BREEDING DISEASE RESISTANT STRAINS

OF PHASEOLUS VULGARIS

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THESIS

Respectfully submitted in partial fulfillment
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THESIS

-Plate I-

-The F_1 Plants 1917.

-T A B L E   O F   C O N T E N T S-

I - INTRODUCTION

Plant pathology or the study of plant diseases has been the subject for a great deal of original research work. Both the cause of disease in plants and their control or elimination have been carefully investigated.

The pathologists have found that certain types of diseases do not readily subject themselves to control or eradication by the practical means available to the producer of our commercial plant products.

Plant breeding, or the improvement of plants by selection, has also been the subject of investigation since man first recognized that variation occurred in the plant forms.

Hybridization has produced the science of genetics during the past twenty years. In the study of plant variation and inheritable characters, geneticists have recently found that strains or varieties of the same species vary in their ability to resist and evade disease attack. In the past few years considerable attention has been given to the production of varieties of farm crops which are resistant or immune to their disease enemies.

The purpose of this investigation is to develop a white pea bean that will carry the commercial importance of the white pea bean trade and the disease resistance of the Wells Red Kidney bean.

II - PREVIOUS INVESTIGATIONS

The most important previous work, bearing on this investigation, is that of Barrus (1915) in demonstrating the practical resistance of the Wells Red Kidney bean, and the work of Emerson (1901-03-05-08-09) in his studies on bean hybrids.

The work of several investigators at the Cornell laboratories on bean diseases and the securing of resistant strains by hybridization started at about the same time as did this investigation now being reported. A report of the Cornell work in which they secured resistant strains by hybfidization has recently been published in Phytopathology and supports many of the conclusions given in this thesis. Burkholder (1918) was able to obtain a resistant Marrowfat strain. McRostie (1919) working with Wells Red Kidney and Robust began his work in the fall of 1916, while the writer using the same materials started work in the summer of 1916.

McRostie studied the inheritance factor of bean anthracnose resistance, and incidentally was looking for a commercial white pea bean resistant to the disease. In his work McRostie started in with the F_1 and tested each succeeding generation for resistance. He and also Burkholder found that resistance was dominant and that approximately seventy-five percent of his F_2 were resistant; twenty-five percent of which were homozygous and fifty percent heterozygous. McRostie states in conclusion that he has been able to isolate several resistant types of white pea beans, but that considerable work is necessary before a commercially important resistant white pea bean can be properly fixed to breed true.

The work of Reddick and Stewart (1919) in their investigations on bean mosaic has established another economic phase of this particular investigation, in that they have determined that the Robust bean is resistant to the mosaic disease, so that strains from the Wells Red Kidney by Robust cross have the possibility of being resistant to both anthracnose and mosaic.

III - MATERIALS AND METHODS

In undertaking this problem the writer has followed the suggestions of Professor F. A. Spragg in selecting varieties

and in method of procedure in the investigation. In determining to what extent the work has been successful, the writer wishes to acknowledge the cooperation of Dr. G. H. Coons, Pathologist, who kindly consented to give the desired strains a test as to their resistant qualities, and whose report is incorporated as a part of this thesis.

The varieties used in the first attempt at this problem consisted of some twenty-eight varieties and strains then on test by Professor Spragg; reciprocal crosses were made and in each case Wells Red Kidney was one of the reciprocals.

A - Varieties Used

The varieties used in the final attempt were Wells Red Kidney, Robust and California Yellow Eye. The following is a complete description of these three varieties:-

I - Wells Red Kidney

This is a typical bush type, green podded, self colored, red kidney bean, which Barrus (1915) demonstrated to be resistant to the A strain of Colletotrichum lindemuthianum, and practically resistant to the F strain. These two strains, according to Dr. Barrus, constitute the occurring disease under any trials that he has made covering a wide range of distribution.

2-Robust

This is a typical navy pea bean of the intermediate indeterminant sort, having originated as a single plant

selection by Professor Spragg. McRostie (1919) states that Dr. Barrus reports it as being resistant to the F strain of Colletotrichum lindemuthianum, but as being very susceptible to the A strain. Reddick and Stewart (1919) report it as being resistant to bean mosaic.

3-California Yellow Eye

This is a self color yellow pea bean of the intermediate indeterminate sort. The name seems to be a misnomer, as the bean has no distinct eye color, but is a sulphur color. To the best of our knowledge it is a pure line, but has not been tested for its resistant qualities. It was used in this investigation because of its reputed excellence as a cooking bean.

