ASSOCIATION
of
ECONOMIC CHARACTERS IN RHIZOMA ALFALFA

by

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A Thesis Submitted in Partial Fulfilment
of the
Requirements for
the Degree of
Master of Science in Agriculture
in the
Department of Agronomy.

Accepted. May 8th

The University of British Columbia

April 1943

ACKNOWLEDGMENT

Grateful acknowledgment if here made to Dr. V. C. Brink, Dr. G. G. Moe, and Dr. A. H. Hutchinson, under whose guidance this paper was written.

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ASSOCIATION OF ECONOMIC CHARACTERS IN RHIZOMA

ALFALFA

Alfalfa, to-day, is one of the world's leading forage crops, in the regions favoring its growth, and these are now many, no crop used for fodder surpasses it in general utility and yield. For thousands of years alfalfa was highly regarded in south-western Asia, in which area it was endemic, as forage sine qua non. Slowly, selected strains made their way into the agricultural regions of North Africa, and southern Europe until by the end of the 19th century all of temperate Eurasia knew it as a valuable addition to the forage resources. The value of the crop was recognized by the early white settlers in the Western hemisphere and, undoubtedly in many separate occasions the plant was introduced to the new agricultural area. However, prior to 1900 it is doubtful if the total acreage in both western continents exceeded 3 million acres. Since that date a truly phenomenal expansion in acreage has taken place especially in the United States, the Argentine, Uraguay, Paraguay, Mexico and in Australia. In the U.S.A. alone present crop acreage must now exceed 20 million acres.

Canada has not been excluded in the "march" of alfalfa over the globe. With the development of winter hardy strains for cooler and more humid areas alfalfa acreage spread in Canada. Ontario, British Columbia and the prairie provinces now maintain a combined crop area of 1½ million acres. In British Columbia alfalfa has become the standard hay and pasture crop in the cattle ranching and irrigated districts.

Alfalfa, unlike many other forage crops which posses a few commendable characteristics, has to its credit many desirable agronomic features. It is this combination or blending of so many desirable agronomic features which account for its popularity. Alfalfa is a legume and as such is a well known source of plant protein and a highly regarded soil enricher. Few crops produce protein so efficiently; from three tons of alfalfa hay (an average crop) 625 pounds of digestable protein are obtained. A comparable crop of timothy (1.5 Tons) produces by comparison only 90 pounds of digestable protein; a crop of clover (3 Tons) , 400 pounds and a barley crop (50 Bushels), 200 pounds. In common with most legumes, alfalfa with associated Rhyzobia, fixes appreciable quantities of atmospheric nitrogen and as such plays an important part in maintaining the soil nitrogen balance on the farm. The significance of this at the present time is well portrayed by Wilson in a recent publication.

Alfalfa has a further marked advantage as a forage crop in that it is a perennial and agressive. Once established a stand competes effectively with weeds and in most localities will maintain itself with little or no care for at least 6 - 7 years. Then again the deep rooting habit of many strains of lucerne has established the crop as a drought tolerant species. As such it is well known in the subhumid and arid regions of this continent. Recent selections of winter hardy strains have further advanced its reputation as a crop for cold dry climates of the north and far south.

Although the desirable agronomic features of alfalfa far outweigh the undesirable, the crop has certain limitations which cause the plant breeder concern. For one thing, the plant, as we know it in commerce is a notoriously poor seed setter. Seed, as a consequence is high priced. In recent years, too, attention has been drawn to the increasing importance of crown injury in standard strains and the failure of injured plants to overcome attacks from insects and fungus pests. Then again coincident, with the expansion of alfalfa acreage in the world has come a growing realization of the handicaps in production resulting from such diseases as wilt, leafspot and virus.

The attention of the plant breeders then has been attracted by the recognition of these imperfections and already many programs for alfalfa improvement have been undertaken.

One of the earliest consious attempts at alfalfa improvement through breeding and selection has much of its history laid at the University of British Columbia from 1918 onwards. The early objects of the program was to produce through interspecific hybridization a strain of alfalfa with a crown and root system suitable for subhumid conditions when a relatively high water table prevailed over much of the year. The early work on this project has been adequately reviewed by Moe (23) and only a brief reference will be made to it. With the growing importance of alfalfa in the agricultural economy and a deeper realization of its problems, it is in conformity that the alfalfa improvement programme should change, should broaden in its scope and purpose.

Field records, some complete, some incomplete, are available in the programme for some twenty years of its history. Therefore it seemed appropriate at this time to survey these records in the light of present knowledge. In addition some records taken by the author on the local material in the summer of 1941-42 and 1942-43 were studied.

Principally the report is a study of the association of economic characters in the alfalfa grown at the University of British Columbia. Characters were chosen such as seed weight, seed number, plant height and the like and their association with general characteristics such as seed setting, plant yield and crown characteristics scrutinized. For plant breeding is not the simple selection of desirable characteristics and the mere incorporation of these in a single desirable strain. It is a study in interaction and linkage, complex to a high degree; the combination of features of merit must intimately be represented in many strains and not in one alone and the procedure used in accomplishing this are at once both an art and a science.

How successful this study in association has been, will be for the reader to decide; difficulties such as incomplete records, poor seed setting and the like, have upset the study from time to time. It is hoped however that something has been added to our knowledge of the behaviour of economic characters in the interspecific hybrid alfalfa such as that at the University of British Columbia.

REVIEW OF LITERATURE

At present the most important problem as far as alfalfa is concerned is the improvement of its seed set. A large amount of work on this problem has dealt with the effect of tripping on seed set. Tripping can be brought about by weather, insects or artificial means. Ofall there insects studies Megachile species have been found the most satisfactory. Lejeune and Olson (19) found that honeybees brought about very little tripping. Hay (13) found that the lack of suitable insects and unfavourable weather conditions for tripping contributed to the low seed yield. Clark and Fryer (7), Carlson (6) and Southworth (5) conclude that tripping increases seed yield. Kirk (17) has developed a self tripping (autogamous) alfalfa which gives promise of overcoming this difficulty.

