

FACTORS RELATED TO THE ISOLATION OF HARD-SEEDED
STRAINS OF CRIMSON CLOVER

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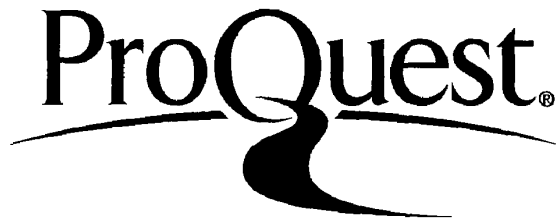
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APPROVAL CERTIFICATE

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STRAINS OF CRIMSON CLOVER

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TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
III. EXPERIMENTAL PROCEDURE	9
I. ANATOMY OF THE SEED COAT	9
Methods and Materials.	9
Results and Discussion	10
II. EFFECT OF INBREEDING	15
Methods and Materials	15
Results and Discussion	17
III. PHYSIOLOGICAL FACTORS	68
<u>SEED SIZE</u>	68
Methods and Materials	68
Results and Discussion	69
<u>MOISTURE SUPPLY</u>	71
Methods and Materials	71
Results and Discussion	72
<u>HUMIDITY</u>	75
Methods and Materials	75
Results and Discussion	78
<u>LIGHT INTENSITY</u>	81
Methods and Materials	81
Results and Discussion	81
<u>FLOWERING SEQUENCE</u>	83

CHAPTER	PAGE
Methods and Materials	83
Results and Discussion.	83
<u>NUTRIENT LEVELS</u>	91
Methods and Materials	91
Results and Discussion	93
<u>MOISTURE CONTENT OF SEED</u>	97
Methods and Materials	97
Results and Discussion	98
IV. SUMMARY AND CONCLUSIONS	100
V. LITERATURE CITED	104
VI. APPENDIX	108
A. GERMINATION OF CRIMSON CLOVER PLANTS	
SELFED IN 1944	108
B. RELATIVE HUMIDITIES AT ATHENS, GEORGIA	
DURING PERIODS OF SEED DEVELOPMENT OF	
CRIMSON CLOVER	115.
C. RELATIVE HUMIDITIES IN CHAMBERS DEVISED	
FOR HUMIDITY STUDY	115
D. GERMINATION OF CRIMSON CLOVER STRAINS	
AT EXPERIMENT AND TIFTON, GEORGIA IN	
1946 and 1947	116.
E. MEAN TEMPERATURES AND RAINFALL AT	
EXPERIMENT AND TIFTON, GEORGIA DURING	
PERIODS OF SEED DEVELOPMENT IN CRIMSON	
CLOVER	116

LIST OF TABLES

TABLE	PAGE
I. PERCENT HARD SEED IN FORTY RANDOM PLANTS . .	18
II. MEAN GERMINATION OF PLANTS SELFED IN 1944 .	19
III. GERMINATION OF SOFT-SEEDED S_I PLANTS SELECTED AS PARENTS	22
IV. GERMINATION OF HARD-SEEDED PLANTS SELECTED AS S_I PARENTS	24
V. GERMINATION OF PROGENY OF SOFT-SEEDED S_I PARENTS	26
VI. GERMINATION OF PROGENY OF HARD-SEEDED S_I PARENTS	31
VII. GERMINATION OF SOFT-SEEDED PLANTS SELECTED AS S_2 PARENTS	37
VIII. GERMINATION OF HARD-SEEDED PLANTS SELECTED AS S_2 PARENTS	39
IX. GERMINATION OF PROGENY OF SOFT-SEEDED S_2 PARENTS	41
X. GERMINATION OF PROGENY OF HARD-SEEDED S_2 PARENTS	47
XI. GERMINATION OF SOFT-SEEDED S_3 PLANTS SELECTED AS PARENTS	53
XII. GERMINATION OF HARD-SEEDED S_3 PLANTS SELECTED AS PARENTS	55

TABLE	PAGE
XIII. GERMINATION OF PROGENY OF SOFT-SEEDED S_3 PARENTS.	58
XIV. GERMINATION OF PROGENY OF HARD-SEEDED S_3 PARENTS.	62
XV. MEAN GERMINATION OF SOFT, AND HARD-SEEDED PARENTS AND THEIR PROGENY.	66
XVI. GERMINATION OF CRIMSON CLOVER AS AFFECTED BY SIZE OF SEED.	69
XVII. ANALYSIS OF DATA IN TABLE XVI.	70
XVIII. EFFECT OF MOISTURE SUPPLY ON SEED SIZE AND PERMEABILITY	73
XIX. EFFECTS OF HUMIDITY ON SEED SIZE AND GERMINATION.	79
XX. EFFECT OF LIGHT INTENSITY ON SEED SIZE AND PERMEABILITY	82
XXI. EFFECT OF HEAD SEQUENCE ON SEED SIZE AND PERMEABILITY OF SEVENTY-FIVE PLANTS IN 1946	84
XXII. EFFECT OF HEAD SEQUENCE ON SEED SIZE AND PERMEABILITY OF SEVENTY-FIVE PLANTS IN 1947	87
XXIII. DESIGN OF NUTRIENT LEVEL EXPERIMENT.	92
XXIV. EFFECT OF NUTRIENT LEVELS ON PERMEABILITY.	93
XXV. ANALYSIS OF VARIANCE OF DATA IN TABLE XXIV	94

TABLE	PAGE
XXVI. CALCIUM CONTENT OF PLANTS AS AFFECTED BY LEVELS OF APPLICATION	96
XXVII. AIR SPACE BETWEEN COTYLEDONS AND SEED COAT AS INFLUENCED BY MOISTURE CONTENT	98
XXVIII. EFFECT OF MOISTURE CONTENT ON PERMEABILITY.	99

LIST OF FIGURES

FIGURE	PAGE
1. Cross Section View Through the Seed Coat of Crimson Clover	12
2. Germination Rate of Total Plant Population Selfed in 1944.	20
3. Rate of Germination of Soft-seeded S_1 Parents	23
4. Germination Rate of Hard-seeded S_1 Parents. .	25
5. Rate of Germination of Progeny of Soft- Seeded S_1 Parents	30
6. Germination Rate of Progeny of Hard-seeded S_1 Parents.	35
7. Germination Rate of Soft-seeded S_2 Parents. .	38
8. Germination Rate of Hard-seeded S_2 Parents. .	40
9. Rate of Germination of Progeny of Soft-seeded S_2 Parents	45
10. Rate of Germination of Progeny of Hard-seeded S_2 Parents	52
11. Germination Rate of Soft-seeded S_3 Parents. .	54
12. Germination Rate of Hard-seeded S_3 Parents. .	56
13. Germination Rate of the Progeny of Soft- seeded S_3 Parents	61
14. Germination Rate of the Progeny of Hard- seeded S_3 Parents	65

FIGURE		PAGE
15.	Germination of Three Parental Generations and Their Progeny	67
16.	Diagram of Humidity Chambers.	77
17.	Regression Line of Seed Impermeability on Head Sequence	85
18.	Temperature Ranges for 1946 and 1947 During Seed Development of Crimson Clover.	88
19.	Rainfall Distribution for 1946 and 1947 During Seed Development of Crimson Clover .	89

INTRODUCTION

Since its introduction into the United States in 1818 and its subsequent distribution by the Patent Office in 1885, Crimson clover (*Trifolium incarnatum* L.) has become increasingly important. It is widely grown along the Atlantic coastal plains and the piedmont sections of the Southeastern States. In Georgia it ranks as the second legume, with approximately 152,000 acres seeded for soil improvement and winter forage in the fall of 1947 (2).

It has been popular as a green manure crop but with stimulated interest in winter grazing, increasing acreages are being seeded for this purpose. Its growth in the fall and early spring, and its ability to make some growth during warm periods in the winter gives it a very high carrying capacity. It has an added advantage in being one of the few winter legumes in the South that produces large yields of viable seed.

Even though it has many desirable qualities as a crop its acceptance has not been as general as would seem desirable. Failures in obtaining stands in the fall tend to discourage further attempts to seed the crop. The failure to obtain stands has been attributed to a variety of causes, chief of which are poor seedbed preparation, inadequate inoculation, and low percentages of hard seed (1). Kephart (13) states that crimson

clover produces practically no hard seed, often germinating 90 per cent in 2 days. Germination of soft seed on soils low in moisture lead to a high mortality when hot fall days dry out the soil. Most southern agronomists agree that the lack of hard seed is the greatest single factor limiting plant survival.

The failure to volunteer when it is used as a grazing crop is also attributed to low seed impermeability. This is probably well founded because it is a common occurrence to find a heavy stand of clover seedlings two weeks after the seed ripen when followed by frequent rains. With few exceptions all these seedlings die out during the summer months.

It appeared that the solution to the problem of insuring a stand of crimson clover which would volunteer over fairly long periods resolved itself into the development of a strain which would produce hard seed in proportions approaching those of other legumes, long recognized as producing an abundance of such. Therefore, the work reported herein was begun in 1942 with the initial objective of isolating lines of crimson clover which would meet the needs of the South for a hard seeded volunteering strain.

REVIEW OF LITERATURE

The structure of the seed coats and possible reasons for impermeability of the seeds of the Leguminosae have intrigued scientists for centuries. Malpighius in 1687 (22), after whom certain cells in the seed coat were named, was apparently the first to study seed coat structure in this family. Most of the investigations of impermeable seeds of cultivated legumes have been confined, almost exclusively, to sweet clover and alfalfa. With few exceptions efforts have been directed toward determining the nature of impermeability and methods of breaking this type of dormancy.

Pammel (22) in his comprehensive review of literature presented all the theories to date concerning the reason for impermeability in the Leguminosae. Opinions were divided as to whether impermeability was caused by an external layer of cutin or the "light line." He found that the light line was permeable to water and attributed impermeable properties to the unequal layer of cutin surrounding the seed.

His investigations of the anatomy of legume seeds included those of Red Clover (*T. pratense* L.) and Buffalo Clover (*T. reflexum* L.), but not of crimson clover. In these he described three separate layers of cells in the seed coat, the first being the macrosclerids, often referred to as "Malpighian"

cells, resembling pallisade cells in arrangement. On the outer part of these cells which he included as a portion of the outer cell wall was a well defined cuticularized layer over which was found an unequal layer of cutin. The radial walls of these cells were found to be greatly but unevenly thickened giving the cell cavity an irregular appearance with portions extending into the radial walls. The uneven thickening, according to Pammel, causes a rupture of the outer cell wall and later development of this layer brings about a deposition of cuticularized material in these ruptures which he termed pore canals. He described the cell cavities of the macrosclerids as being filled with protein grains, tanin, pigments, and an occasional large chromophore.

The next layer verified by Pammel are the osteosclerids or hour-glass cells. These were described as thick-walled cells with longitudinal canals. His description of these canals gives the impression that he refers to the projections of the outer cell wall into the cell cavity. He called the next layer of compressed parenchymous tissue the nutrient layer because in this tissue was found the vascular system before its collapse.

The last two layers of cells he included under the endosperm. The outermost of these were designated as aleurone cells, with thick walls, and containing fats and protein grains. The aleurone was found to be underlain by cells with thin

primary walls with a thickening of reserve cellulose.

Winton (30) did not consider crimson clover in his investigations but his description of red clover is in agreement with that of Pammel.

Hamly (5) demonstrated the true nature of impermeability in *Melilotus alba* and noted that water absorption by soft unscarified seed was by way of the strophiole. The same structure was found to perform in this manner in *T. agrarium*, *T. arvense*, *T. hybridum*, and *T. pratense*. His observations on the function of the strophiole in *Melilotus* were confirmed by Martin and Wyatt (17).

Anatomical studies of clover seeds show no structures analogous to the cones or Malphigian caps of *Melilotus*. Nelson (21) and White (29) agree with Pammel that impermeability of some legume seeds can be attributed to a waxy coating on the exterior of the seed. This would explain the impermeability of the true clovers. Earlier theories supporting this view have already been mentioned (22).

A survey of the literature reveals only three investigations of limited scope into the possible inheritance of impermeability. Lute (15) as a result of her investigations over a period of two years, classifies alfalfa into three groups. One group produced approximately the same percentage of hard seed in two consecutive years. Another a much lower,

and the third a much higher percentage than the parents. She postulates a genetic difference which may be obscured by climatic factors.

Harrington (6) definitely states that hard-seededness is not a varietal characteristic of red clover. The percentage of hard seed produced by progeny was unaffected whether the parent plants ran 95 per cent hard seed or 5 per cent.

Peiters (24) studied the seasonal production of hard seed in five plants of *Lespedeza sericea* and five years of selection for soft lines resulted in no progress. Descendants of plants which produced a high percentage of permeable unhulled seed, were no better in permeability than the average.

It may be noted that the experiments cited above concerned only perennials and with the exception of Harrington's work was confined to very small populations. Apparently no effort has been made to prove or disprove genetic differences in annuals by selfing and selection over a period of years.