B-Method of Investigation

The method pursued in this investigation of obtaining resistant strains of beans has been somewhat different from other investigators working with similar material, in that they have subjected their early generations to epidemics of the disease, and then proceeding to isolate desirable commercial strains from these resistant types. The writer has attacked the problem in reverse order to this, that is, by first selecting out of the segregating progenies desirable commercial types, and then applying the disease test. In this way as soon as a resistant strain has been found among the desirable commercial types it is a simple problem of increasing for distribution.

IV - TECHNIQUE OF HYBRIDIZATION

The method of hybridizing is so well understood that it hardly seems necessary to go into detail, and yet every plant and every new set of field and climatic conditions gives to each problem and to each investigator an individual case. Beans are naturally self-fertilized, though the writer observed a condition reported by Techermak and others, which accounts for some cross-fertilization in beans. In a few instances, cases were observed where the pistile had penetrated the keel of the flower and thus become separated from any chance of receiving its own pollen, and liable to receive the wind blown pollen of mature flowers, or contamination from the pollen carried by visiting insects.

A - Hybridizing in the Field

The problem of securing "sets" is a good deal more difficult under field conditions than under greenhouse conditions. Under field conditions so much depends on the weather. When the writer arrived at East Lansing in July of 1916 to begin this hybridizing, the weather was decidedly unfavorable,as a great many of the normal flowers were turning yellow and dropping because of excessive heat. The emasculated flowers were even more susceptible to the heat and considerable difficulty was encountered before any pods would set.

During the first three weeks crossing was attempted on a great many flowers without obtaining a single pod. In desperation an attempt was made to use a weak sugar solution (one-half of one percent) to aid in securing fertilization. Extreme care was used to emasculate just before the anthers would normally discharge their pollen, in order to faciliate fertilization. Fresh pollen was collected at frequent intervals but with no avail. In early August there came a week of cool, moist weather and the difficulty was solved as the pods began to set. A total of fifteen pods was obtained during the summer from five hundred and six attempted crosses. Because of the lateness of the cool weather and of the sets obtained, fourteen of the fifteen were caught by frost before reaching maturity; and the one that matured proved to be a self, so that the summer had given some valuable experience in technique but no beans.

B - Hybridizing in the Greenhouse

Realizing that it was necessary to get some crosses made during the winter if the problem was to get underway, arrangements were made with the Lansing Floral Company of West Barnes Avenue, Lansing, Michigan, for space to make some winter plantings, no space being available in the college greenhouse.

On October 17, 1916, sixty-five pots of beans were planted in three inch pots, filled with ordinary greenhouse potting soil, and these pots set in flats and filled in around with dirt to better hold the moisture. The sixty-six pots were planted as follows:-

25 pots of 630302. (Robust)
13 pots of 60901. (Wells Red Kidney from Cornell)
12 pots of Crimson Rummer.
4 pots of 613801. (California Yellow Eye)
4 pots of 613902. (California Yellow Eye)
4 pots of 614102. (California Yellow Eye)
4 pots of 614103. (California Yellow Eye)

On January 20, 1917, seventy-three additional pots were planted as follows:-

20 pots of 60501 - 3 - 4 - 6. (Wells Red Kidney)
20 pots of 613700. (California Yellow Eye)
28 pots of 630501. (Robust)
5 pots of Crimson Rummer.

With the controlled conditions of the heat and moisture in the greenhouse it is a much easier matter to

get "sets" than in the field, except as the weather happens to be favorable out-of-doors. Some difficulty was encountered in the greenhouse because the house was used largely for the propogation of flowers and was usually too cool for ripening beans. The technique was the same as used in the field in the summer, except no sugar solution was used.

Crossing was faciliated by placing the plants side by side, thus saving the carrying of the pollen of the male plant around. A special pair of forceps, obtained from a dentist's set, was used, which enabled the removing of anthers from the mother flower with a minimum of mutilation. A camels-hair brush was found convenient for dusting on the pollen, care being used to avoid any contamination by sterilizing when changing pollen parents.

V - DATA

In working up the data of this thesis it has been found rather difficult to offer it in table form, so that most of it is given by steps in the order in which the results were obtained.

A - Breeding

The method of hybridizing having been discussed, we are now concerned with the results obtained from the

crosses made in the greenhouse during the winter of 1916-17.