Various studies have been done on hybridizations as a means to increasing seed yield. Dwyer selected high yielding strains by inbreeding, then crossed them to regain vigor. Englebert (10) found that the seed yield of any single hybrid varied with the environmental conditions. A number of papers have been written on the development of an inbred strain which will give a good seed yield Kirk (16) found that seed yield was inherited to a certain degree. Tysdal and Ckark (29), Bolton and Fryer (2) and Southworth (25) found that the general seed yield went down on inbreeding but a few high yielding plants segregated which bred true for this quality.

Self sterility in alfalfa has given rise to many studies. Brink and Cooper (3) found that there were fewer fertile ovules in selfed material and that the lower ovules were rarely fertile. Bolton and Fryer (2) working on pollen sterility divided it into two classes: (a) clear empty grains, (b) normal appearing which did not germinate. Brink and Cooper (3) found a failure of fertilization even after tripping. Pollen tubes were produced and in some cases fertile ovules started to develop but failed to mature. The embryos of low seed yielders developed more slowly and there was a large percentage of abortive ovules. Brink and Cooper (3) found that fertilization was prevented due to abnormal positional relationship between anthers and stigma.

The part of enviromental effect must not be overlooked in relation to seed set. Bolton and Fryer (2) found that soil moisture and the stage, of the seasonal development contributed a great deal to seed set. They found that the normal appearing pollen, mentioned above, would germinate under favorable climatic conditions. Freeman (11) cautions that hereditary factors can only show up to advantage in a suitable environment. Tysdal and Clark (7) emphasize the effect of temperature and light on seed production. Southworth (25) stresses the effect of moisture, he concludes that there should be sufficient moisture to fill the seed after it has set but in the early stages of development abundant moisture will stimulate the development of leaves and height at the expense of seed set. Englebert (10) substantiates Southworth's work, finding that seed set was better in years when there was a limited rainfall in July and poor when there was excessive rainfall for this month.

Hybridization has been used as another method of improving alfalfa. Among the characteristics which have been given special emphasis are disease resistance, root types, winter hardiness, seed yield and weight of plants. The cytology of these hybrids has yielded a great deal of information of interest genetically. The cross in all cases was only successful if M. falacata was used as the pistillate parent. Dwyer (9) found that a cross between M. sativa and M. Lupulina gave a poor forage yield. Southworth (23) in trying to develop a self tripping variety used M. lupilina as the pistillate parent because of its self tripping characteristics. The F1 and F2 gave a great variety of types but a very poor seed yield. In the F3 a few good seed producing plants appear. A few self tripping varieties appeared in the F4 but were not self fertile. It was not until the F6 that one self tripping, self fertile plant appeared.

The flower color in these interspecific cross has provoked considerable interest. The M. falacata is pure breeding yellow and the M. sativa blue. The F1 and succeeding generations give a wide variety of color from white to yellow and deep purples. Burton (5) found that flower color had no positive correlations with any of a number of other characteristics. Hay (13) found that color had no effect on seed set, while Moe (23) on the other hand suggested that white flowered plants were poor seed yielders. Lepper and Odland (20) conclude that flower color in alfalfa was due to three factors.

During the many breeding experiments involving alfalfa, a number of abnormalties have come to light. Lepper and Odland (19) mention a crinkly leaf mutation. Stewart (24) mentions a peculiar vegitative proliferation which replaces the alfalfa flowers. McVicar (22) found white seeds were due to a homozygous recessive factor as a result of the absence of a factor for yellow. Black seed required at least three factor pairs and arose originally as a single gene mutation.

This only very superficially touches on a few of the lines of investigation being carried on with alfalfa. Winter hardiness and disease resistance are problems which are receiving a great deal of attention in other parts of the continent. In this vast improvement work with alfalfa many interesting facts are being brought to light which are contributing greatly to the improvement of alfalfa and plant improvement work in general.

MATERIALS AND METHODS

The materials used in these studies were the highly heterozygous population which resulted from six hybrids of a cross M. falacata (♀) X M. sativa (♂). Details of the hybridization and subsequent treatment of the progeny are given by Moe (23).

A few of the more important features of this plant material might be briefly emphasized. It should be noted that the pistillate parent in the cross was usually the low growing yellow flowered M. falacata Var. Don. and that the pollen parent was a tall purple flowered variety of Grimm one of the Ontario Variegated type. Seed obtained from the hybrids was grown out and six tall growing hybrids were differentiated from the low growing hybrids. The hybrids proved to be considerably fertile and produced some seed in good seed years both from selfed and open pollinated racemes.

The flower color of the hybrids was variegated but showed a preponderance of yellow pigment.

The hybrids were selfed and the seed thus produced was subjected to progeny row tests. The seed was then taken from these selected plants and itself set out in progeny rows. At this time little or no attempt was made to control pollination but there was no opportunity for admixture of pollen from other strains or varieties.

The progeny from the F2 and subsequent generations showed an enormous degree of segregation. Many of the selected plants showed a great variation as to type of growth, vigor and degree of sterility.

Careful selection of individual plant progeny was carried on for five generations but at the end of that time there was still a high degree of segregation; i.e. no stability of type had, as yet been established.

From the F2 and succeeding generations the populations were the subject of a mass selection program. In this work emphasis was placed on seeking a higher yielding alfalfa with the spread characteristics of the M. falacata parent and the quality characteristics of the M. sativa .

Records were taken by the Department of Agronony on many morpholigical characteristics of both the hybrids and plants of subsequent generations. From these records data on spread, height, seed yield, pod shape and flower color were used.