As indicated above, all investigators noted a marked difference in hard seed production from year to year, but investigations to correlate climatic and physiological factors with seed permeability have been very limited. Lute (15) found that alfalfa at an altitude of 7,000 feet produced 15 per cent more hard seed than the same variety grown at 6,200 feet. Temperature and moisture relationships were not reported. Weather records (4) show, however, that the higher altitude received, annually, 7.6 inches more

precipitation than the lower altitudes. The higher altitude also had a 6.2 degree lower July temperature. The difference observed may have been due to altitude, precipitation, temperature, humidity or to their interactions.

Harrington (6) discounts soil as having any effect on hard seed production; plants on clay soils and sandy soils produced seed with the same degree of hardness. Tests over six years led him to conclude that large quantities of hard seed are produced by red clover, alsike clover, white clover, and white sweet clover regardless of season. Samples of seed grown from the same original lot planted at seven locations in the United States all contained a large percentage of hard seeds. His observations on season and climate do not seem to be in full accord with his study on yearly variation as previously quoted.

Hiltner & Kinzel (8) reported their observations on the hardness of the seed of several legumes and stated that red clover generally contains from five to ten per cent of hard seeds but in some years the proportion may run as high as 60 per cent. They attribute the difference between years to weather variations.

The need for hard seed in crimson clover is emphasized by the fact that better stands are obtained when unhulled seed is sown. Unhulled seed germinate more slowly than hulled seed.

Stitt (27) found that hulled seed absorbed 18 per cent more water in 8 hours than unhulled seed when placed on blotting paper. On a mulched area unhulled seed produced a 25 per cent better stand than hulled. Hulled and unhulled seed gave equal stands with late rains. McKee (20) reports no serious damage, if the seed sweated and dried out, but if the radicle had emerged, injury increased with the length of time of drying.

Several farmers in the southeast have succeeded in maintaining a stand of crimson clover for periods of 18 to 20 years. This seed is reported as being hard. Recently the most promising of these strains were bulked and released by the Georgia Agricultural Experiment Station (28) under the name of Dixie. In view of the investigations which follow it is doubtful whether they are justified in classing this variety as hard seeded.

EXPERIMENTAL PROCEDURE

Three phases relating to impermeability are reported herein, namely: the anatomy of the seed coat, effect of inbreeding, and physiological factors. They will be considered in this sequence.

I. ANATOMY OF THE SEED COAT

Methods and Materials

The most satisfactory method found to prepare seeds for sectioning was to glue the ends to small depressions in wooden blocks and then imbed them in parafin. Sections 4 to 5 microns in thickness were successfully made with a sliding microtome. Seed coat sections were removed from the parafin under a binocular stereoscopic microscope, or by dropping the sections into a corner of a porous filter paper and dissolving the parafin with carbon tetrachloride or carbon bisulfide. Seed coat sections were then transferred to slides with a moistened needle.

Methylene blue was found to be a satisfactory stain for cursory examination. More specific indicators were required for tissue identification. With one exception the tests used by Hamly (5) were followed.

Results and Discussion

Except for minor differences the observations made were in accord with those of Pammel (22) and Winton (30). The composition of the tissue, when present in crimson clover seed coats, is essentially the same as for similar tissue in *Melilotous alba* as identified by Hamly (5).

Five separate layers which seem to be characteristic of all small legumes were identified, namely the macrosclerids, the osteosclerids, collapsed parenchyma, the aleurone, and finally the thick-walled parenchyma. Figure 1 illustrates those layers drawn to scale. The cuticle on Crimson clover seeds and the subcuticular layer are well defined. The cuticle is insoluble in either concentrated sulfuric or hydrochloric acids. Sulfuric acid, however, does penetrate the cuticle. Impermeable seed become permeable when treated with the acid for 5 minutes. Treatment for this period dissolves the subcuticular layer down to the light line. It is not affected by 2 per cent hydrochloric acid and 2 per cent ammonium hydroxide. Sulfuric acid dissolves the subcuticular layer, and with sections leaves the cuticle as a thin ribbon of about 2 to 3 microns in thickness. The cuticle is not penetrated by a 1 per cent solution of osmic acid. The absence of a suberized area as found in sweet clover would suggest the possibility that impermeability in crimson clover might be attributed to an impervious cuticle.

The subcuticular area dissolves in a 2 per cent hydrochloric acid followed by 2 per cent ammonium hydroxide indicating the pectic nature of this material. The faint tinge of blue resulting from chlorzinc iodide shows some hemicellulose to be present. This faint blue may be discerned to extend into the open ends of the macrosclerids to the depth of the light line.

Reeve (25), and Hamly (5) have clarified the nature of the macrosclerids and tests with crimson clover substantiate their observations. Winton (30) describes the macrosclerids of *Trifolium* as being flat on the outer ends. This was found to be true but in no mature seeds examined was the outer wall found to be intact, unless the subcuticular area is considered as the outer wall. The difference in composition would make this unlikely, but the absence of an outer wall may be explained on the basis of a complete rupture as shown by Hamly (5). These cells are somewhat smaller than those of red clover, measuring 35 to 45 microns in length and 13 to 18 microns in cross section. The cell contents were not determined but it was observed that considerable time was required for decomposition by sulfuric acid. Reaction to sulfuric acid and iodine showed that the walls were composed of cellulose. The light line which occurred 7 to 9 microns below the surface disappeared when macerated with hydrochloric acid and ammonia. This confirms the findings of Hamly (5), and Reeves (25) that the light line is caused by a

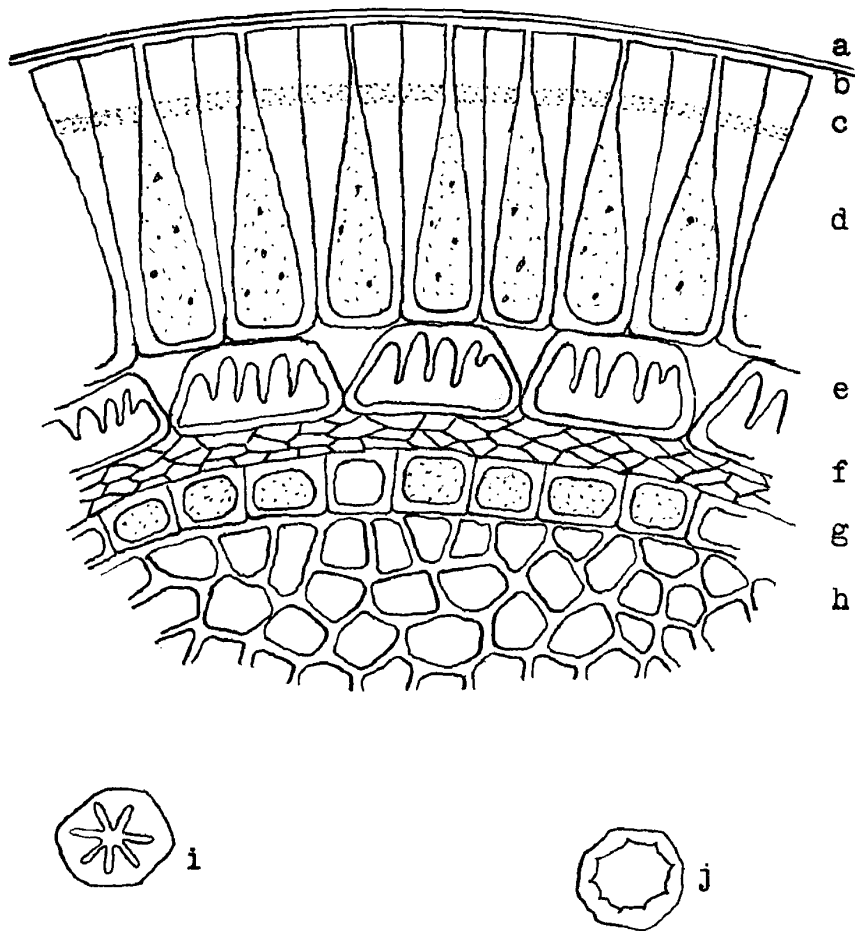


Fig. 1. Cross Section View Through the Seed Coat of Crimson Clover. Scale 1 mm. = 1 micron.
a - Cuticle, b - Subcuticular layer, c - light line, d - Macrosclerids, e - Osteosclerids, f - Nutrient layer, g - Aleurone, h - Endosperm, i - Cross section of upper part of macrosclerid, j - Cross section through lower part of macrosclerids.

difference in refractive index bringing about an optical illusion rather than a structural difference as stated by Coe and Martin (3), and Martin (17) in their studies of sweet clover.

The osteosclerids appeared to be only slightly larger than those of other clovers examined by Pammel (22) but agree more closely with Winton's (30) description of these cells in red clover. These cells are not as clearly defined as Figure 1 shows but seem to be somewhat compressed or fused with the thin-walled parenchyma underneath. Measurements of occasional cells in numerous seed coats revealed their dimensions as approximately 20 to 25 microns in length and 13 to 18 microns in depth. The structure of these cells in other species has been well illustrated by Pammel (22), Winton (30), and Martin and Wyatt (17) and their composition given as pectic and cellulose-like materials by Hamly (5).

The remaining cells are not usually considered as a part of the spermoderm but as $3n$ endosperm tissue. The endosperm appears to be identical with similar layers of cells in sweet clover and alfalfa. A marked difference was observed, however, in the thickness of the thick-walled parenchyma which measured approximately 40 to 50 microns at the ends of the seed, to 70 to 90 microns at the sides.

To test the hypothesis that impermeability in crimson clover may be attributed to the cuticle, halves of seed coats

were cemented to the ends of glass tubing drawn out to a little less than the diameter of a seed. The internal structure of the seed coat was thoroughly disorganized, before mounting, with 2 per cent hydrochloric acid and ammonia. The cement used was a water-proof type and when applied extreme care was taken to leave the major portion of the seed coat exposed. The tubes were partly filled with a saturated sugar solution and immersed in water. This technique was successful with two of those prepared. In 48 hours there was no rise of the water level in these two. In tubes where the cementing procedure failed to seal the ends the water rose to the same height as the capillary rise of the open ended check. On the basis of the test it appears safe to conclude that impermeability in crimson clover is due in part to the well developed cuticle layer.

Treatment of unscarified seed with osmic acid confirmed the observations of Hamly (5) , and Martin and Wyatt (17) that water enters permeable seed through a rupture in the strophiole, but an intact strophiole prevents the absorption of water. Since no differences could be found in the cuticle layer of hard and soft seeds, it is evident that the condition of the strophiole is the dominant factor affecting water absorption, and would materially affect any efforts to isolate lines breeding true for permeability.

II. EFFECT OF INBREEDING

Methods and Materials

Seed from forty plants, selected at random, were harvested in the spring of 1942 and germination determined after hulling by hand. Tests were run in accordance with the official method (26) with all live seed not germinating in 6 days being classed as hard. With the establishment of plant difference the remaining seed was discarded in favor of inbred material.

New material was obtained in the spring of 1944 from three sources: volunteer plants growing around the Agronomy farm at Athens, Georgia, plants from a lot of seed imported from Europe in 1938 and combined during the intervening years for seeding purposes, and seed of a volunteering strain known as Allen's.

Six hundred random plants were selfed by placing small envelopes of onion skin paper over the heads. These envelopes were made with water-proof cement and the closed corners clipped slightly to provide for aeration. The bottom of the envelopes were folded around the plant stems and held with a paper clip. Every two or three days the envelopes were removed in order to roll the heads to assist in pollination. At harvest time the selfed heads were kept separate from the open pollinated heads.

A violent rain storm caused such lodging that it was difficult to locate all the plants selfed in this manner. Considering the fact that tests in the seed germination laboratory at the College of Agriculture showed crimson clover to run 5 to 10 per cent hard, it was felt that if three hundred plants were recovered, enough material would be at hand to provide fifteen to thirty plants for parents with impermeable seed. Consequent work showed that time would not permit the hulling and germination of a larger number of plants.

Seed of this crop and of the following crop were hulled with a huller designed and built by the author (10). The design and operation of this machine has been previously reported and was found to be an excellent substitute for hand hulling, but not as rapid as desired. Hulling of the crops of 1946 and 1947 was done with an improvement of the huller reported by Higbee (7). The improvements consisted of lining the drum with corrugated rubber matting and installing a built-in blower for removing the hulls. Rubber impregnated canvas belting was used for beaters and a rubber valve was installed below the hopper to prevent seed from being thrown out by the beaters. The extent of scarification was felt to be small with either machine.

Germination tests were run at 25 degrees Centigrade on the open pollinated seed, and the selfed seed were saved

for planting purposes.

Selfed seed from plants producing the largest percentages of soft, and of hard seed were planted in the fall of 1944. The progeny of these were selfed and processed in the same manner as the original parents. Hard and seeded parents for the S_2 and S_3 generations, with an occasional exception, were chosen only from the progeny of the initial hard-seeded lines. The soft-seeded S_2 , and S_3 parents were likewise selected from the progeny in the soft-seeded lines. The small change in germination developing between 21 and 63 days led to the reduction of the germination period to the 21-day period.

Results and Discussion

As shown in Table I the season of 1942 was apparently favorable for the development of soft seed. The mean of 4.4 per cent hard seed appears to confirm the observations of Kephart (13) that crimson clover produces a small amount of impermeable seeds.

The variation from no hard seed to 24.7 per cent indicates that crimson clover plants differ in the amount of hard seed produced. This is in agreement with the results obtained by Lute (15) with alfalfa, and the variation between plants of lespedeza, as noted by Peiters (24).