I - Type of Cross Made and Pods Set

Mother Parent		Male Parent	Number of Pods Set
613902	x	630501	1
613902	x	Crimson Runner	1
60901	x	630501	1
60503	x	630501	5
60503	x	Crimson Runner	2
630501	x	605 (Tag blurred)	1
60504	x	630501	4
630501	x	Tag blurred	2
630501	x	60506	2
60506	x	630501	1
613801	x	630501	2
60504	x	Crimson Runner	4
60506	x	613801	1

In the spring of 1917 those plants in the greenhouse that had crosses setting on them were taken up and transplanted in the alfalfa nursery plat east of the M. A. C. Dairy Building. The writer found it necessary to give up his investigation for a time and, on leaving,

arrangements were made with Professor Spragg to have the beans harvested when mature and planted. The writer was unable to return to this investigation until the winter of 1918-19. In the meantime Professor Spragg had kept the material going, having raised the F_2 seed in 1917 and the F_3 seed in 1918. The writer was stationed at the Upper Peninsula Experiment Station during the summer months of 1919, 1920 and 1921, but was at the college during the winter months, at which time the bean material was studied and the next year's seed made ready. By this arrangement much more complete data has been obtained than is usually possible in an investigation of this kind.

2-Accession Numbers Assigned

Early in June, 1917, the F_1 beans were harvested and given accession numbers. They were then ready for planting. A register was made up and the following

notes taken:-

Register Number.	Cross	Accession No.	Type of bean.	Color of bean.	Date planted.	Date harvested.
728000	60506 / 630501	165	Navy	White	6/22	10/5
728100	630501 / 60504	166	Kidney	Red	6/22	10/13
728300	613801 / 60506	167	Kidney	Red	6/22	10/15
728300	630501 / ? Tag blurred	168	Navy	White	7/13	Frosted
728400	60501 / 630501	169	Kidney	Colored	7/13	Frosted
728500	60506 / 630501	170	Kidney	Colored	7/13	Frosted
728600	? Tag blurred / ? Tag blurred	171	Navy	White	7/13	Frosted
728700	60506 / 630501	172	Kidney	Colored	7/13	Frosted

It is observed that five of the plots were caught by frost and only three were harvested. Of these three, accession number 165 proved to be a self, as it bred true to the Robust type in 1918.

There were left two crosses with which this paper is concerned. They are accession number 166, the Robust pollen on a Wells Red Kidney flower and accession number 167, California Yellow Eye pollen on a Wells Red Kidney flower. These two crosses will be referred to as

accession 166 and 167, through succeeding generations of the resulting progeny.

3-Progeny of F_1--1917

a. Accession 166--Ten red Kidney beans produced ten red kidney viney plants. The F_2 seeds had the appearance of red kidney beans because the F_1 plants that produced them were of the Red Kidney type and, since the bean seed coat is mother tissue, they appear to be red kidney.

b. Accession 167--Three red kidney beans produced three vine plants that developed 120 F_2 seeds.

4-Progeny of F_2--1918

a. Accession 166--Eight of the 10 F_1 plants were selected in 1918. The seed of each selected plant was planted in a separate plot. In all there were 550 F_2 seeds planted. Some of these produced bush type and others indeterminate plants. There were in all 4,655 F_3 seeds saved from these F_2 plants.

The writer, returning to the investigation during the winter of 1918-19 had an opportunity of studying the F_3 seed in detail. One of the interesting characters

-Plate II-

The 1919 Bean Nursery.

The F_3 plants of accession 166 and 167 are located at the back of the nursery.

brought out in this generation is a black color factor. Emerson explains this condition by assuming that the white bean carries either the pigment factor or the color factor; in the absence of one the pigment fails to develope. The red kidney carries another color factor and the pigment factor. In the recombination of characters any bean that carries the black pigment factor and also the color factor would develop into a black bean. In working up these F_3 seeds for 1919 planting, something of the complexity of the problem could be ascertained by the great diversity of types into which this generation could be grouped.

b. Accession 167--The seed of the three F_1 plants were planted in separate plots in 1918. The one hundred and twenty F_2 seeds produced vine type plants that developed only 370 F_3 seeds. Many of these F_2 plants were very weak and failed to mature seed which accounts for the small increase in this particular cross.