During the summer of 1941 plants were selected at random from the fifth generation material and Roger's alfalfa. From there plants data on pod shape, number of seeds per pod, seed set, foliage color, flower color, flower fall, stem thickness, plant height and degree of leaf spotting, were taken.

The following statistical analysis was used as the best method of organizing and interpreting this data.

EXPERIMENTAL WORK

I Association of Pod Shape and Number of Seeds Per Pod.

(a) Self pollinated alfalfa

It was thought there might be some association between the seed yeild and the size of the pod, i.e. the number of twists in it.

Cooper and Brink (3) have found that strains which produce a large number of seeds continue to do so and strains giving a small amount of seed tend to continue this low seed yeild in future generations. Bottom and Fryer (2) are of the opinion that the number of seeds per pod is a better index of inherent fertility than the percentage of flowers which give rise to fully developed pods. They also state that seed set is due to genetic factors and the number of seeds per pod is a good indication of this inherent capacity. A good seed setting strain will tend to continue this abundant seed setting capacity, even of selfing.

Table I gives the distribution of the number of seeds per pod against the number of twists per pod.

The number in brackets in each case is the expected number the number above the the actual number. The number of twists per pod were divided into three catagories curved (semi-circle), one circle, and more than one circle. The number of seeds per pod were also divided into three categories, 2, 4, and 6 seeds per pod.

Material used for this study was data taken by the author from Rogers (24) alfalfa.

The total number of samples taken here is fifty-nine. A larger number might yeild somewhat different results.

The chi square for 4 degrees of freedom at the 1% level is only 13.28; the chi square for expectation is 28.10. Independence is therefore unlikely and a strong association of highly curled seeds and a large number of seeds per pod is likely.

Table I

	2	4	6	Total
a	16 (9.4)	7 (9.1)	0 (3.5)	23
b	8 (9.4)	14 (9.1)	1 (3.5)	23
c	0 (5.3)	5 (5.8)	8 (2.9)	13
Total	24	26	9	59

a -- curved b -- one circle c -- more than one circle.

(b) Open pollinated

This study is the same as the previous one in all respects except that the material was open pollinated instead of selfed.

Table 2 gives the distribution of the seeds and the shape of the pods. The categories are the same as Table 1.

Table 2

	2	4	6	Total
a	9 (2.5)	2 (4.3)	0 (2.5)	11
b	3 (2.6)	16 (7.5)	0 (7.1)	19
c	0 (5.3)	3 (7.1)	20 (8.6)	23
Total	12	21	20	53

The chi square for 4 degrees of freedom at the 1% level of significence is 13.28 which shows an association between the the number of seeds per pods and the number of twists per pod.

Kirk (17) found that the seed yield upon open pollination was greater than upon selfing but high yeilding strains tended to remain good seed producers whether open or selfed.

As in the previous case the number of samples are small, making these results far from conclusive.

(c) Seed set in grams

Larger number were available for this study. There were 26 progeny rows (open pollinated) from all of the F_1 hybrids (1928), giving a total of 770 plants.

Table 3 gives the distribution of seed yield in grams compared with the shape of the pod. The categories have been enlarged in both cases.

Table 3

Table 3

Seed weight in grams

No. of twists	0-2	2-4	4-6	6-8	8-10	10-12	12+	Total
0-$\frac{1}{2}$	12 (7.7)				1 (.4)			13
$\frac{1}{2}$-1	144 (110.3)	22 (25.9)	8 (4.4)	4 (9.8)	1 (6.4)	2 (6.2)	4 (12.7)	185
1-1$\frac{1}{2}$	190 (199.8)	52 (47.1)	25 (24.8)	18 (17.9)	14 (11.7)	13 (11.3)	24 (23.1)	336
1$\frac{1}{2}$-2	98 (116.5)	30 (27.5)	17 (14.5)	14 (10.4)	10 (6.8)	8 (6.6)	19 (13.5)	196
2-2$\frac{1}{2}$	14 (23.7)	4 (5.1)	7 (2.9)	5 (2.1)	1 (1.4)	3 (1.1)	6 (2.7)	40
Total	458	108	57	41	27	26	53	770

The number of twists bears no statistical relation to the weight of seed set per plant. This however does not imply that there is no correlation between the number of twists per pod and the number or weight of seed set per pod.

In table 4 the categories have been reduced to 3 for yield and 3 for number of twists per pod.

Table 4

No. of twists	Amount of Seed Set (Grams)			Total
	0 - 4	4 -8	8 - 14	
0 - 1	178 (145.5)	12 (25.2)	8 (27.25)	198
1 - 2	370 (391)	74 (67.7)	88 (73.23)	532
2 - 3	18 (29.4)	12 (5.09)	10 (5.5)	40
Total	566	98	106	770

The chi square for 4 degrees of freedom at the 1% level of probability is 13.28.

One might conclude that there is a significant association between low yield of seed and the small amount of twisting in the pod.

In this connection Brink and Cooper (3) found that the lower ovules in the carpel did not develop into mature seeds due to (a) failure of fertilization (b) ovule abortion or (c) infertility of the ovule.

2. Association of Flower Abundance and Number of Seeds

The F_4 plants differed greatly in the number of flowers produced. A chi square was run on this characteristic to see if it had any bearing on the seed yield.

The flower abundance was put in 3 categories, poor, fair and good. The individaul seeds produced were counted and divided into 3 categories 0 - 30, 30 - 60, 60 and more.

Table 5 gives the distribution.

Table 5

Flower Abundance	Number of seeds 0 - 30	30 -60	60 +	Total
Poor	10 (7.9)	5 (4.8)	1 (3.2)	16
Fair	14 (13.3)	11 (8.2)	2 (5.4)	27
Good	10 (12.8)	5 (7.9)	11 (5.5)	26
Total	34	21	14	69

The chi square at the 5% level of probability and 4 degrees of freedom is 9.49.