The low percentage of hard seed produced by the forty plants corresponds to amount obtained in germination

TABLE I

PER CENT HARD SEED IN FORTY RANDOM PLANTS

Plant No.	: Per cent : hard seed	: Plant No.	: Per cent : hard seed
1	7.2	21	0
2	12.7	22	Dead seed
3	1.7	23	.7
4	6.3	24	0
5	0.4	25	0
6	2.2	26	1.6
7	8.7	27	3.4
8	5.8	28	2.1
9	8.4	29	5.9
10	6.0	30	2.9
11	5.0	31	11.8
12	0.7	32	24.7
13	1.4	33	Insufficient seed
14	2.1	34	15.0
15	1.1	35	1.3
16	2.7	36	17.8
17	2.8	37	0.8
18	1.1	38	1.3
19	1.6	39	3.6
20	.0	40	2.4
Mean		4.4	

tests of combined seed. The gentle method of hulling, however, should have kept scarification at a minimum. The fact that plant 32 yielded seed 24.7 per cent hard is evidence in support of this assumption.

None of the plants used in this study yielded a high percentage of hard seed. The variation present, however, suggested the possibility that selection in a larger population might reveal plants with a higher degree of impermeability. Subsequent work showed this to be possible.

The mean germination of the three-hundred and three plants selfed in 1944 is presented in Table II and illustrated graphically in Figure 2. The germination record of all plants may be found in the Appendix. The mean germination of 57.8 per cent represents all seed which softened within 63 days in the germinator. The degree of impermeability conflicts with earlier observations relative to the amount of hard seed produced by crimson clover (13).

TABLE II

MEAN GERMINATION OF PLANTS SELFED IN 1944

Days in germinator	:	Per cent germination	:	Days in germinator	:	Per cent germination
2		27.4		12		42.4
4		34.0		14		43.5
6		37.1		21		49.1
8		39.5		63		57.8
10		41.4				

Extreme variation may be noted with one plant yielding only 6 per cent, and others as high as 100 per cent permeable seed.

The failure of many plants to set seed when selfed eliminated many potential parents. Twenty-two plants with a mean permeability of 85.7 per cent produced enough selfed seed to be used as soft-seeded parents. The range in permeability of these parents, as shown in Table III was from 71 to 100 per

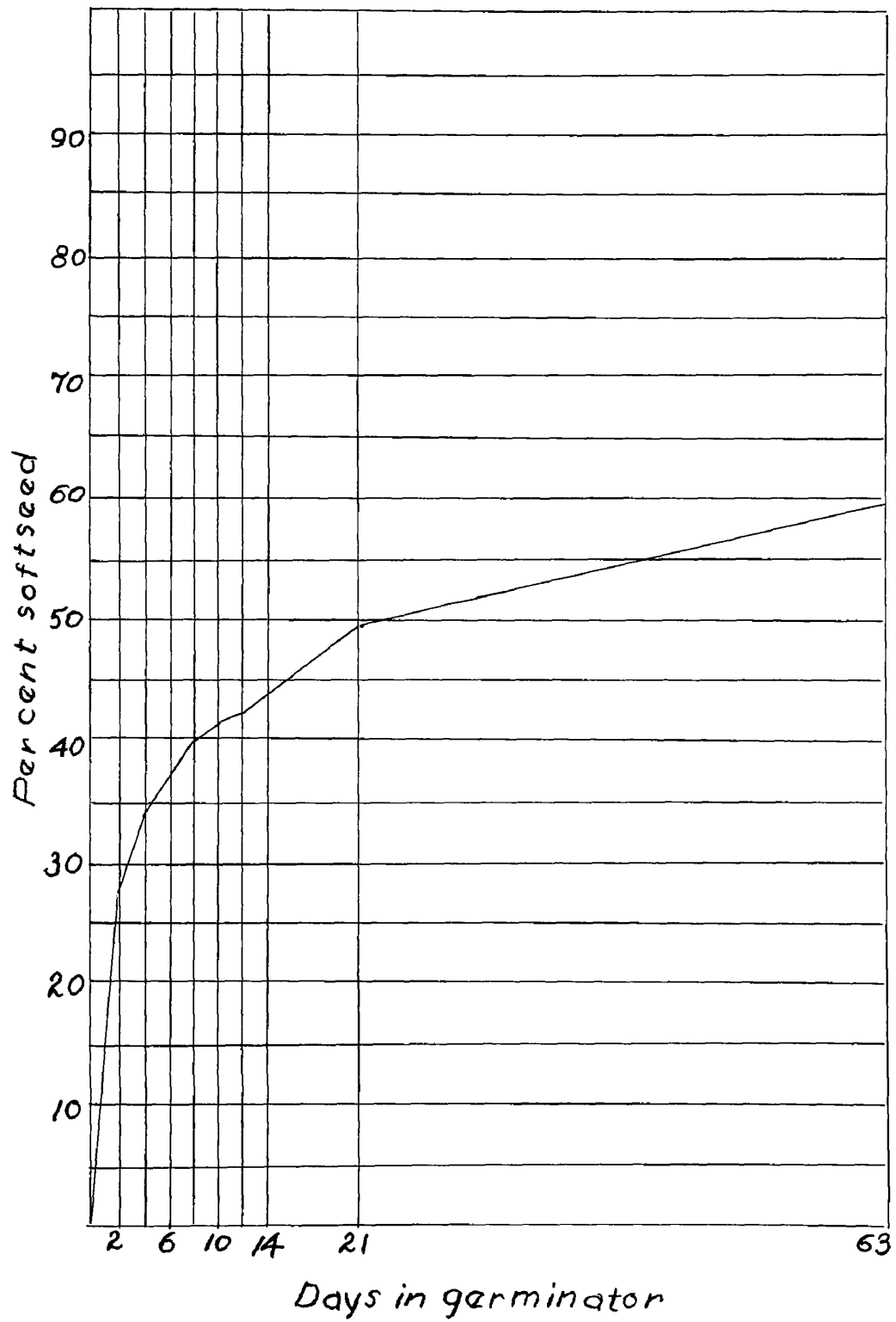


Fig. 2. Germination Rate of Total Plant Population Selfed in 1944.

cent based on the 63-day germination test. The rate of germination of these parents is also illustrated in Figure 3.

Table IV and Figure 4 presents the germination record of twenty-nine plants which set enough selfed seed to allow their use as hard-seeded parents. It may be observed that the average permeability of these parents was 25.4 per cent with a range of 8 to 41 per cent.

If the degree of impermeability were an inherited or varietal characteristic, parents differing so widely could be expected to produce similar progeny, unless this quality is of such heterozygous nature that several years would be required to isolate pure lines.

The 21-day germination record of the first generation progeny is given in Tables V and VI and illustrated in Figures 5 and 6. When the mean difference of 0.3 per cent in germination of the two groups is considered, it would appear that the characteristics of the parents were not evident in the progeny. In general this is true, but three soft-seeded progeny lines, 44, 49, and 63 fell within the range of the permeability of the parents selected on the basis of the 63-day test. Progeny group 49 was the only one softer than the parents.

It may be seen that six lines, 14, 117, 131, 175, 198, and 234 from the hard seeded parents fell within the range

TABLE III

GERMINATION OF SOFT-SEEDED S_1 PLANTS
SELECTED AS PARENTS

Plant number	Germination by days								
	2	4	6	8	10	12	14	21	63
1	11	44	64	64	68	68	69	72	82
7	46	62	67	70	72	72	73	75	80
20	91	94	94	94	94	94	94	97	100
35	24	37	47	53	57	59	59	76	90
44	20	36	45	54	70	72	74	79	88
49	50	62	64	64	66	66	67	69	77
57	42	54	59	62	62	65	65	72	84
61	23	32	41	46	52	59	61	75	89
63	25	47	66	76	88	92	92	94	95
72	20	35	42	45	51	54	54	61	84
84	9	14	23	28	35	36	40	54	77
110	41	51	51	53	57	59	59	64	94
159	32	69	78	79	82	84	84	92	100
190	35	50	52	52	59	59	59	64	78
205	20	37	44	47	47	50	50	55	71
213	25	62	72	73	75	81	84	87	90
249	70	75	76	76	77	77	77	78	79
266	69	70	70	70	70	70	71	71	72
279	83	84	84	84	84	84	84	84	84
285	70	84	84	84	84	84	84	85	86
289	84	90	90	90	90	90	90	90	90
300	97	97	97	97	97	97	97	97	97
Mean	44.8	58.4	64.0	66.5	69.6	71.4	72.1	76.8	85.7

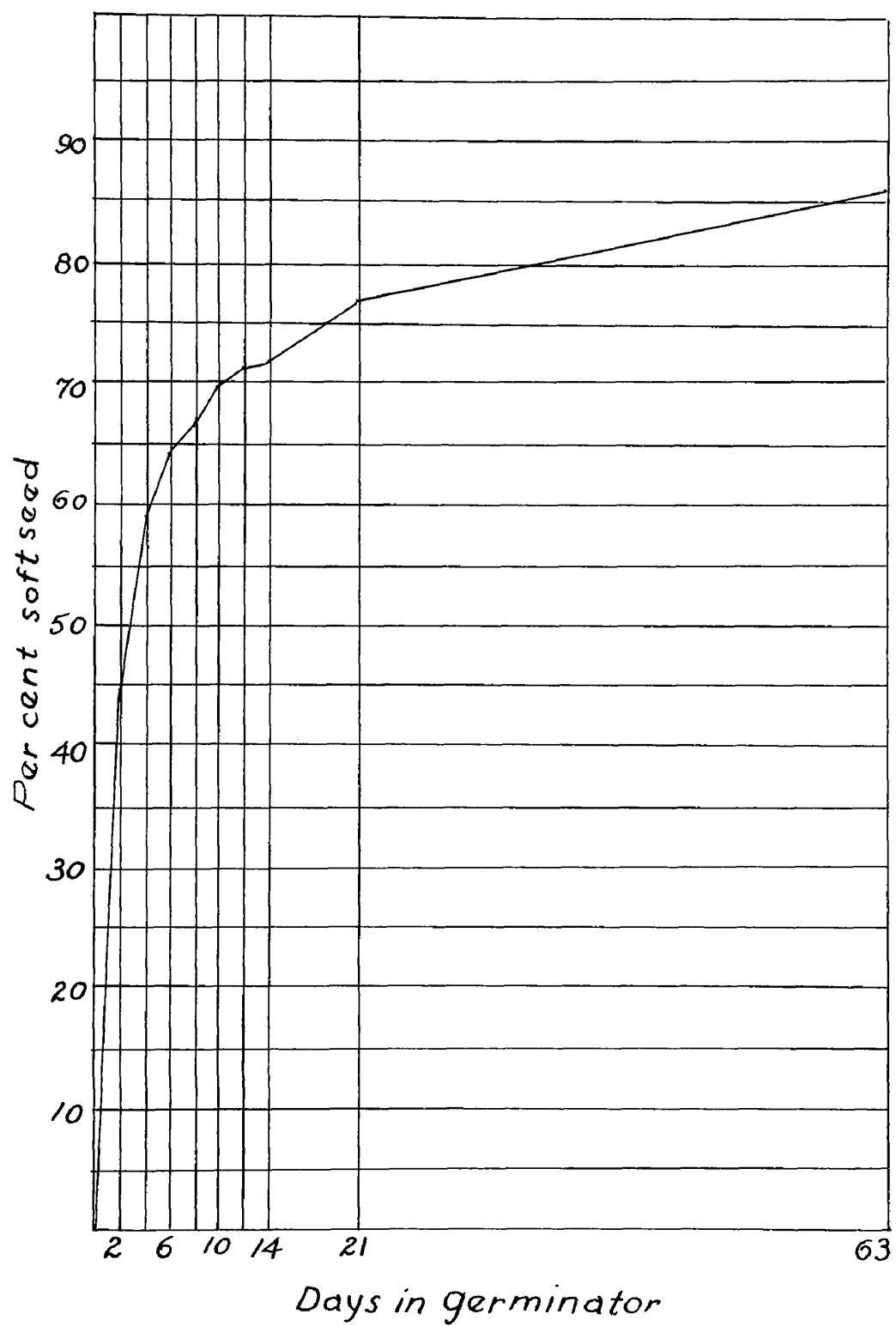


Fig. 3. Rate of Germination of Soft-seeded S₁ Parents

TABLE IV

GERMINATION OF HARD-SEDED PLANTS
SELECTED AS S₁ PARENTS

Plant number	Germination by days								
	2	4	6	8	10	12	14	21	63
5	17	17	17	17	17	17	19	19	23
14	6	8	8	8	8	9	9	9	17
66	4	5	7	7	10	13	14	17	22
92	5	3	14	18	20	22	26	32	41
93	4	4	4	5	7	7	7	7	17
94	12	15	16	17	17	18	18	22	27
97	4	4	4	4	4	4	4	6	8
99	5	9	9	9	9	10	10	13	22
100	3	11	15	17	22	25	25	29	33
106	14	16	17	17	17	18	18	22	32
117	3	4	5	5	5	5	5	7	15
119	18	24	26	27	27	27	27	29	38
131	20	22	23	26	27	28	28	31	34
137	2	4	7	7	7	7	7	9	14
146	1	6	7	8	10	11	11	16	31
148	19	24	28	29	29	31	31	37	40
152	13	20	22	22	23	24	24	29	40
167	8	11	12	12	12	12	12	15	18
172	8	11	11	11	12	12	12	15	15
175	9	15	19	19	19	24	24	25	27
179	2	3	4	4	5	5	5	7	17
181	6	8	9	9	9	9	10	13	17
183	13	15	17	17	18	18	18	19	25
196	13	13	14	15	16	16	16	17	28
198	2	7	10	12	13	16	19	22	32
215	5	6	6	6	6	6	6	7	9
219	11	14	14	15	15	17	17	23	30
221	9	14	16	19	19	23	23	29	35
234	24	28	29	29	29	29	29	29	30
Mean	9.0	11.9	13.4	14.1	14.8	15.9	16.3	19.1	25.4