5-Progeny of F_3--1919

a. Accession 166--Of the 151 F_2 plants saved in 1918 for 1919 planting, eighteen produced white beans, sixty-five produced red kidney and sixty-eight produced some

other color of bean. Since the problem was to select out desirable commercial types, all of the variable characters had to be considered. Many of those that had apparently become homozygous for color were still heterozygous for size and shape, also heterozygous for bush and vine character. In arranging the Register for the 1919 planting, the F_2 plants of 1918 with their progeny of F_3 beans were grouped together for the purpose of field study into homozygous whites, homozygous reds and heterozygous other colors. Since part of the population had been dropped it was not necessary to classify them into any Mendelian ratio. Something over 4,000 F_3 plants were planted in 1919. The plants were carefully studied during the summer of 1919. It was observed that many of the strains had apparently taken on all of the weak characters of both parents. These strains were dropped because they gave very little if any promise of having commercial value. Fifty-three of the 151 plats planted in this year were discarded in the field. Ninety-three strains, representing 1260 F_3 plants were harvested for study during the winter.

b. Accession 167--The 370 F_3 seeds of 1918 developed something over 300 F_3 plants in 1919. Many of these plants were very weak and failed to produce seed. Only fifty-eight

-Plate III-

The 1920 Bean Nursery. Many of the apparently desirable strains of 166 and 167 were retained for increase.

plants were harvested for study during the winter.

6-Progeny of F_4--1920

a. Accession 166--With the evidence of some strains having segregated into a homozygous condition for the more important characters involved, it seemed desirable to study the material during the winter of 1919-20 with the view of making the final test to determine whether or not resistance had been fixed in any of the types that gave evidence of having commercial value. The 1,260 F_3 plants of 1919 were classified according to the way in which the characters had segregated into a fairly well fixed type. It was found that 188 were intermediate white navy type, 175 were bush type red kidney, 897 were still breaking up, some were white navies on bush plants, some red kidneys on vine plants, and in fact all gradations that we would expect coming from a population of this sort. Seventy progenies of F_3 plants, that were apparently breeding true, were turned over to Dr. G. H. Coons, Pathologist, to be tested for their resistance to disease. These F_4 seed were tested during the summer of 1920 by Dr. Coons and under date of September 13, 1920, he submitted a report as to their performance.

b. Accession 167--The fifty-eight F_3 plants and their progeny of F_4 seed of this cross were carefully studied during the winter of 1919-20 to determine whether or not this population should be discarded or preserved for further study. Nothing of importance had come out of this cross in the previous generation, but in studying these F_3 plants it was observed that some of them were producing white F_4 seed. Since this is a cross between two pigmented varieties and since the California Yellow Eye is reputed to have exceptional cooking qualities, it was thought that a white strain might have some commercial importance, so that this population along with the remnant of the 166 was turned over to Professor Spragg for further consideration as a station problem.

B-Disease Resistance

It is only in the past few years that investigators have been working on this problem of disease resistance. Even yet it is not very clearly understood as to what constitutes resistance. Fortunately there has been considerable work on the bean diseases in the past few years. McRostie (1919) states that there is a single factor difference between resistance and susceptibility to bean

anthracnose and that resistance acts as a dominant. If this is the case, then we would expect it to act as any other single factor difference and under autogamous no selection, segregate itself into homozygous pure lines at about the fourteenth generation. By applying the formula $\frac{2^{n-1}-1}{2n}$ where n refers to F_n, we find that in the fourth generation we have a ratio of seven homozygous resistant to two heterozygous resistant to seven homozygous susceptibles.

By analyzing Dr. Coons' report, we find 55 showing resistance and 15 susceptible to bean anthracnose. If these 70 strains have been uninfluenced, so far as resistance susceptibility is concerned, by the selections previously made and if they are in the fourth filial generation, so far as this disease character is concerned, the genetic expectation would be that approximately 39 would show resistance and 31 susceptibility. If this is the case we would expect that 16 of the 55 on further test would prove to be susceptible. Considering the data from another angle, it is probable that in discarding certain strains from time to time, whole series of homozygotes have been eliminated and probably some of the seventy strains tested are actually in the F_2 generation so far as the segregating of this disease character is concerned. We find in the F_2 generation, the genetic expectation would be 52.5 showing resistance and 17.5 showing susceptibility.

-Plate-IV-

The 1920 Bean Nursery at harvest time. The vacant spots in the rows show where the desirable strains have been harvested. The bulk of the material was found unsuited for further increase.

It is probable that all of these influences are at work and the population does not coincide with any one genetic expectation.

REPORT ON PUTNAM'S BEAN CROSSES-SEPT. 13, 1920

Made by Dr. G. H. Coons

Register No.	Anthracnose	Blight	Yield	Maturity	Type	Remarks
90203	0	+	Good	Good	Navy	Save
90206	0	+	Heavy	Fair	Navy	Save
90213	0	+	Fair	Poor	Navy	
90219	+	+	Poor		Navy (Broad) (Pods)	Two plants only.
90402	0	+	Poor			No pods set. One plant.
90515	0	+	Excellent	Good	Navy	Broad Pods
90508)) 90501)	One poor plant only on each.					
90417			Poor		Navy	Stand Poor
90412	+	+	Poor	Late		Leaves lived on vines.
90407	0	+	Heavy	Late	R. K.	
90524	0	Slight	Fair	Late	R. K.	
90702	0	Severe	Poor	Late	R. K.	
90811	+	+	Poor	Fair	R. K.	
90708	0	+	Heavy	Fair	Navy	
90713	+	One weak plant.				
90716	+	Few weak plants.				