An association is indicated, however, data are too few to place much confidence in these results. Additional work using larger numbers might yield different results.

Bolton and Fryer (2) are of the opinion that flower abundance is no indication of seed yield. And Cooper and Brink (3) found that tripping increases the number of flowers forming seed.

3. Seed Setting (expressed as seeds per pod per plant) Compared in Racemes Which Have Been Selfed and Open Pollinated.

Rogers (24) alfalfa was used for this correlation. Plants were selected on the basis of flower colour. Six colour designations were given, white, pale yellow, yellow, variegated purple yellow, dark purple, pale purple. Open pollinated racemes were tagged, while self pollinated ones were rolled in the fingers and tagged.

Table 6 gives the seed yield per raceme in number of seeds. There too few seeds to weigh.

Table 6

Seed Setting (expressed as seeds per pod per plant) Compared in Racemes Which Have Been Selfed and Open Pollinated.

PLANT No.	No. Seeds per Raceme (average) Selfed (x)	Open (y)	PLANT No.	Selfed (x)	Open (y)
1	6	2	15	4	1
2	2	3	16	3	5
3	2	4	17	4	1
4	3	3	18	3	1
5	4	4	19	2	2
6	3	4	20	2	2
7	2	2	21	5	5
8	4	3	22	5	3
9	6	8	23	7	3
10	4	2	24	9	2
11	3	3	25	7	4
12	4	3	26	6	6
13	4	3	27	3	1
14	6	4	28	6	3

29	4	4	43	2	4
30	4	4	44	6	6
31	4	4	45	2	2
32	4	2	46	5	2
33	6	2	47	4	4
34	5	5	48	1	2
35	6	2	49	4	4
36	2	2	50	4	3
37	4	4	51	6	5
38	5	6	52	4	2
39	3	2	53	2	2
40	4	3	54	6	3
41	6	2	55	5	4
42	5	1			

One may safely conclude that the open pollinated racemes set more seed per pod than did the self pollinated racemes. The difference, however, is not large and would indicate that the degree of self sterility in the F_5 plants used is not high.

Self incompatability factors etcetera might be set forth and the influence of tagging andlhandling might have been deleterious. Abnormal pollen is as abundant in the open as in the selfed plant material. It could not therefore be a reason for the lower yield in the first case. However, plants which tend to set seed abundantly in the open pollinated material tand to set seed well in the selfed.

The association is not strong biologically although statistically it is highly significant. It would seem therefore that there is some evidence to support the indications of self incompatability factors.

Kirk (17) on comparing self and open pollinated material found a general decrease in yield with selfing, however, a fewe strains segregated which gave consistently good yield. Tysdal and Clark (28) and Clark and Fryer (7) substantiates Kirk's work.

4. Association of Flower Color and Seed Yield

Materials used here were the 1928 records of the F_4 progeny.

There were a great many flower color types exhibited in the field and it was thought that the seed yield might be associated with flower~~s~~ color. Although there were about 15 different color designations they were either predominantly purple or yellow. The flower color was therefore divided into 4 classes on the basis, purple, variegated purple, yellow, and variegated yellow. The seed yield was taken in grams per plant and divided into 4 categories as shown in Table 7

Table 7

F

Flower Color	Seed Yield in grams per plant				Total
	0-1	1-6	6-10	10 -	
Purple	13	15	4	6	38
Var. Purple	17	23	5	7	52
Yellow	2	2			4
Var. Yellow	29	29	6	12	76
Total	61	69	15	25	170

Many ratios weretried to determine the genetics of flower color inheritance but none fitted. No doubt these could be worked out with controlled pollination. Two factors (a) failure of controlled pollination (selfing) and (b) the probability of complex polyploid ratios complicated things.

Lepper and Odland (20) set forth a 3 factor basis for flower color inheritance in alfalfa., They only took their work to the F_2, but had they carried their investigations on to the F_3 they might have found flower color inheritance more complex, i.e. polyploidy was involved.

In connection with flower color and seed yield, Moe (23) found that white flowered plants tended to give a low seed yield while Hay (14) on the other hand, found no association between flower color and seed yield.

Table 8 gives the distribution of seed yield (in grams) and flower color using more color designations than Table 7.

Table 8

Flower Color	Seed yield (Grams per plant) 0-1	1-6	6-10	10-	Total
Blue	7	12	5	2	26
Purple	13	15	4	6	38
Variagated	51	59	11	20	141
Green	7	9	7	7	30
Yellow	2	2			4
White		1			1
Total	80	98	21	35	240

In both Tables 7 and 8 no association was found between seed yield and flower color. In the above studies formation of classes for flower color was difficult due to various field workers idea of color. The flowers tended to change color during the blooming period which made an accurate color designation difficult. In this connection it was noted that the darker colors - blues and purples tended to predominate. Whether this is due to dominance or a greater gene frequency is unknown.

5. Comparison of Seed Yield for Two Successive Years.

A comparison of the seed yield of the progeny of the hybrids for two different years was made.