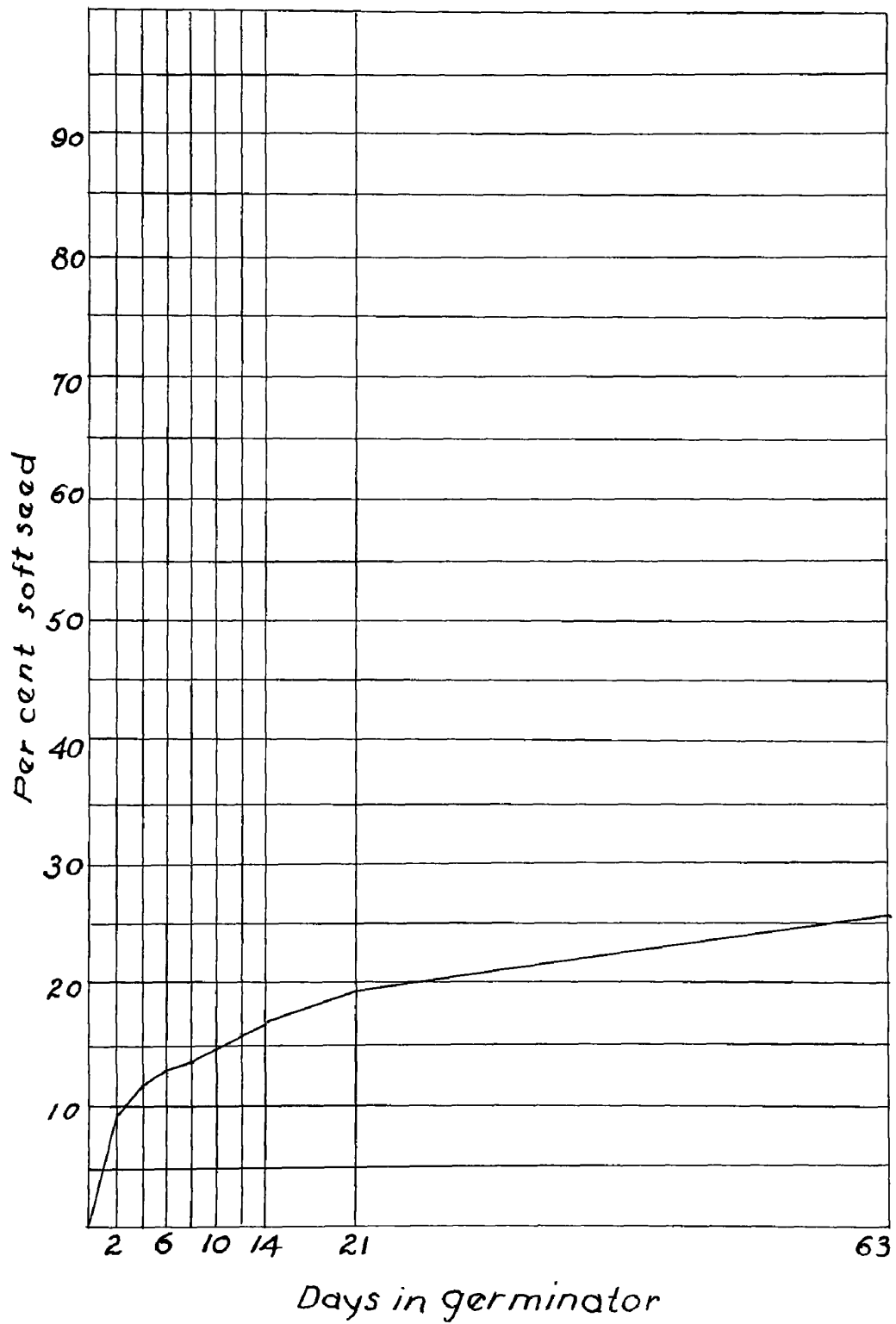


Fig. 4. Germination Rate of Hard-seeded S_1 Parents.

TABLE V

GERMINATION OF PROGENY OF SOFT-SEED S₁ PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
1-1	1	8	11	12	14	15	17	21
1-2	2	8	12	15	17	19	19	20
1-3	6	8	11	12	16	18	19	19
1-4	3	9	10	13	13	16	18	19
Mean	3	8.2	11	13	15	17	18.2	19.7
7-1	41	43	43	43	44	44	44	45
7-2	49	53	53	53	54	54	55	58
7-3	28	31	31	32	33	33	34	39
7-4	62	65	70	75	81	81	87	91
7-5	47	50	53	53	60	60	62	68
7-6	59	66	66	66	66	66	67	67
7-7	36	42	42	42	42	42	42	44
7-8	23	24	24	25	26	26	28	31
7-9	49	52	52	53	54	54	54	57
Mean	43.7	47.3	48.2	49.1	51.1	51.1	52.5	55.5
20-1	5	19	20	20	20	20	23	26
20-2	7	12	14	17	17	18	22	27
20-3	4	14	20	20	21	21	22	25
20-4	3	12	13	14	16	20	20	24
20-5	6	13	16	17	17	18	18	25
Mean	5.0	14.0	16.6	17.6	18.2	19.4	21.0	25.4
35-1	36	54	56	56	56	58	58	60
35-2	30	54	54	54	54	55	56	58
35-3	36	53	53	53	53	53	53	56
35-4	40	46	48	48	49	50	50	57
Mean	35.5	51.75	52.7	52.7	53.0	54.0	54.2	57.7
44-1	69	83	84	85	86	90	91	92
44-2	60	80	81	82	82	83	83	86
44-3	68	78	78	79	79	80	82	86
44-4	60	75	76	78	78	79	79	84
44-5	62	80	80	80	80	80	82	85
Mean	63.8	79.2	79.8	80.8	81.0	82.4	83.4	86.6

TABLE V (Cont'd.)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
49-1	45	79	83	83	83	83	86	88
49-2	41	67	71	71	73	74	76	76
49-3	40	74	76	76	76	77	77	80
49-4	42	67	70	70	71	71	72	82
49-5	43	70	73	74	74	74	75	76
Mean	42.2	71.4	74.6	74.8	75.4	75.8	77.2	80.4
57-1	3	45	46	46	46	46	52	56
57-2	6	15	18	22	22	24	29	30
57-3	10	13	15	17	17	17	17	17
Mean	6.3	24.3	26.3	28.3	28.3	29.0	32.6	34.3
61-1	23	66	70	71	71	72	73	85
61-2	9	27	28	30	33	34	37	44
61-3	6	21	22	23	26	27	32	40
61-4	11	14	16	16	16	16	17	21
61-5	27	47	50	50	55	57	58	65
Mean	15.2	35.0	37.2	38.0	40.2	41.2	43.4	51.0
63-1	39	83	93	93	93	93	93	93
63-2	20	62	62	62	65	67	68	77
63-3	53	66	69	72	77	77	78	86
63-4	31	49	54	61	77	77	84	89
63-5	12	19	21	21	23	23	23	24
63-6	19	40	44	49	57	62	66	74
63-7	17	38	40	42	45	46	48	54
Mean	27.5	51.0	54.7	57.1	62.4	63.5	65.7	71.0
72-1	24	31	35	42	57	61	66	78
72-2	11	18	21	22	25	28	31	41
72-3	16	26	29	29	33	34	35	39
Mean	17.0	25.1	28.3	31.0	38.3	41.0	44.0	52.6
84-1	11	24	28	28	28	31	39	60
84-2	9	26	31	31	32	32	39	42
84-3	12	22	30	31	33	36	45	53
84-4	10	20	31	35	39	43	47	52
84-5	7	22	34	38	44	44	50	56
84-6	12	24	28	32	36	39	45	49
Mean	10.1	23.0	30.3	32.5	35.3	37.5	44.1	52
110-1	41	51	51	53	57	59	59	64
110-2	40	52	52	52	54	54	56	63
Mean	40.5	51.5	51.5	52.5	55.5	56.5	57.5	63.5

TABLE V (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
159-1	82	100	100	100	100	100	100	100
159-2	20	44	51	51	52	54	57	59
159-3	15	23	23	23	24	27	32	36
159-4	32	36	40	41	41	42	42	45
159-5	18	28	29	31	31	32	37	41
Mean	33.4	46.2	48.6	49.2	49.6	51.0	53.6	56.2
190-1	26	32	36	37	37	42	42	52
190-2	21	38	43	46	50	52	53	59
190-3	19	35	42	42	45	47	49	58
190-4	25	40	47	51	55	58	63	69
Mean	22.7	36.2	42.0	44.0	46.7	49.7	51.7	59.5
205-1	29	52	54	55	57	58	58	62
205-2	34	50	50	50	52	54	54	55
205-3	2	2	6	7	9	9	9	10
205-4	63	67	69	73	74	76	78	83
205-5	39	45	45	45	45	47	47	51
205-6	12	13	17	19	22	25	27	50
205-7	21	22	22	22	25	25	26	36
205-8	39	41	46	46	51	54	64	73
205-9	17	23	23	23	25	25	25	26
205-10	48	52	54	54	55	55	55	55
205-11	70	74	76	76	77	78	79	81
205-12	43	51	54	54	56	61	63	74
Mean	34.7	41.0	43.0	43.8	45.6	47.2	48.7	54.6
213-1	2	2	3	3	3	3	3	3
213-2	20	20	20	20	20	20	20	23
Mean	11.0	11.0	11.5	11.5	11.5	11.5	11.5	13.0
249-1	0	1	2	7	18	24	26	51
249-2	1	5	7	10	28	41	46	58
Mean	0.5	3.0	4.5	8.5	23.0	32.5	36.0	54.5
266-1	2	12	26	42	50	58	58	62
279-1	1	7	13	29	40	48	50	57
279-2	0	0	0	0	35	40	40	50
279-3	0	3	5	13	19	25	31	33
Mean	0.3	3.3	6.0	14.0	31.6	37.6	40.3	46.6

TABLE V (Cont'd.)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
285-1	0	6	11	11	12	14	14	15
285-2	4	16	30	40	40	50	52	58
285-3	3	10	20	24	36	42	42	50
Mean	2.3	10.6	20.3	27.3	29.3	35.3	36.0	41.0
289-1	1	6	10	20	25	27	27	31
289-2	35	58	62	65	66	66	68	68
289-3	1	6	11	13	13	15	16	16
Mean	12.3	23.3	37.6	32.6	34.6	36.0	37.0	38.3
300-1	1	3	6	16	28	34	44	57
300-2	5	12	21	43	52	59	61	71
300-3	7	10	14	23	28	35	35	38
Mean	4.3	8.3	13.6	27.3	36.0	43.6	46.6	55.3
General Mean	24.4	36.0	39.1	41.3	42.8	46.4	48.4	53.5

of parental selection. Only two progeny groups, 131 and 175 were harder than their parents. Further study of Tables IV, V, and VI will show that the hardest parent, 97, produced progeny with a mean permeability of 77.5 per cent which is only 9 per cent softer than progeny group, 44, in the soft-seeded lines. It may be noted, also, that this hard-seeded parent produced progeny with the highest percentage of permeable seeds in the hard seeded group.

