
9
45
55
65
75
85
95
105
115
125
135
145
155
165
PLANT INDICES

cluded in the same tests. On one inoculation in the fall of 1942 this selection scored 69. Plate IX shows some leaves and a section of a stem from this plant on the left, and the leaves and stem from a susceptible plant on the right. Another plant from the F_2 generation of a Semipalatinsk by Ladak cross averaged 48 for two inoculations. Cuttings of each of these plants have been made and will be used in future breeding work. No progeny have been tested to date.

Prediction of the Reaction of the F_1 Generation Plants

It is generally accepted that the average of the selfed progeny from a plant is a better index of a plant's transmitting ability than the actual plant score of that plant. For this reason the means of the selfed progeny of each parent in the cross as reported in Table 12 were averaged to obtain an expected index intermediate between the parents. The correlation of the means of the F_1's and the expected indices was significant at the five percent level. This indicated that the F_1's were intermediate between the parental transmitting abilities. The F_2 generation has not been tested. Table 13 shows the means of F_1's and the index numbers that would be expected based on an average of the selfed progenies of each parent in a cross.

It would have been possible to predict the reaction of these hybrids with fairly good accuracy by averaging the means of the selfed progeny of the parents in each cross. If this

EXPLANATION OF PLATE IX

The section of stem and the leaves to the left were taken from a highly resistant Kansas Common plant. Those on the right are from a susceptible Hairy Peruvian plant. Note the difference in the size of the lesions on the two plants.

PLATE IX

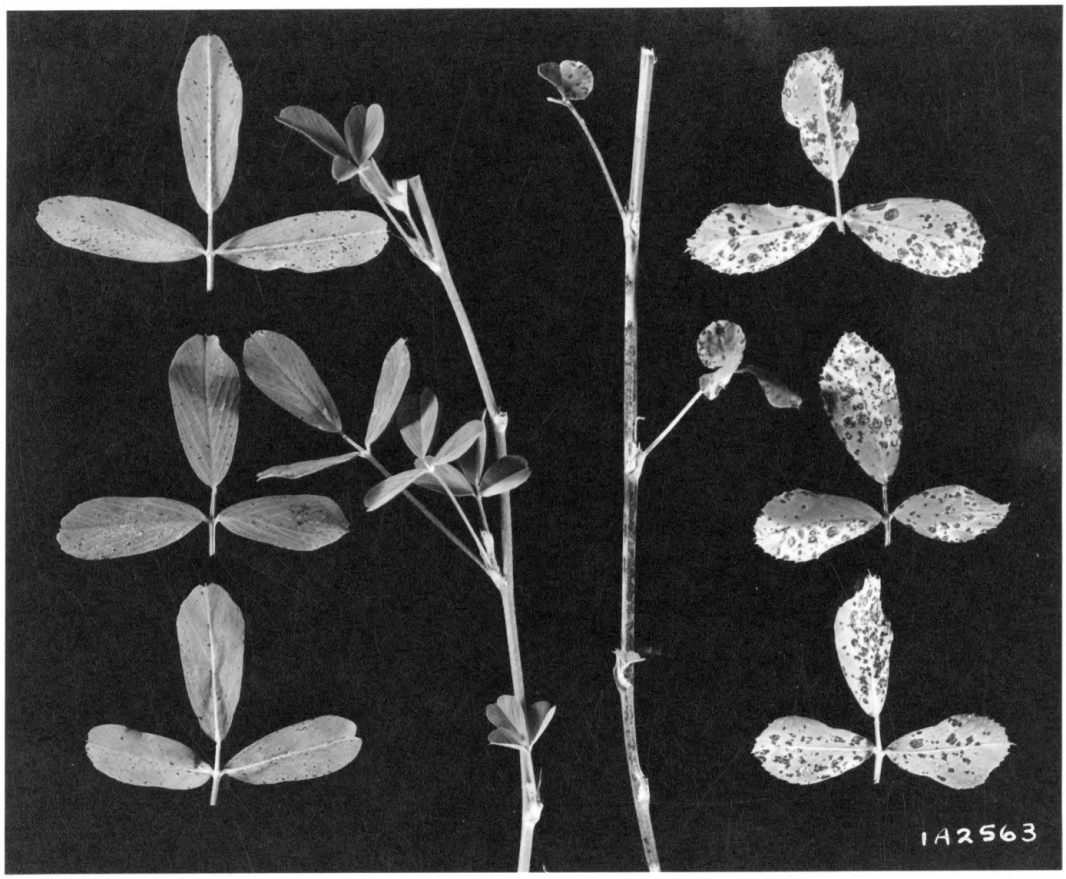


Table 13. Means of infection of the F_1's and the calculated or expected index numbers.