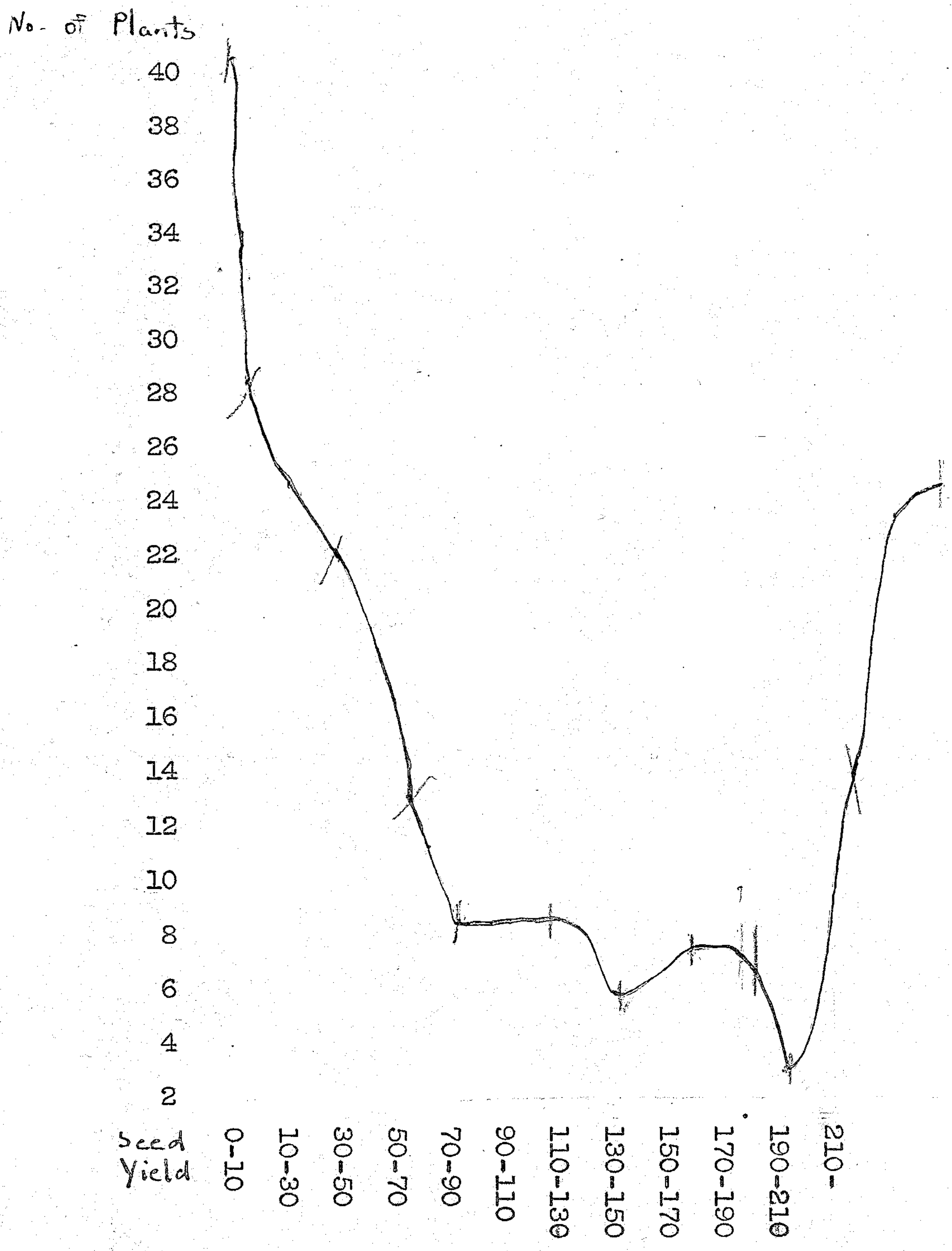
The mean yield for the first year was 1.52 grams and for the second .60 grams.

This lower seed yield is probably due to environmental causes and bears out the work of Hay (14), Bolton & Fryer (2) Freeman (11), Southworth (23) and Englebert (10)

In both years though, good seed yielders tend to yield seed well and poor seed yielders to give poor seed yields. This consistancy of seed yield suggests the operation of heredity factors.

6. Distribution of Seed Yield Classes.

The accompanying graph shows the distribution of plants as to seed yield.



Ordinarily it would be expected to find the seed yield falling in a normal distribution curve. This distribution is highly skewed, there are distinctly more high yielding plants than expected in a normal distribution. This would seem to indicate the operation of hereditary factors.

7 Comparison of Seed Yield, Plant Heights and Plant Weights in F_1 and F_2

The materials used for this study were the original 7 hybrids and their F_1 and F_2 progeny. The seed yield and plant weight were taken in grams and the height in inches.

Table 9 gives the seed yield, plant weight, and plant height for the 7 hybrids, also the average seed yield, plant weight and plant height for the F_2 progeny of the hybrids.

Table 9

Designation of Plants	Seed Yield (g)	Plant Wt. (g)	Plant Ht. (")
Hybrid -7	7.0 1	172	28
" -56	1.4 4	148	24
" -68	0.9 5	123	20
" -71	0.3 6	136	23
" -156	2.4 3	100	24
" -190	3.5 2	263	30
F_2 of H-7	1.5 (ave.) 3	92.4(Ave)	25.4 (ave.)
" " H-56	1.6 " 2	57.2 "	20.3 "
" " H-68	0.8 " 4	55.7 "	19.7 "
" " H-71	0.4 " 6	24.9 "	13.5 "
" "H-156	0.8 " 5	32.7 "	14.8 "
" "H-190	1.7 " 1	48.9 "	14.9 "

Kirk (17) found a reduction of variability in the F_2 with selfing.

The above figures would indicate hybrid vigor. Comparisons of previous tables on seed yields indicate that the seed yield and plant vigor are correlated, i.e. the same factors which are responsible for low seed yield are in all probability responsible for a lessening of vigor. From Table 9 can be seen the striking reduction in height and weight of the F_2 over the F_1 but a few plants in the F_2 gave an increased yield over the F_1.

Throughout there seems to be a small correlation in yield of the F_1 and the yield of their progeny. This may be a significatn observation in terms of their chromosome number.

8. Comparison of Seed Yield in F_1 Plants and F_2 Progeny (Open pollinated prevaling)

The F_1 here used were the original 7 hybrids.