Reversal of permeability is also evident in the soft lines. Three very soft parents, numbers 20, 213, and 289 produced offspring with 25.4, 13.0 and 38.3 per cent permeable

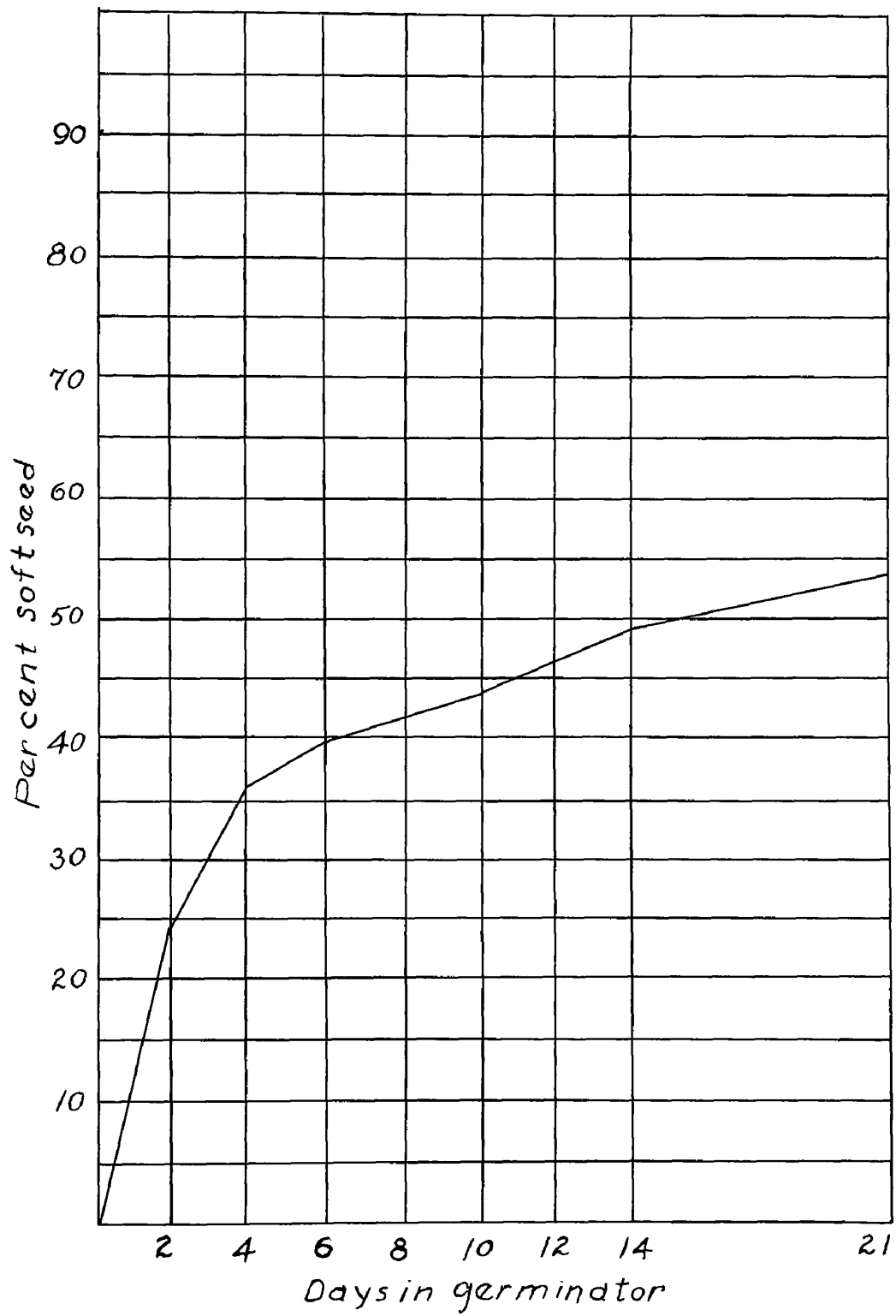


Fig. 5. Rate of Germination of Progeny of Soft-seeded S₁ Parents

TABLE VI

GERMINATION OF PROGENY OF HARD-SEED S₁ PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
5-1	34	47	47	47	47	47	47	47
2	29	31	32	32	32	32	32	37
3	47	49	49	49	49	49	49	49
4	69	73	73	74	74	74	74	75
5	47	49	49	49	50	50	51	62
6	32	39	40	40	42	44	44	50
7	35	38	40	41	41	41	41	46
8	55	69	74	76	76	79	79	82
Mean	43.5	49.3	50.5	51.0	51.3	52.0	52.1	56
14-1	12	13	16	16	16	16	18	18
2	20	27	28	28	29	29	30	32
3	17	21	21	22	23	24	24	25
Mean	16.3	20.3	21.6	22.0	22.6	23.0	24.0	25.0
66-1	63	79	79	79	79	79	79	79
2	11	16	16	16	18	19	20	24
3	5	26	38	40	49	49	51	62
4	14	69	71	73	81	81	81	86
5	26	57	57	57	57	57	57	58
6	11	45	46	46	49	50	50	50
Mean	21.6	48.6	51.1	51.8	55.5	55.8	56.3	59.8
92-1	10	24	27	29	31	31	34	40
2	15	21	21	22	23	25	29	34
3	26	30	31	32	34	34	36	40
4	15	19	22	22	23	23	24	30
5	48	49	51	52	52	55	57	65
Mean	22.8	28.6	30.4	31.4	32.6	33.6	36.0	41.8
93-1	54	65	65	65	65	68	68	75
2	40	50	50	51	58	60	67	77
3	26	35	36	36	37	37	37	40
Mean	40.0	50.0	50.3	50.4	53.3	55.0	57.3	64.0
94-1	43	46	47	47	47	47	47	50
2	56	64	65	68	68	70	72	76
3	46	48	50	53	54	54	54	55

TABLE VI (Cont'd.)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
94-4	56	56	56	57	57	58	58	61
5	23	41	54	57	65	61	73	78
6	87	88	92	92	92	92	92	92
7	33	33	33	33	33	36	36	39
Mean	49.1	53.7	56.7	58.1	59.4	60.5	61.7	64.4
97-1	52	54	62	64	72	72	80	86
2	49	57	63	64	67	71	74	81
3	31	52	59	60	61	63	67	70
4	24	36	54	58	63	64	67	73
Mean	39.0	49.7	59.5	61.5	65.7	67.5	72.0	77.5
99-1	36	41	48	49	49	51	53	56
2	18	22	22	22	24	24	25	28
3	4	18	18	18	19	20	20	22
4	23	27	27	27	29	29	31	32
5	9	47	47	47	47	47	47	51
6	26	57	65	70	73	79	82	87
7	22	22	22	22	33	33	33	38
Mean	19.7	23.4	35.5	36.4	39.1	39.5	41.5	44.8
100-1	57	64	71	72	72	72	72	72
2	42	52	56	57	57	59	60	67
3	31	45	48	48	49	51	52	54
4	34	49	52	52	54	54	57	61
Mean	41.0	52.5	56.7	57.2	58.0	59.0	60.2	63.5
106-1	54	54	56	56	56	56	56	63
2	49	56	58	58	59	59	60	62
3	44	48	48	48	48	50	50	52
4	53	58	65	65	65	65	65	66
5	52	53	53	54	55	56	57	64
Mean	50.4	53.8	56.0	56.2	56.6	57.2	57.6	61.4
117-1	22	31	38	38	39	52	62	65
2	12	14	14	14	14	14	15	20
3	17	19	22	28	34	34	36	42
4	15	19	21	25	31	31	32	36
5	16	21	25	27	27	29	30	38
Mean	16.4	20.8	24.0	26.4	29.0	32.0	35.0	40.2

TABLE VI (Cont'd.)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
119-1	25	26	26	26	27	28	28	32
2	30	58	60	60	64	66	68	69
3	24	26	28	28	28	28	31	35
Mean	26.3	37.0	38.0	38.0	39.6	40.6	42.3	45.3
131-1	40	41	41	41	42	42	42	42
2	20	20	23	23	23	23	24	26
3	28	28	29	29	29	29	30	30
Mean	29.3	29.6	31.0	31.0	31.3	31.3	32.0	33.3
137-1	27	39	44	46	46	50	53	61
2	46	56	58	62	62	62	62	64
Mean	36.5	47.5	51.0	54.0	54.0	56.0	57.5	62.5
146-1	2	12	20	21	24	37	44	49
2	57	60	61	61	61	63	63	70
3	27	54	57	57	59	60	60	62
4	21	41	45	45	47	48	49	59
5	19	45	49	49	52	55	60	71
6	27	47	57	57	58	59	62	65
Mean	25.5	43.1	48.1	48.3	50.1	53.6	56.3	62.7
148-1	83	87	87	87	88	88	90	94
152-1	1	9	28	33	45	51	51	56
167-1	51	66	69	69	69	69	69	69
172-1	12	40	49	52	53	56	68	81
175-1	2	9	9	9	10	10	10	10
2	5	11	11	11	11	11	11	11
3	2	2	2	2	2	2	2	2
Mean	3.0	7.3	7.3	7.3	7.6	7.6	7.6	7.6
179-1	24	80	84	84	86	86	87	89
181-1	16	54	58	61	61	65	69	72
2	21	66	73	74	76	78	78	82
Mean	18.5	60.0	65.5	67.5	68.5	71.5	73.5	77.0
183-1	19	21	24	24	24	24	24	24
2	64	66	67	69	69	71	73	78
3	11	20	23	23	27	30	32	34
Mean	31.3	35.6	38.0	38.6	40.0	41.6	43.0	45.3

TABLE VI (Cont'd.)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
196-1	39	78	81	81	82	85	86	92
198-2	3	23	26	26	29	35	35	39
215-1	7	12	12	13	14	14	14	15
2	83	86	87	87	87	87	87	88
3	31	34	35	36	36	36	36	36
4	23	35	37	37	40	41	41	42
5	27	29	32	35	35	35	35	39
6	69	69	69	81	81	81	81	81
7	22	31	39	43	43	47	53	65
8	40	40	40	40	40	40	44	48
Mean	37.7	42.0	43.8	46.5	46.8	47.6	48.8	51.7
219-1	7	12	16	18	19	20	26	33
2	20	69	73	77	77	79	84	91
3	18	24	24	24	24	24	24	26
Mean	15.0	35.0	37.6	39.6	40.0	41.0	44.6	50.0
221-1	9	11	11	11	11	11	11	11
2	20	34	34	34	34	34	35	36
3	46	72	73	73	73	74	74	77
4	56	64	64	64	64	64	64	67
5	7	23	23	23	23	27	31	34
Mean	27.6	40.8	41.0	41.0	41.0	42.0	43.0	45.0
234-1	13	21	21	26	28	31	33	33
2	6	14	14	14	14	14	14	14
3	18	42	45	45	47	50	50	51
Mean	12.3	25.6	26.6	28.3	29.6	31.6	32.3	34.3
General Mean	30.4	40.9	44.1	45.1	46.6	47.9	49.4	53.2

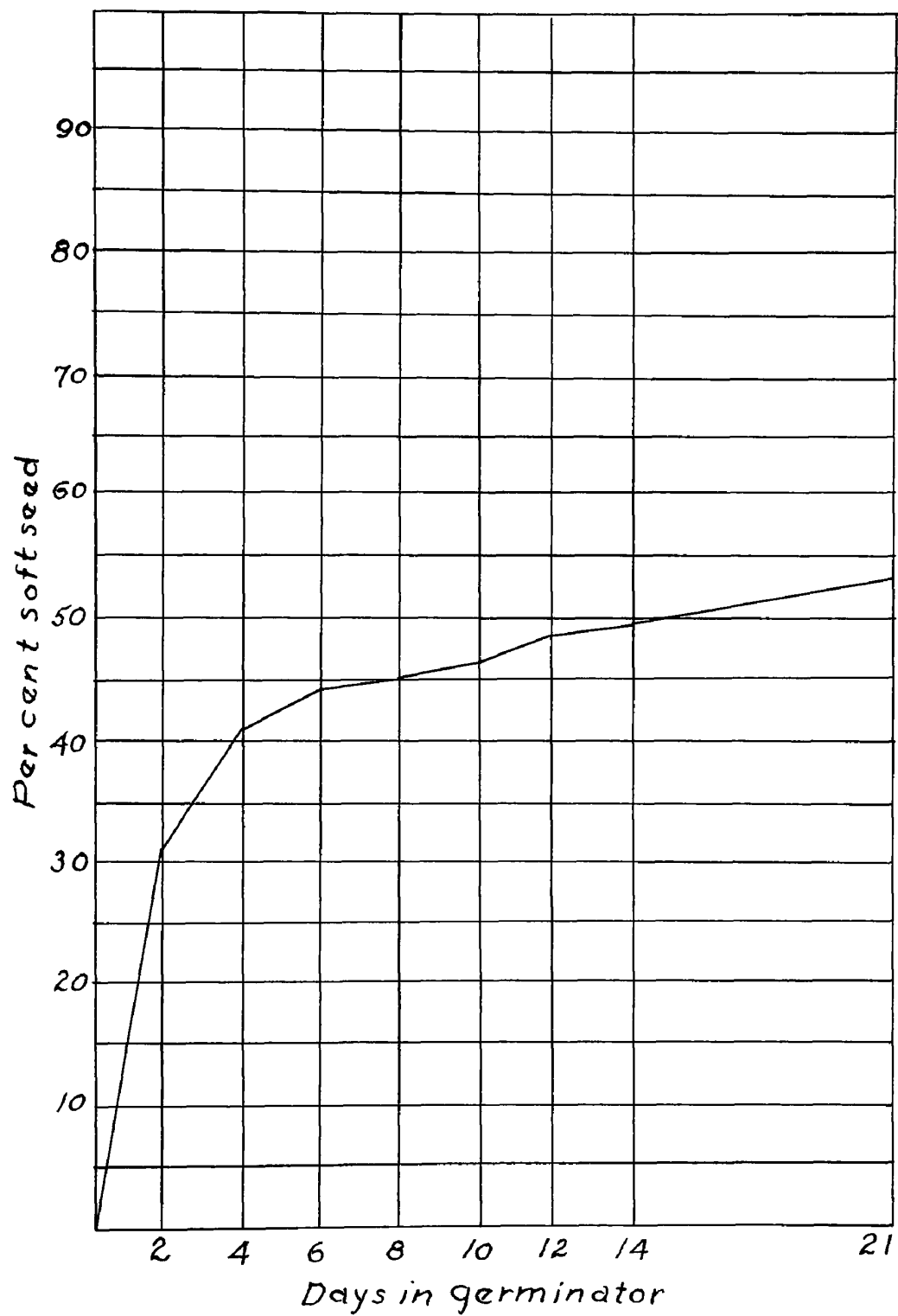


Fig. 6. Germination Rate of the Progeny of Hard-seeded S₁ Parents

seed, respectively, all within the range of permeability of the hard seeded parents. The first two were harder than any of the hard-seeded progeny with the exception of line 175.