Register No.	Anthracnose	Blight	Yield	Maturity	Type	Remarks
90801	on 3 pods	Slight	Good	Good		Strong plants by leaves (out)
90806	0	+	Low	Fair	R. K.	
90814	0	+	Poor	Late		Low growth
91104	0	0	Low	Fair	R. K.	
91107	+	0	Fair	Late		Upright.
91111	0	+	Good	Late	Navy	
91115	+	+	Fair	Fair	R. K.	
91402	0	+	Fair	Good	R. K.	
91406	0	+	Fair	Good	R. K.	
91409	0	+	Fair	Good	R. K.	
91414	0	+	Good	Late	Navy	O. K.
91602	0	+	Low	Early	Navy	
91801	0	+	Fair	Early	Navy	
91805	0	+	Fair	Good	R. K.	
91811	0	+	Low	Early	Navy	
91821	0	Slight	Low	Early	Navy	
92704	+	+	Fair	Fair	R. K.	
92713	0	+	Good	Good	R. K.	
93208	+	+	Poor		R. K.	
93211	+	+	Poor		R. K.	Out.
93213	+	+	Fair	Late		Three strong plants.
93302	0	+	Good	Good	R. K.	
93309	0	+	Good	Good	R. K.	

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Register No.	Anthracnose	Blight	Yield	Maturity	Type	Remarks
93507	0	+	Good	Good		
93511	+	+	Good	Late	R. K.	
95003	0	Very Severe	Poor	Late	R. K.	Out.
95005	0	Severe	Poor	Late	R. K.	Out.
95008	+	+	Poor	Late	R. K.	Out.
95009	0	Severe	Fair	Late	R. K.	
95506	0	+	Fair	Late		One plant only
95508	0	Severe		Med-early	R. K.	Out.
95509	0	Severe	Good	Fair	R. K.	Strong growth.
97001	0	Severe	Poor	Early	R. K.	Out.
97009	0	+	Fair	Fair	R. K.	
97304	0	+	Poor	Late	R. K.	
97308	0	+	Good	Good	R. K.	
97317	0	0	Poor	Late		One plant only.
97404	0	+	Fair	Late	R. K.	4 plants only.
97408	0	+	Poor	Late	R. K.	
97409	+	+	Good		R. K.	
97601	0	+	Good	Early		
97605	0	+	Fair	Fair	R. K.	
97608	0	Slight	Good	Late	R. K.	Sturdy plants.
97613	0	+	Excellent	Good	R. K.	Sturdy plants.

Register Number	Anthrac-nose	Blight	Yield	Matur-ity	Type	Remarks
97701	+	+	Poor	Late	R. K.	5 weak plants.
97708	0	+	Poor	Fair	R. K.	Do
97805	0	+	Fair	Fair	R. K.	4 fair plants.
97807	0	+	Poor		R. K.	7 weak plants (out)
97902	0	+	Poor		R. K.	Small (out)
97905	0	+	Good	Late	R. K.	
97907	0	+	Fair		R. K.	Slight viney ten-dency. Strong growth
97908	0	+	Good	Late	R. K.	
Common Beans	On pods	+	Fair	Near-ly mature now	Navy	
Mexican Tree	+	+	Good	Fair		
Robust	+	+	Fair	Late		
Wells Red Kidney		Severe	Good	Good		

CONCLUSIONS

In this investigation two very difficult problems have been encountered--that of being able to select out a strain that has retained the desirable commercial qualities of the Robust and then of having such a strain show the resistance of the Wells Red Kidney. Bringing the inheritance of two such distinct types together for recombination involves so many pairs of characters that it is necessary to study such large classes of individuals through so many generations in order to obtain the desired combination, and when such a commercial type is fixed it is still a problem to select from this the desired resistant type.

It has been fortunate that circumstances have made it possible for the writer to follow this cross through four generations instead of but two as would ordinarily have been the case had it been possible to finish up the work on schedule time.

The one year's disease report is not sufficient to say definitely just what strains are homozygous for resistance, but with the work already reported by other workers on similar material in obtaining resistant strains and with several strains of desirable commercial characteristics fixed, it is believed that some of the strains developed in this investigation may have the desired qualities for which the problem was intended.

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