The weight in each case was taken in grams. The seed yield of the progeny is listed in 2 columns, the average seed yield and the maximum seed yield.

Table 10 gives these seed yields.

Table 10

	Hybrid No.	Hybrid Seed Yield (grams)	Progeny Sample Average gms. per plant	Seed Yield Maximum gms. per plant.
	# 7	7.0	1.5	4.7
	56	1.4	1.6	11.5
	68	0.9	0.8	6.0
	71	0.3	0.4	6.2
	56 168	2.4	0.8	4.0
	190	3.5	1.7	7.0
Average		2.56	0.9	6.6

The progeny on the average tended to repeat the seed yielding abilities of the parental hybrids.

The maximum seed yielded by any one individual in progeny bears little relationship to the constitution of its hybrid.

The data were too few for γ_{xy} but the $\gamma_{xy} = +.45$ which was significant for columns a and b.

ASSOCIATION OF FACTORS NOT INVOLVING SEED YIELD.

1. Comparison of Height and Weight Between F_1 and F_2.

Materials used for this study were the weights and heights of the hybrids and the average heights and weights of random samplings of their progeny.

Both Kirk (17), Stewart (26), Tysdal & Clark (28) and Southworth (25) found a reduction of variability on succeeding generations.

Table 12 gives the distribution of height and weight for the F_1 and F_2.

The lower number for the F_2 would point to the action of hybrid vigor. The F_1 certainly gives high figures than the parent M. falacata plant which is low grown and low yielding. Table 12 indicates that the vigor of the plants decreases on selfing, which is shown by the striking decrease in height and weight of the F_2 over the F_1.

Table 12A

Wt. Comparisons			
Hybrid number	Wt. of hybrid (in grams) Plants F.	Av. Wt. of progeny (samples in grams) F_2	Maximum Wt. for progeny sample F_2
7	172	92.4	208
56	148	57.2	167
68	123	55.7	91
71	136	24.9	41
156	100	32.7	76
190	263	48.9	79
Average	157	52.1	110.3

Table 12B

Ht. Comparisons			
Hybrid number	Ht. of hybrid (in inches) Plants	Av. Ht. for progeny sample (in inches)	Maximum Ht. for progeny sample (in inches)
7	28	25.4	32
56	24	20.3	29
68	20	19.7	27
71	23	13.5	20
156	24	14.8	21
190	30	14.9	22
Average	24.8	18.1	25.1

2. Yield and Height

A great amount of data was available for this correlation. The material used was the fourth generation plants from the original 7 hybrids. Plants were selected at random from this large group and correlations run on them. Unfortunately there was only available the data for 1928 making impossible to make a year to study.

The results are givenin Table I

Table I

Correlation Numbers	No. of Plants	Field Designation	Correlation Coefficients
1	33	7-7	+ 0.86
2	32	5-56	+ 0.68
3	31	156-0	+ 0.88
4	31	190-6	+ 0.35
5	30	3-2	+ 0.76
6	30	3-3	+ 0.45
7	28	5-68	+ 0.86
8	30	71-0	+ 0.75

The correlations are all positive and range from + 0.35 to + 0.88. By far the larger number have a high correlation which would seem to indicate that there is a high correlation between height of plant and yield of plant. The same results were obtained by Hacbarth and Ufer (29), Burton (5) and Kirk (17).

This correlation, however, is not absolute as seen in the two values which give + 0.35 and + 0.45.

3. Association Leaf Spotting Incidence and % Ovules Developing on Racemes.

Leaf spotting is very prevalent and is due to a disease Pseudopeziea medicaginis. It was thought that there might be an impairment of physiological activity due to leaf spotting. If there is any it does not manifest itself in the number of ovules developing on the racemes. Table 2 shows the leaf spotting incidence plotted against the % ovule development.

Table 2

Leaf Spot Incidence	0 -50%	50% -80%	80% - 100%	%
1	3 (4.2)	21 (19.6)	11 (11.2)	35
2	6 (4.8)	21 (22.4)	13 (12.8)	40
Total	9	42	24	75

No. 1 denotes high incidence and 2 low. The top value is the actual number while the lower is the theoretical.

The chi square for 2 degrees of freedom at the 5% level of significance is 5.99, whichhindicates that there is no association between the degree of leaf spot present and the number of ovules developing.

4. Association of Leaf Spotting and Leaf Color.

Little is mentioned in the literature regarding the effect that leaf spot may have on alfalfa. Present indications are that it is increasing.

Table 3 gives the distribution for the incidence of leaf spot and leaf color.

Table 3

Leaf Spot Incidence*

		1	2	
Foliage Color	Dark	23 (24.1)	26 (15.4)	49
	Light	15 (13.8)	13 (14.1)	28
		38	39	77

* Leaf Spotting: 1 high incidence
2 med. & low incidence

The materials used here were 77 plants selected at random from the F_4 progeny.

The chi square for 1 degree of freedom at the 5% level of probability was 3.84 . This indicates there is no special significance between foliage color and the amount of leaf spotting.

It should be noted in this connection that a boron deficiency will often give a paler leaf. However, in this case the different colors of leaves could hardly be attributed to nutritional factors.

5. Association leaf spot and Flower Abundance.

Instead of affecting the seed yield directly the leaf spot might have reduced the number of flowers, thereby decreasing the seed yield. A comparison of flower abundance and leaf spotting is given in Table 4.

Random selections from the F_4 progeny were used for these studies.

Table 4

	Poor	Fair	Good	
1	13 (9.8)	13 (14.3)	13 (13.8)	38
2	7 (10.1)	16 (14.7)	16 (14.2)	39
Total	20	29	28	77

The leaf spot incidence was divided into 2 categories 1 denoting high degree of leaf spotting and 2 low. The flower abundance was divided into 3 classes, poor, fair, and good.