In general, as much variation may be found within individual lines as between lines. Only nine progeny groups 1, 20, 35, 44, 106, 110, 137, 181, and 249 exhibit any degree of uniformity within lines.

The tendency for progeny to be the reverse of their parents or to approach the mean of both groups made it impossible to select S₂ parents as hard or as soft as the S₁ parents. Failure to set selfed seed again disposed of many plants which might have been used as S₂ parents. Sixteen individuals from the soft-seeded progeny were selected as S₂ parents. As shown in Table VII and Figure 7 these parents had a mean permeability of 77.1 per cent and a range of 62 to 100 per cent.

Twenty-five plants from the hard-seeded group were selected as parents. These had a mean permeability of 32.3 per cent as shown in Table VIII and Figure 8. One plant in this group, Number 175-3, had only 2 per cent permeable seed.

The mean difference between these two groups was 44.8 per cent which is considerably less than the spread between the S₂ parents. In the absence of any criteria to differentiate between permeability and impermeability, below 40 to 42 per cent germination was arbitrarily selected as impermeability and above 60 per cent as permeable. Exceptions to this rule may be observed in parents 5-1, 99-1, and 117-1 which germinated 47, 56, and 65

TABLE VII

GERMINATION OF SOFT-SEEDED PLANTS
SELECTED AS S₂ PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
7-4	62	65	70	75	81	81	87	91
7-5	47	50	53	53	60	60	62	68
7-6	59	66	66	66	66	67	67	67
35-1	36	64	56	56	56	58	58	60
44-1	69	83	84	85	86	90	91	92
49-1	45	79	83	83	83	83	86	88
49-2	41	67	71	71	73	74	76	76
61-1	23	66	70	71	71	72	73	85
63-1	39	83	93	93	93	93	93	93
63-2	20	62	62	62	65	67	68	77
72-1	24	31	35	42	45	46	48	78
84-1	11	24	28	28	28	31	39	60
110-1	41	51	51	53	57	59	59	64
159-1	82	100	100	100	100	100	100	100
205-8	39	41	46	48	51	54	64	73
266-1	2	12	26	42	50	58	58	62
Mean	39.9	58.4	62.1	64.2	66.6	68.3	70.6	77.1

per cent respectively, but which were included because of the high degree of impermeability of their progenitors.

The lower production of seed by selfed heads and the poor survival of seedlings in the greenhouse even when planted in sterilized soil reduced progeny numbers in every generation. The germination record of the descendants of the S₂ parents is given in Tables IX and X and graphically in Figures 9 and 10.

The second generation produced more soft seed than the

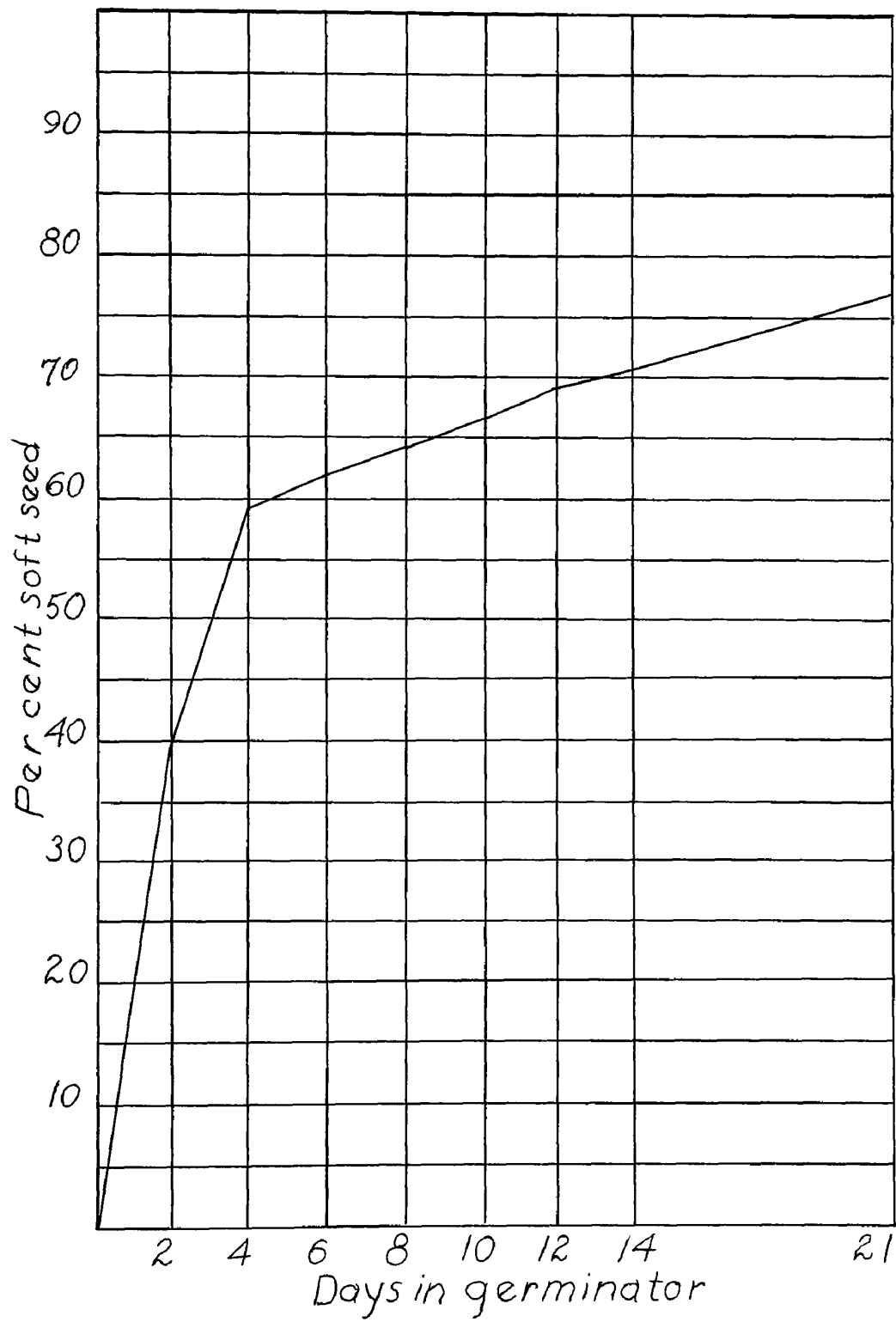


Fig. 7. Germination Rate of Soft-seeded S₂ Parents

TABLE VIII

GERMINATION OF HARD-SEEDED PLANTS SELECTED
AS S₂ PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
5-1	34	47	47	47	47	47	47	47
5-2	29	31	32	32	32	32	32	37
5-7	35	38	40	41	41	41	41	46
14-1	12	13	16	16	16	16	18	18
14-2	20	27	28	28	29	29	30	32
66-2	11	16	16	16	18	19	20	24
92-1	10	24	27	29	31	31	34	40
92-2	15	21	21	22	23	25	29	34
92-3	26	30	31	32	34	34	36	40
93-3	26	35	36	36	37	37	37	40
99-1	36	41	48	49	49	51	53	56
99-2	18	22	22	22	24	24	25	28
99-3	4	18	18	18	19	20	20	22
117-1	22	31	38	38	39	52	62	65
119-1	25	26	26	26	27	28	28	32
119-3	24	26	28	28	28	28	31	35
131-1	40	41	41	41	42	42	42	42
131-2	20	20	23	23	23	23	24	28
131-3	28	28	29	29	29	29	30	30
175-2	5	11	11	11	11	11	11	11
175-3	2	2	2	2	2	2	2	2
183-1	19	21	24	24	24	24	24	24
183-3	11	20	23	23	27	30	32	34
215-1	7	12	12	13	13	14	14	15
219-3	18	24	24	24	24	24	24	26
Mean	19.9	25.0	26.5	26.8	27.5	28.5	29.9	32.3

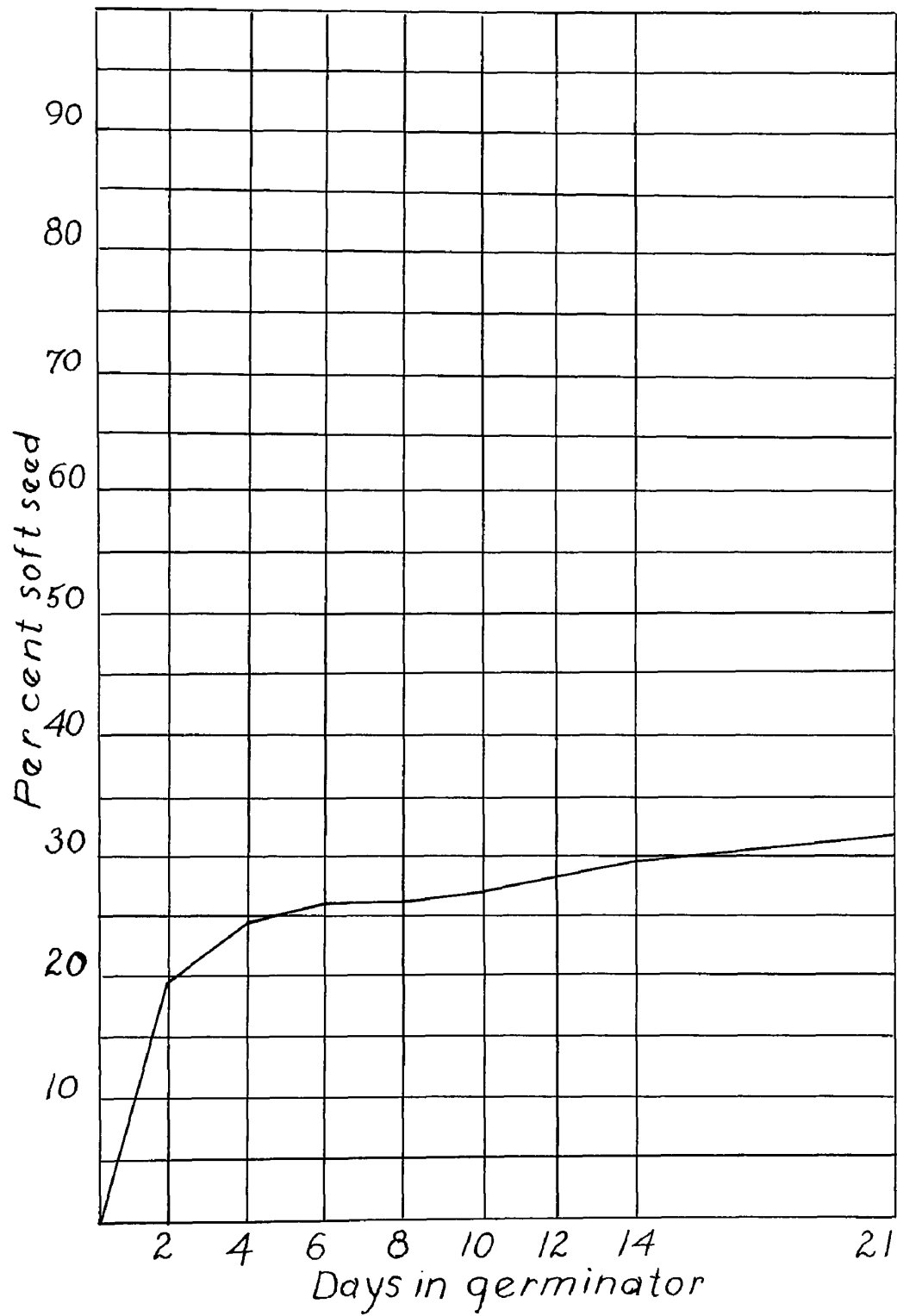


Fig. 8. Germination Rate of Hard-seeded S₂ Parents

TABLE IX

GERMINATION OF PROGENY OF SOFT-SEEDED S2 PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
7-4-1	86	91	95	95	99	99	99	99
7-4-2	95	99	99	99	99	100	100	100
7-4-3	7	14	18	58	78	88	96	99
7-4-4	90	93	93	100	100	100	100	100
7-4-5	80	84	88	92	92	92	92	92
7-4-6	84	88	92	92	92	92	92	96
7-4-7	70	70	77	93	93	93	97	97
Mean	73.1	77.0	80.3	89.9	93.3	94.8	96.6	97.6
7-5-1	40	42	44	50	72	72	76	78
7-5-2	48	52	60	64	68	80	84	84
7-5-3	10	25	45	70	70	75	80	80
7-5-4	30	30	35	40	50	60	60	70
7-5-5	56	63	69	76	80	83	85	88
7-5-6	37	40	40	40	53	60	60	67
7-5-7	43	50	52	55	62	62	63	63
7-5-8	45	56	56	61	63	68	70	72
Mean	38.6	44.7	50.1	57.0	64.7	70.0	72.2	75.2
7-6-1	26	34	36	50	66	66	68	74
7-6-2	18	31	33	40	40	40	43	45
7-6-3	37	57	57	64	64	64	64	67
7-6-4	81	94	97	99	100	100	100	100
7-6-5	78	90	94	97	98	98	99	99
7-6-6	46	52	52	66	75	78	81	81
7-6-7	65	75	80	85	85	85	85	85
7-6-8	26	33	38	51	54	54	70	70
7-6-9	40	43	50	60	63	63	80	86
7-6-10	17	31	54	65	76	80	80	86
7-6-11	50	55	66	70	70	72	72	75
7-6-12	40	40	40	40	40	40	40	50
Mean	43.7	52.9	58.1	65.6	69.2	70.0	73.5	76.5
35-1-1	26	34	34	38	40	41	43	49
35-1-2	11	23	25	25	27	27	27	29
35-1-3	78	78	78	78	78	78	78	79
35-1-4	46	52	52	52	52	52	52	52
35-1-5	17	24	25	28	31	31	32	40
35-1-6	29	35	40	41	43	44	44	50
35-1-7	60	63	63	65	65	65	66	71