Cross	Average of F_1's	Expected index number
7- x F	80.7	99.2
C3 x C4	86.7	100.8
B2 x C3	89.6	95.4
B8 x C5	94.2	104.5
C3 x D2	96.8	106.6
B8 x C4	96.9	108.4
B2 x B8	99.4	98.7
C4 x C5	103.0	111.0
6- x D	105.8	119.6
3- x B	111.3	106.8
D x 5-	112.8	110.2

holds true for all hybrids one could select the parents for the desired crosses on the basis of the selfed progeny of the parents. The number of crosses made could be reduced considerably.

Field Readings

The spring of 1942 was exceptionally cool and wet; thus, climatic conditions were very favorable for plant growth as well as the black-stem disease in the field. Readings were taken on 66 strains of alfalfa planted in 20-foot rows with two replications in the uniform nursery on the Agronomy Farm at Manhattan. It contained improved strains from Rhode Island, New Jersey, New York, Michigan, Wisconsin, Nebraska, Kansas, and Colorado as well as some of the standard varieties such as Ladak, Kansas Common, Grimm, Meeker Baltic, Orestan, Hardistan, Dakota Common, and Arizona Chilean. A few foreign introductions were grown here also. Black-stem notes were taken on April 30 or about a month before the crop was cut for hay. At this time black-stem was the predominant disease present. Four weeks later leaf blotch caused by Pyrenopeziza medicaginis (Lib.) Sacc. was present in greater abundance than black-stem. Severe defoliation had occurred from the time of the first reading. A large share of this was attributed to black-stem. Analysis of variance of the readings showed the variation due to strains highly significant, far exceeding the one percent level of significance, thus showing that there was a real dif-

ference in the reaction of these strains to black-stem. The variation between replications was quite high but not significant at the five percent level. Strains A-169 from Nebraska, A-155 from Rhode Island, A-128 and A-131 from Wisconsin were the most resistant strains according to these readings. All of the foreign introduction grown here and Arizona Chilean were very susceptible. Of the standard varieties Grimm and Dakota Common ranked the highest in resistance.

Miscellaneous Observations

It was observed while making crosses that some plants crossed more readily than others. In one Kansas Common line, C, in controlled crosses between sister S_1's, three percent of 430 flowers pollinated formed pods when C5 was used as the male parent. When C5 was used as the female parent 25 percent of 59 flowers pollinated set pods. There was very little selfed seed on this plant. When pollen from C5 was applied to the stigmas of another Kansas Common inbred line B, 25 percent of 40 flowers pollinated set pods. Thus this plant was a very poor parent when used as the male parent in crosses with related plants but was a good parent when crossed onto unrelated plants. When C5 was used as the female parent in crosses of sibs it set pods equally as well as when it was used as the male parent in crosses of unrelated material. This is some form of sterility, but it cannot be called male sterility as it was not male sterile when crossed to unrelated material. Further study

should be made to find the real cause of this sterility.

One cross of Semipalatinsk by Kansas Common produced one plant with very abnormal shape of leaflets in the F_2 generation. The leaflets were long and extremely narrow. Although there was some variation in length of these leaflets the width was never more than one-eighth inch at its widest place, narrowing to the width of the midrib of some leaflets. This characteristic probably was a mutation as it occurred on one plant in an F_2 population of 12 in 1941, and over 400 F_2 generation plants from the same cross were grown in 1942 without the appearance of a similar plant. This plant was not examined cytologically. Although it did not possess much vigor it grew to eight to 10 inches tall, but never blossomed. Cuttings of it were made several times without success.

Two plants, C3-1 and C3-5, appeared in the S_2 progeny of a Kansas Common plant with extremely predominant serrations of the margin of the leaflets. Eight S_3 plants from C3-1 were secured. Four of them were like the parent, and four were normal. The numbers were too small to determine the manner of inheritance. Apparently it is not a simple recessive or it would have bred true.

Three Medicago falcata plants were found to have a varying number, three, four, or five, leaflets for many of the leaves. The extra leaflets were at the terminal end of the petiole and not laterally as sometimes are found in common alfalfa. Some of the leaves had the normal number of three

leaflets. Whether this character is inherited or not is not known as no flowers were produced on either plant in the six-month period that they were grown in the greenhouse. If this character is found to be inherited it may be of value in distinguishing varieties.