The chi square at 2 degrees of freedom at the 5% of probability was 5.99, which would indicate that leaf spotting has not seriously influenced the number of flowers. The number~~s~~ of samples used was small and a different association might be obtained using larger numbers.

6. Association of Stem Thickness and Plant Height.

The materials used for this association were selections from Rogers (24) alfalfa. 75 plants were selected at random and a chi square run on these. The stem thickness was given 3 designations, thin, medium, and thick; the height was also divided into 3 categories, 25 inches and less, 25-30 inches, and 30 inches and more. Table 5 gives the distribution.

Table 5

Plant Height(Inches)

		0 - 25	25- 30	30 & over	Total
Stem thickness	Thin	18 (8.9)	3 (7.5)	0 (4.48)	21
	Medium	13 (14.9)	18 (12.6)	4 (7.4)	35
	Thick	1 (8.1)	6 (6.8)	12 (4)	19
	Total	32	27	16	75

The chi square at the 5% level of probability and 4 degrees of freedom was 9.49 which would indicate avery strong association between thickness of stem and plant height. However, it might be possible to select a tall growing plant with a thing stem: Burton (5) found a strong association between height and number of stems.

7. Association of Height and Spread.

Materials used were random selections from the progengy involving 500 plants. Records were taken by the Department of Agronomy in the early summer.

Kirk (17) and Armstrong and White (1) found a positive correlation between these 2 characters.

Table 6 gives the distribution.

Table 6

	xy
1-17	+.04
2-15	-.13
3-13	+.03
4-3	+.30
5-1	+.16
6-7	+.15
7-9	+.35
8-25	+.24
9-28	+.58
10-19	-.07
11-15	+.23
12-11	+.26
13 -21	+.23
14-23	+.24

The correlation is positive but low though in some lines the association is quite strong. It should therefore be possible to select tall growing plants which are spreading.

The stage of growth at which these correlations are taken is important. The above mentioned plants were relatively mature, earlier correlations may have been better.

8. Association of Flower Abundance and Raceme Supporting Ovules.

It was thought that the flower abundance would have a considerable effect on seed development. The following studies deal with the effect of flower abundance on various characteristics affecting seed yield.

Table 7.

FLower Abundance	Racemes % Remaining.			
	0-50	50-80	80-100	Total
Poor	0 (2.63)	13 (11- 37)	7 (6.1)	20
Fair	3 (3.4)	15 (16.9)	12 (9.1)	30
Good	7 (3.4)	15 (14.2)	4 (7.8)	26
Total	10	43	23	76

The flower abundance was divided into 3 categories, poor, fair, andgood and the percentage of flowers remaining were divided into 3 classes according to percentage 0-50%, 50-80%, 80-100%. There is no association between the flower abundance. and the number of racemes remaining. The chi square at the5% level of significance is 9.49 which indicates no relationship.

9. Association of Foliage Color and Flower Abundance.

Rogers alfalfa (24) was used for this correlation. The foliage color was divided into light and dark and the flower abundance into 3 classes poor, fair and good.

Table 8

Foliage Color	Flower Abundance Poor	Fair	Good	Total
Dark Green	10 (12.0)	18 (17.7)	19 (17.1)	47
Light Green	9 (6.9)	10 (10.2)	8 (9.8)	27
Total	19	28	27	74

As would be expected there was no relationship in this association. The chi square for 2 degrees of freedom at the 5% level of probability is 5.99.

Here again the possibility of the effect of a boron deficiency must not be overlooked.

10. Association of Flower Abundance and Flower Fall.

In some cases there were a large number of flowers that fell early in development i.e. long before pods had begun to form. In Table 9 the flower abundance is divided into 3 classes poor, fair and good, and the amount of flower fall into 3 classes 0-50, 50-80 and 80 and over.

Table 9

Flower Abundance	Amount of Flower Fall			Total
	0- 50	50-80	80-	
Poor	(2.63)	13 (11.3)	7 (6.1)	20
Fair	3 (3.9)	15 (16.9)	12 (9.1)	36
Good	7 (3.4)	15 (14.7)	4 (7.8)	26
Total	10	43	23	76

Materials used were random selections from the F_4 progeny.

The chi square at the 5% of probability and 4 degrees of freedom is 5.81 which would indicate no association.

FLOWER COLOR INHERITANCE STUDIES

Flower Color Inheritance Studies in Alfalfa.

The flower colors have been placed in 15 categories. Although the variegated colors are predominately blue or purple there appeared variegated flowers which were basically yellow or green or a mixture of this with blue and purple.

The material used was the F_2 of the original 7 hybrids. Table 1 gives their distribution. The first column indicates the hybrid from which the plants were derived.

Table 1

F_1 Parent	Blue	Purple	Var. Blue	Var. Purple	Var. Purple-blue	Var. Pale-Purple	Variegated	Variegated Green-blue	Var. Green-purple	Var. Green	Var. Yellow-blue	Var. Yellow-purple	Var. Green-Yellow	Var. Yellow	Variegated Purple-White
# 7		2	3	3	3		13							12	
# 56	1	3			6	1	20			1				1	2
# 68		3	2	2	2	2	2	3	1	1			3	5	
# 71					1		6	4		1	2		2	13	
# 156		1					8			1			8	12	
# 190		1					7				1	1	18	3	

The designation of actual colors presented a great difficulty. Some plants were different colors during the various stages of their blooming period.

A large number of ratios were tried but none fitted.

Color inheritance is probably further complicated due to the polyploid nature of the material.

In table 2 the color classeswere cut down to 3. The flowers wer basically purple, green or yellow and were classified on that basis.

Table 2

Parent Plant	Purple & Blue	Green	Yellow
#7	21	0	12
#56	30	1	1
#68	17	8	5
#71	8	7	13
#156	10	9	12
#190	8	20	3

Lepper and Odlands (20) put flower color inheritance in alfalfa on a 3 factor basis. Their hypothesis was not disproved.