TABLE IX (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
35-1-8	28	54	59	64	64	67	67	70
35-1-9	27	31	32	33	33	33	34	45
Mean	35.8	43.8	45.3	47.1	48.1	48.7	49.2	53.9
44-1-1	21	23	24	24	24	24	24	26
44-1-2	71	76	76	76	76	76	76	78
44-1-3	90	92	96	96	100	100	100	100
44-1-4	78	82	85	91	94	96	96	98
44-1-5	43	45	48	52	56	61	62	79
44-1-6	26	28	29	29	29	32	33	49
44-1-7	23	24	28	29	32	39	40	57
44-1-8	49	51	53	54	57	59	59	73
44-1-9	29	57	76	78	84	87	88	95
44-1-10	45	62	66	69	69	72	74	78
44-1-11	16	24	24	24	24	25	26	30
44-1-12	44	49	49	49	50	53	53	57
Mean	44.5	51.0	54.5	55.9	57.8	60.3	60.9	68.3
49-1-1	83	84	86	86	86	86	87	87
49-1-2	76	81	84	86	88	89	89	90
49-1-3	89	94	95	95	96	97	98	98
49-1-4	77	79	83	85	88	91	96	98
49-1-5	32	37	42	42	42	42	46	50
Mean	71.4	75.0	78.0	78.8	80.0	81.0	83.2	84.6
49-2-1	75	76	77	79	80	82	87	97
49-2-2	61	64	66	67	68	69	73	78
49-2-3	72	80	83	84	84	85	86	86
49-2-4	20	24	24	26	26	28	29	37
49-2-5	73	82	86	87	87	88	90	93
49-2-6	37	41	44	47	47	48	49	68
49-2-7	74	87	91	93	96	97	98	98
49-2-8	81	86	88	88	90	90	91	92
49-2-9	19	26	29	30	32	33	36	45
49-2-10	72	72	72	72	72	74	75	85
Mean	58.4	63.8	66.0	67.3	68.2	69.4	71.4	77.6
61-1-1	51	54	56	56	56	58	60	62
61-1-2	52	52	52	56	56	56	56	64
61-1-3	15	17	20	21	21	22	23	33
61-1-4	39	41	45	45	45	47	61	54
Mean	39.2	41.0	43.2	44.5	44.5	45.7	47.5	53.2

TABLE IX (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
63-1-1	56	60	61	62	62	62	63	68
63-1-2	28	38	42	42	43	48	49	57
63-1-3	97	99	99	99	99	99	99	99
63-1-4	58	61	64	64	68	70	70	87
Mean	59.7	64.5	66.5	66.7	68.0	69.7	70.2	77.7
63-2-1	68	80	88	92	92	92	92	96
63-2-2	58	90	93	93	93	95	95	98
63-2-3	40	90	90	95	95	100	100	100
63-2-4	61	98	98	98	98	98	98	98
63-2-5	45	96	100	100	100	100	100	100
63-2-6	65	85	90	95	100	100	100	100
Mean	56.2	89.8	93.2	96.3	96.3	97.5	97.5	98.7
72-1-1	8	68	68	68	68	68	68	72
72-1-2	46	94	94	100	100	100	100	100
72-1-3	50	95	95	100	100	100	100	100
72-1-4	65	75	75	75	75	80	80	85
72-1-5	13	98	98	98	98	98	98	100
72-1-6	64	80	80	80	80	84	84	92
Mean	41.0	85.0	85.0	86.8	86.8	88.3	88.3	91.5
84-1-1	48	48	48	48	48	49	49	51
84-1-2	60	61	62	63	63	64	64	65
84-1-3	74	74	74	74	74	74	75	77
84-1-4	18	19	20	20	21	21	22	23
84-1-5	39	39	43	43	43	44	44	45
84-1-6	82	82	82	82	82	83	83	87
84-1-7	44	47	52	55	61	65	66	74
Mean	52.1	52.8	54.4	55.0	56.0	57.1	57.6	60.3
110-1-1	30	35	38	38	40	45	54	76
110-1-2	48	57	58	60	62	63	63	67
110-1-3	42	44	45	45	45	45	45	45
Mean	40.0	45.3	47.0	47.7	49.0	50.3	50.3	62.7
159-1-1	31	39	45	47	52	57	62	77

TABLE IX (Cont'd)

	Germination by days								
Plant number	2	4	6	8	10	12	14	21	
205-8-1	89	100	100	100	100	100	100	100	
205-8-2	96	96	100	100	100	100	100	100	
205-8-3	88	92	96	96	96	96	96	100	
205-8-4	65	75	80	85	90	95	95	95	
Mean	84.5	90.7	94.0	95.2	96.5	97.8	97.8	98.7	
266-1-1	49	72	78	82	85	86	89	95	
266-1-2	6	6	6	6	6	6	6	9	
Mean	27.5	39.0	42.0	44.0	45.5	46.5	46.5	52.0	
General									
Mean	50.3	60.1	63.1	66.2	68.2	70.0	71.4	76.5	

previous generation regardless of the permeability of the parents. Plants, from the soft-seeded parents, with a mean germination of 76.5 per cent were only 3.7 per cent softer than those, of the hard-seeded parents, which had a mean germination of 72.8 per cent. It may be noted that the progeny of the permeable group differed from their parents by only 0.6 per cent, but the fact that the hard-seeded and soft-seeded plants were so near alike in permeability appears to indicate that environmental factors were more contributory to this condition than inheritance.

There was, in some instances, a slight tendency for the plants from the hard-seeded parents to perform as did their antecedents but in all cases these plants were softer than

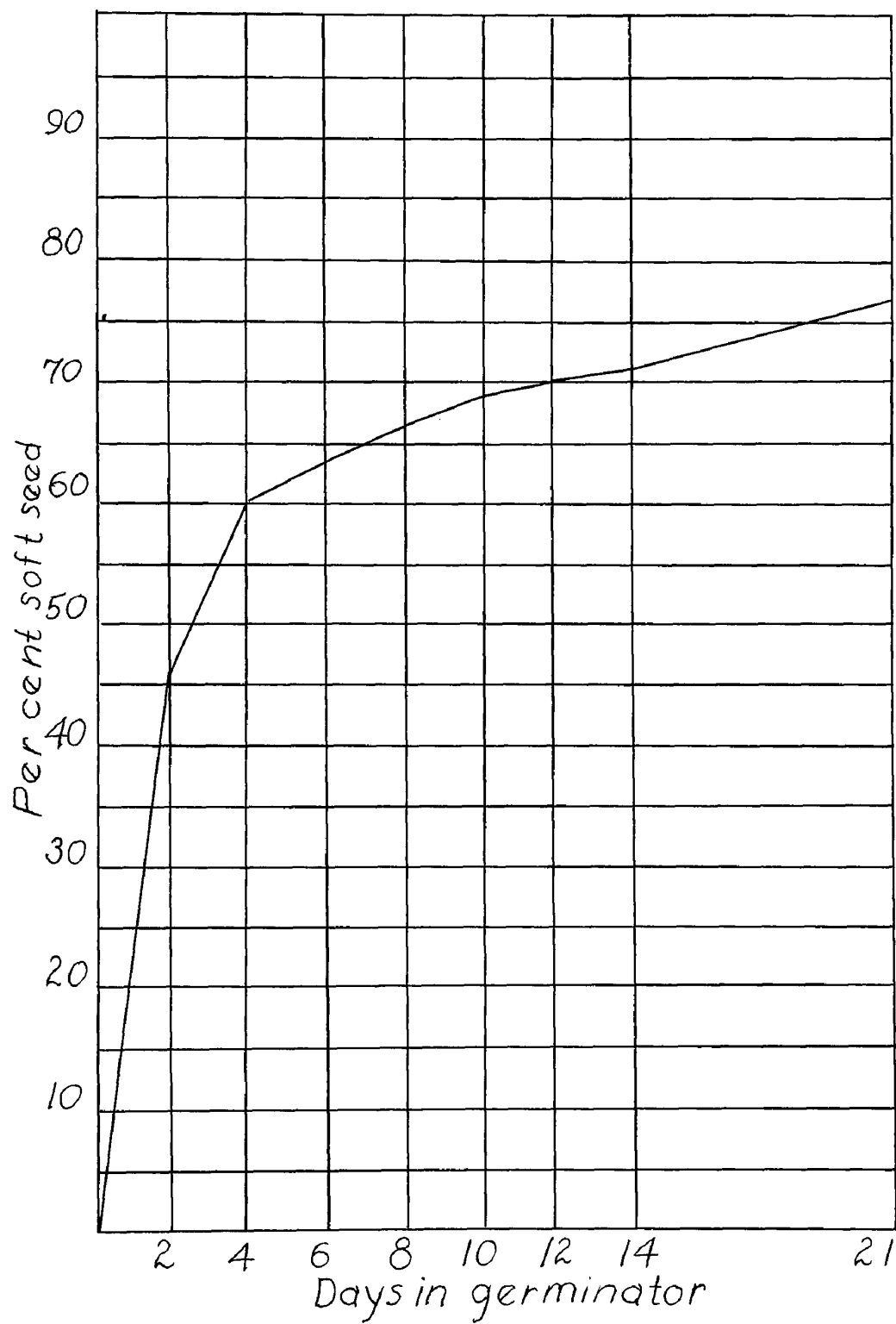


Fig. 9. Rate of Germination of Progeny of Soft-seeded S_2 Parents

their parents. Plant 175-3 produced offspring with the lowest percentage of permeability. It would appear, also, that the impermeability of parent 175-2 was passed on to its offspring, which had a mean germination of 38.7 per cent. By the same token, parent 117-1 should have had the softest seeded progeny, but this was not the case.

In this generation seven of the sixteen soft-seeded progeny lines were softer than their parents, but only one hard-seeded line, 119-3 was as hard as its parent. Variability within lines was again the trend with only three soft-seeded lines proving uniform. Nine lines from the hard-seeded parents were reasonably uniform, but all of these were soft or very soft.