DISCUSSION

It is not always possible to prove that actual hybrids have been secured when alfalfa plants are cross pollinated. All of the plants used for the black-stem disease study had purple or variegated flowers and were similar in the morphological characters observed except as mentioned. It was noticed in two crosses where a vigorous upright Kansas Common plant C3 was used as the male parent, the F_1's exhibited the same vigor and upright type of growth as when C3 was used as the female parent. They appeared similar to the selfed progeny of C3, and it was possible to pick out these plants without looking at the label stakes. Thus, in this case the cross seemed to have been successful. A technic experiment was performed on the efficiency of emasculation for making crosses. A clone not used in the black-stem disease work which was a homozygous recessive for white flowers was pollinated with pollen from a purple flowered plant. Seventy flowers were crossed and all of the F_1 progeny showed purple flowers. Approximately 50 F_1 plants were secured. Therefore, the emasculation was satisfactory in this case. At the same

time about 10 racemes with six to eight flowers in each were emasculated to which no pollen was applied. No pods formed in these flowers. Thus it seems safe to conclude that the majority of intended crosses actually resulted in hybrids if any seed was produced.

The results obtained in the experiment on root reserves indicated that resistance may be more internal than environmental. The practical application of the greater resistance of plants when low in root reserves is limited. Other things being equal, a field well managed as to cutting treatments may be subject to more black-stem than an improperly managed field because of the difference in root reserves. Conversely, plants with high root reserves would tend to make more vigorous regrowth following defoliation by this disease. All of the implications of these relationships and the actual nature of resistance to black-stem are not known.

Much variability was found to exist in the selfed progeny from one generation inbreds. Intergrading classes were observed in all populations. In addition, the fact that plants upon selfing produce offspring which may vary greatly shows that inheritance of resistance to black-stem is not simple. Since the reactions of the F_1's from crosses of inbreds and back crosses could be predicted by calculating expected indices from the reaction of the selfed progeny of the parents there is an indication of the lack of dominance of resistance or susceptibility, unless a large number of factors concerning resistance

is involved. In that case resistance could be dominant or recessive. Thus the reaction of the F_1's substantiates further the fact that resistance is not simple.

SUMMARY

A study was made of the reaction of alfalfa strains to the black-stem disease caused by the fungus Ascochyta imperfecta Peck. While making this study it was found that there was much variability in infection of plants at different times. Thus several factors which may cause this variability of infection were studied also.

It was found that neither the age of the culture nor the age of the top growth had any effect on the degree of infection. There was a significant positive correlation favorable for infection between the average temperature in the inoculation chamber and the amount of black-stem. The optimum temperature during the three-day infection period was 72.8° F. Very little infection was obtained in late spring when the temperatures were high. Thus the relationship of temperature to black-stem infection was curvilinear. Plants that were high in root reserves were significantly more susceptible than plants which were low in root reserves.

There was no correlation between resistance to black-stem and resistance to bacterial wilt as found in 30 Kansas Common lines.

Classes of resistance formed an intergrading series in

all inoculation work, and the plant indices of infection were distributed in a normal curve.

Significant differences in resistance were found between 30 open-pollinated Kansas Common lines though there was much variation within lines.

There was a significant correlation of resistance between nine S_2 and S_1 lines from five varieties. The variation was much lower in the S_2 generation than in the S_1.

Although 10 crosses of open-pollinated plants reacted similarly to black-stem in the F_1 generation, significant differences were found between lines in the F_2 generation. Significant differences existed between crosses of inbred and back crosses with the F_1's intermediate between their parents' transmitting abilities. Thus the reaction of the F_1's could be predicted with fair accuracy by averaging the means of the selfed progeny of the parents.

Inbreeding followed by selection proved to be valuable in raising the resistance of both resistant and susceptible lines. No increase in resistance was obtained where there was no selection following inbreeding.

Two plants, a Kansas Common selection and an F_2 plant from a Semipalatinsk x Ladak cross were found to be highly resistant. These plants will be used in future breeding work.

Strains A-169 from Nebraska, A-155 from Rhode Island, A-128 and A-131 from Wisconsin were the most resistant strains according to field readings made in the spring of 1942.

ACKNOWLEDGMENT

This study was conducted through the cooperation of the Division of Forage Crops and Diseases, Bureau of Plant Industry of the United States Department of Agriculture, the Department of Agronomy, and the Department of Botany and Plant Pathology of Kansas State College. Special acknowledgment is given to Mr. C. O. Grandfield for helpful suggestions and use of equipment; to Associate Professor L. P. Reitz for encouragement, helpful suggestions, and constructive criticism throughout the course of this study; to Dr. E. D. Hansing of the Botany Department for advice regarding plant pathological phases of the investigation; and to Dr. H. C. Fryer of the Mathematics Department for help regarding statistical treatments. Professor Reitz and Dr. Hansing acted in the capacity of major instructors.

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