However, "pure yellow" occurs much less frequently than expected. Out of 185 F_2 plants, whites and yellows are not recovered at all. They are recovered fairly frequently in later generations. Pollination control may not have been satisfactory.Either some crossing may have taken place or the numbers of progeny received were not sufficient for accurate conclusions. The hybrids are polyploid and therefore not likely to yield many yellows and whites till later generations.

The general segration distribution, however, suggests an inheritance pattern involving several principal mendelian factors, with factors for purples and blues epistatic to those for yellow and white.

DISCUSSION

The results obtained from this work are of interest in relation to the practical bearing which they may have on the problem of alfalfa improvement. When selection is desired for a certain character, it is often desirable to determine, if possible, which other characters of the plant, if any, are associated with it. By selecting for one, it may be possible to secure the other also. However, in crop improvement work a breeding program must go hand in hand with correlation studies.

It is possible to generalize on the possibilities of inbreeding and hybridyation as a means of improving alfalfa. As in most normally cross fertilized crops there is a reduction of vigor upon inbreeding. However, some lines showed no lessening of vigor upon self-fertilization, which seems to indicate that the differential effect of self-fertilization is due to the genetic constitution.

In this investigation emphasis has been placed upon seed production. At present the major problem is to develop a vigorous plant which is also a good seed yielder. An abundance of flowers would appear to have some bearing on the final seed production. Many factors both genetic and environmental affect these characteristics of the plant. High and low fertility and vigor are apparently inherited. However, environmental conditions must be satisfactory before a plant will function to its optimum. It is hoped that the correlation established, both positive and negative, between seed yield and other characteristics will prove of help in future alfalfa improvement work.

Pigmentation does not appear to have any effect on any of the characters studied. Both flower color and the degree of pigmentation of the leaves yielded no definite associations.

Leaf spot was the only disease of which there was any evidence. Other than its appearance on the leaves it did not appear to have any effect either morphologically or physiologically.

In some of these studies sufficient numbers were available but in others due to the lack of time and facilities, the number of samples taken were few. It should therefore be cautioned that in these cases where limited numbers were used conclusive results are impossible. Further work may yield different conclusions.

CONCLUSIONS

Selection within self fertilized lines appears to provide a primary mode of attack for the breeding of improved varieties of alfalfa. As indicated in these and other studies there is a general reduction of vigor upon self pollination. However, a few plants retain their good characteristics even upon selfing. When superior inbred strains have been obtained which are vigorous enough to replace the heterogenous variety now grown, the breeding program becomes relatively simple. These studies show that there is a general reduction of seed yield, plant yield and plant height between the F_1 and F_2. But a few plants tend to retain the good qualities of the parent.

Seed yield was studied in some detail and it was shown that high yielding plants had a greater number of twists per pod. Open pollination and good weather had a beneficial effect on the amount of seed set. The abundance of flowers seemed to be an indication of final seed yield i.e. an abundance of flowers would give a good seed set. However, due to the small number of samples involved and some difficulties encountered in the field, this result can by no means be taken as absolute. Leaf spot and flower color, on the other hand, gave no association with seed yield at all. Self fertilization tended to decrease the seed yield. Whether handling of the racemes in the processes effecting fertilization increased or decreased the final seed yield, is uncertain.

These results indicate that flower abundance is no indication of a superior plant. Associations of this character with foliage color, leaf spot and percentage of ovules developing on the raceme gave no positive results.

The stem, on the other hand, is a fairly reliable indication of a superior plant. Correlations between the height of the stem and stem width, plant yield and spread all gave positive results.

Leaf spot did not appear to hinder the activity of the plant at all. There was no association between leaf spot and flower abundance, leaf color or percentage of ovules developing on the raceme.

Several unsuccessful attempts were made to reach some conclusion regarding the mode of flower color inheritance. Various workers have suggested hypothesis for flower color inheritance of alfalfa but the author was unable to fit any of these to her results.

It is hoped that this work will contribute something to future alfalfa improvement work. It was unfortunate that in some cases data was very limited. These results can only be used as indications. However, further investigation using larger numbers may contribute some definite conclusion.

SUMMARY

I There was a significant association between pod shape and number of seeds per pod for self pollination material.

2 There was a highly significant correlation between number of seeds per pod and pod shape in the open pollinated material.

3 Low seed yielding plants tend to have straight pods; high seed yielding plants tend to have twisted pods.

4 There is probably some association between many flowers with a large seed set but data are too scanty to put much reliance on it.

5 There is a significant difference in seeds

(a) per pod between self and open pollinated racemes.

(b) there is a significant correlation between pods with abundant seed inboth open pollinated and self pollinated.

6 There is no association between flower color and seed yield.

7 Comparisons between seed yield of successive years showed a decline. However, a good seed yielder tended to remain good seed yielders.

8 There are distinctly more high yielding plants than expected in a normal distribution curve.

9 Comparisons of seed yield, plant yield and plant height between the F_1 and F_2 showed a reduction in general.

10 There is no association of flower abundance and flower color.

11 There is no association between leaf spotting incidence and flower abundance.

12 There is no association between flower abundance and the percentage of ovules developing on the racemes.

13 There is no association between flower abundance and flower fall.

14 There is no association between flower abundance and leaf color.

15 There is a strong correlation between stem thickness and stem height.

16 There is a positive correlation between plant height and plant yield. The positive correlation is high but not absolute.

17 There is a correlation between plant height and spread.

18 There is no association between leaf spotting incidence and leaf color.

19 There is no association between leaf spotting incidence and the percentage of ovules developing on the racemes.

20 No conclusions were arrived at regarding the mode of flower color inheritance.

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