With the gradual reduction of plants which duplicated their parents and the failure of many to produce selfed seed, the selection of S_3 parents in both groups was limited. Sixteen plants were found suitable for soft-seeded parents and thirteen for hard-seeded parents. The S_3 parents and their germination record is shown in Tables XI and XII and also in Figures 11 and 12. In one case descendants of plant 266-1 appear in both S_3 parental lines which was originally classed as soft seeded. The selection of plant 266-1-2 as a parent for the hard-seeded group was justified by the possibility that segregation might be taking place and by the fact that this

TABLE X

GERMINATION OF PROGENY OF HARD-SEEDED S2 PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
5-1-1	52	72	76	76	76	76	84	92
5-1-2	68	84	88	92	92	92	92	96
5-1-3	4	36	40	52	64	72	72	84
Mean	41.3	64.0	68.0	73.3	77.3	80.0	82.6	90.6
5-2-1	80	87	87	94	97	97	97	97
5-2-2	96	98	100	100	100	100	100	100
5-2-3	40	68	72	80	80	84	84	84
5-2-4	60	73	76	89	90	90	90	90
5-2-5	77	90	90	93	93	100	100	100
5-2-6	63	74	76	82	83	84	84	86
5-2-7	85	93	93	98	100	100	100	100
5-2-8	38	48	50	60	70	75	83	90
5-2-9	80	94	98	99	99	99	100	100
5-2-10	80	95	95	100	100	100	100	100
5-2-11	48	78	91	100	100	100	100	100
5-2-12	41	58	73	99	99	99	99	99
Mean	65.6	76.6	83.4	91.1	92.5	94.0	94.7	95.5
5-7-1	48	56	66	71	73	78	80	80
5-7-2	60	63	63	65	65	65	65	65
5-7-3	28	28	32	32	36	36	36	48
5-7-4	50	60	65	70	72	72	72	72
5-7-5	42	52	57	59	61	64	66	67
Mean	45.6	51.8	56.6	59.4	61.4	63.0	63.8	66.4
14-1-1	0	2	6	22	33	40	42	44
14-1-2	8	38	44	48	50	66	66	70
14-1-3	6	52	54	55	57	68	74	80
14-1-4	3	36	36	39	55	68	68	68
14-1-5	5	37	37	39	42	50	50	52
14-1-6	0	8	8	12	16	24	28	44
14-1-7	0	10	15	30	60	75	80	95
14-1-8	0	5	5	15	22	45	48	53
Mean	2.75	23.5	25.6	32.5	41.8	54.5	57.0	63.2
14-2-1	9	40	42	42	44	46	48	58
14-2-2	42	59	59	67	74	89	91	84
14-2-3	49	80	83	86	87	87	87	94
14-2-4	66	79	82	83	85	92	94	94

TABLE X (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
14-2-5	47	67	72	77	85	95	95	98
14-2-6	68	79	83	85	87	91	91	95
14-2-7	79	85	86	86	86	86	86	86
14-2-8	73	74	74	76	80	80	80	80
14-2-9	22	52	52	54	56	61	61	61
14-2-10	20	40	46	46	49	58	58	68
14-2-11	32	76	80	82	82	82	82	82
14-2-12	26	60	65	74	75	78	78	83
Mean	44.4	65.9	68.6	71.5	74.1	78.7	73.1	82.7
66-2-1	65	65	65	65	65	65	65	70
66-2-2	30	70	80	80	80	80	80	85
66-2-3	15	45	45	45	45	55	55	75
66-2-4	20	35	40	45	45	50	50	55
Mean	32.5	53.7	57.5	58.7	58.7	62.5	62.5	71.2
92-1-1	76	98	98	98	98	100	100	100
92-1-2	30	50	60	60	60	60	60	60
92-1-3	40	68	72	72	72	72	72	72
92-1-4	25	60	67	68	68	70	70	80
92-1-5	49	58	63	65	69	73	73	75
92-1-6	57	77	77	77	80	80	83	86
Mean	46.1	68.5	72.8	73.3	74.5	75.8	76.3	78.8
92-2-1	90	91	91	91	91	91	91	91
92-2-2	85	90	95	95	95	95	95	95
92-2-3	70	100	100	100	100	100	100	100
92-2-4	65	90	90	90	90	90	90	95
Mean	77.5	92.7	93.7	93.7	93.7	93.7	93.7	95.2
92-3-1	54	60	69	72	72	84	84	87
92-3-2	50	62	68	70	74	78	80	80
92-3-3	56	60	69	69	69	76	80	84
Mean	53.3	60.6	68.6	70.3	71.6	79.3	81.3	83.6
93-3-1	77	82	82	82	82	82	82	86
93-3-2	84	84	84	88	88	88	88	92
93-3-3	57	57	57	57	57	60	60	60
93-3-4	35	40	40	40	45	45	45	65
93-3-5	47	53	53	56	56	56	60	60
93-3-6	93	93	93	93	93	93	93	93
93-3-7	77	77	77	77	77	77	77	80

TABLE X (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
93-3-8	92	100	100	100	100	100	100	100
Mean	70.2	73.2	73.2	74.1	74.5	75.1	75.6	80.7
99-1-1	10	93	93	95	97	100	100	100
99-1-2	45	85	85	95	95	95	100	100
99-1-3	6	86	89	94	98	100	100	100
99-1-4	20	70	70	70	72	74	77	87
99-1-5	30	97	97	97	97	100	100	100
99-1-6	5	65	69	84	86	88	89	91
Mean	19.3	82.6	83.8	89.1	90.6	92.8	94.3	96.3
99-2-1	4	35	37	40	45	50	55	85
99-2-2	6	60	60	63	67	67	70	87
99-2-3	15	55	55	75	75	75	75	85
Mean	8.3	50.0	50.6	59.3	62.3	64.0	66.6	85.7
99-3-1	8	82	82	85	85	95	95	95
99-3-2	8	52	52	60	68	72	72	80
99-3-3	5	50	55	55	60	70	70	75
99-3-4	3	45	45	60	71	74	74	80
99-3-5	17	95	95	97	98	98	100	100
99-3-6	4	84	84	92	94	100	100	100
Mean	7.5	68.0	68.8	74.8	79.3	84.8	85.1	88.3
117-1-1	14	21	21	21	21	21	21	25
117-1-2	0	10	16	20	24	30	40	45
117-1-3	2	22	30	30	36	40	48	62
Mean	5.3	17.1	22.3	23.6	27.0	30.3	36.3	44.0
119-1-1	70	76	76	76	76	76	76	79
119-3-1	24	26	28	28	28	31	31	35
131-1-1	75	80	85	85	85	86	86	89
131-2-1	77	80	80	80	80	82	82	87
131-2-2	71	74	74	76	76	77	79	83
131-2-3	67	72	72	72	76	77	79	83
131-2-4	28	32	33	33	33	33	33	39
131-2-5	33	36	36	36	36	36	36	37

TABLE X (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
131-2-6	41	60	61	61	61	61	61	62
131-2-7	64	80	80	80	80	80	80	81
131-2-8	53	61	63	64	64	64	64	64
131-2-9	63	64	64	64	64	64	64	68
131-2-10	44	53	55	55	55	55	55	56
Mean	54.1	61.2	61.8	62.1	62.5	62.9	63.3	66.0
131-3-1	48	48	49	50	51	52	55	58
175-2-1	22	24	28	28	33	40	40	53
175-2-2	16	29	37	42	52	57	57	70
175-2-3	37	39	40	41	44	47	49	53
175-2-4	16	19	20	20	20	22	23	29
175-2-5	17	18	18	18	18	18	18	19
175-2-6	10	11	12	14	15	16	17	27
175-2-7	12	12	13	14	15	16	16	20
Mean	18.5	21.7	24.0	25.2	28.1	30.8	31.4	38.7
175-3-1	24	32	34	35	39	40	43	46
175-3-2	25	29	30	31	34	35	35	36
175-3-3	22	26	26	27	28	30	30	33
Mean	23.6	29.0	30.0	31.0	33.6	35.0	36.0	38.3
183-1-1	12	24	28	33	34	36	37	44
183-1-2	27	37	39	41	47	53	54	59
183-1-3	20	31	34	37	44	45	45	52
183-1-4	19	28	30	36	40	44	46	48
Mean	19.5	30.0	32.7	36.7	41.2	44.7	45.5	50.7
183-3-1	26	28	30	31	33	35	35	39
183-3-2	10	22	22	22	22	25	25	30
183-3-3	36	38	40	40	40	42	42	42
183-3-4	44	48	48	52	52	56	60	60
Mean	29.0	34.0	35.0	36.2	36.7	39.5	40.5	42.7
215-1-1	86	90	94	95	97	97	97	98
215-1-2	85	88	90	91	94	96	96	97
215-1-3	64	76	82	84	86	88	88	92
215-1-4	51	55	61	63	67	69	69	71
215-1-5	50	57	57	57	64	64	64	64

TABLE X (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
215-1-6	77	77	77	77	80	86	86	90
Mean	68.8	73.8	76.8	77.8	81.3	83.3	83.3	85.3
219-3-1	30	30	32	35	39	42	45	72
219-3-2	30	34	36	36	40	40	42	47
Mean	30.0	32.0	34.0	35.5	39.5	41.0	43.5	59.5
221-1-1	24	26	31	31	31	33	36	36
221-1-2	22	28	32	33	35	35	36	44
Mean	23.0	27.0	30.5	32.0	33.0	34.0	36.0	40.0
General Mean	40.6	57.3	59.6	62.4	64.6	67.5	68.4	72.8

plant was the most impermeable in either group. A fairly wide margin of permeability between parents was obtained with a difference of 47.5 per cent, with the hard-seeded parents, running only 4.7 per cent softer than the S₂ parents.

The germination of the descendants of the S₃ parents is given in Tables XIII and XIV and presented also in Figures 13 and 14. It will be noted that the mean germination of both groups was again practically the same with a difference of only 1.6 per cent. Furthermore both groups

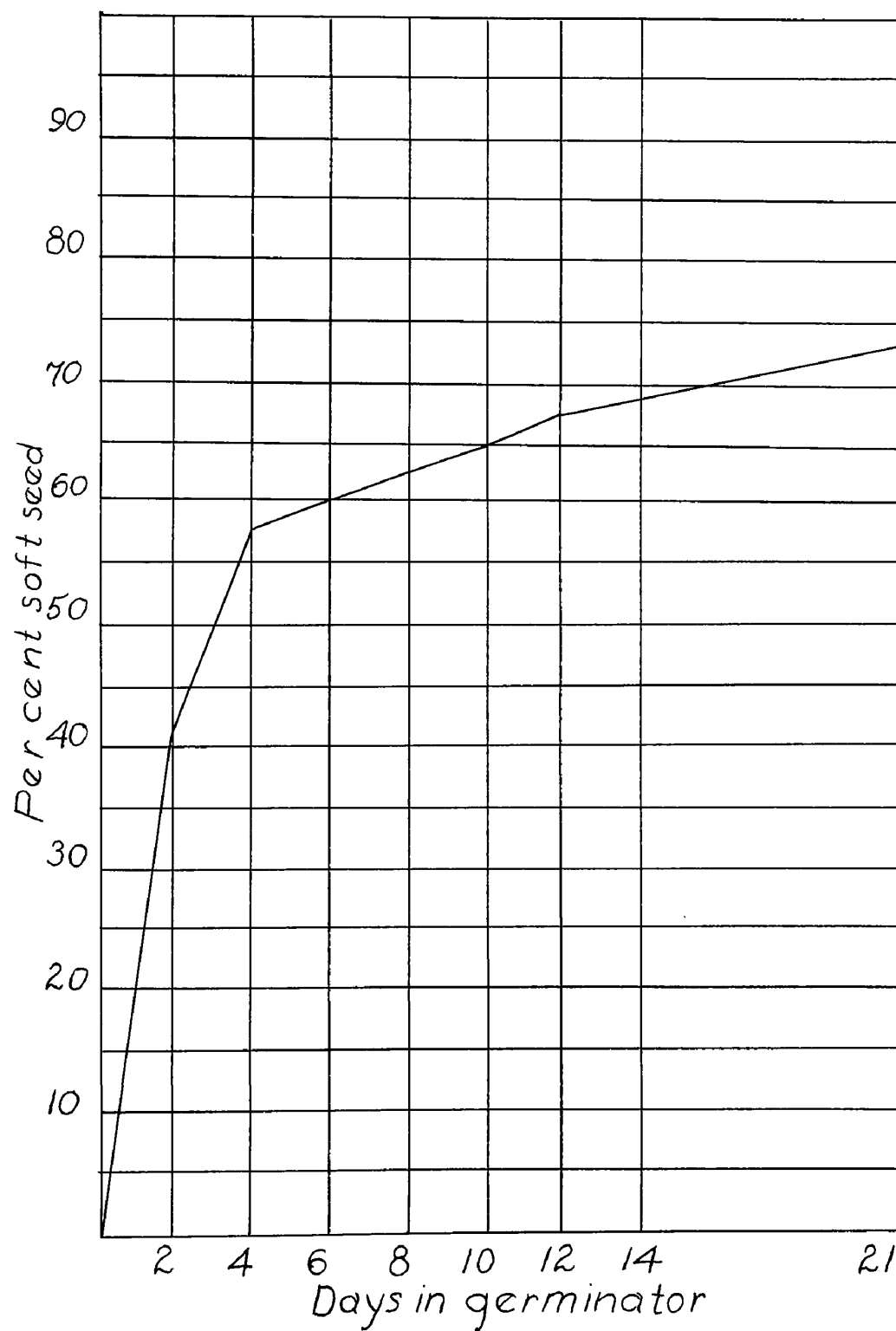


Fig. 10. Rate of Germination of Progeny of Hard-seeded S₂ Parents

TABLE XI

GERMINATION OF SOFT-SEEDED S_3 PLANTS SELECTED AS PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
35-1-3	78	78	78	78	78	78	78	79
35-1-7	60	63	63	65	65	65	66	71
44-1-3	90	92	96	96	100	100	100	100
44-1-4	78	82	85	91	94	96	96	98
44-1-9	29	57	76	78	84	87	88	95
49-1-1	83	84	86	86	86	86	87	87
49-1-2	76	81	84	86	88	89	89	90
49-1-3	89	94	95	95	96	97	98	98
49-1-4	77	79	83	85	88	91	96	98
49-2-3	72	80	83	84	84	85	86	86
49-2-5	73	82	86	87	87	88	90	93
49-2-7	74	87	91	93	96	97	98	98
49-2-8	81	86	88	88	90	90	91	92
84-1-4	18	19	20	20	21	21	22	23
84-1-5	39	39	43	43	43	44	44	45
266-1-1	49	72	78	82	85	87	89	95
Mean	66.6	73.4	77.2	78.6	80.3	81.1	82.4	84.2

were as permeable as the original S_1 soft parents regardless of parental selection. Table XIV also shows that only five individuals were obtained which met the arbitrary criteria originally set up for impermeability.

Plant 266-1-2 which germinated only 9 per cent as a parent produced plants which ranked second in permeability in both groups.

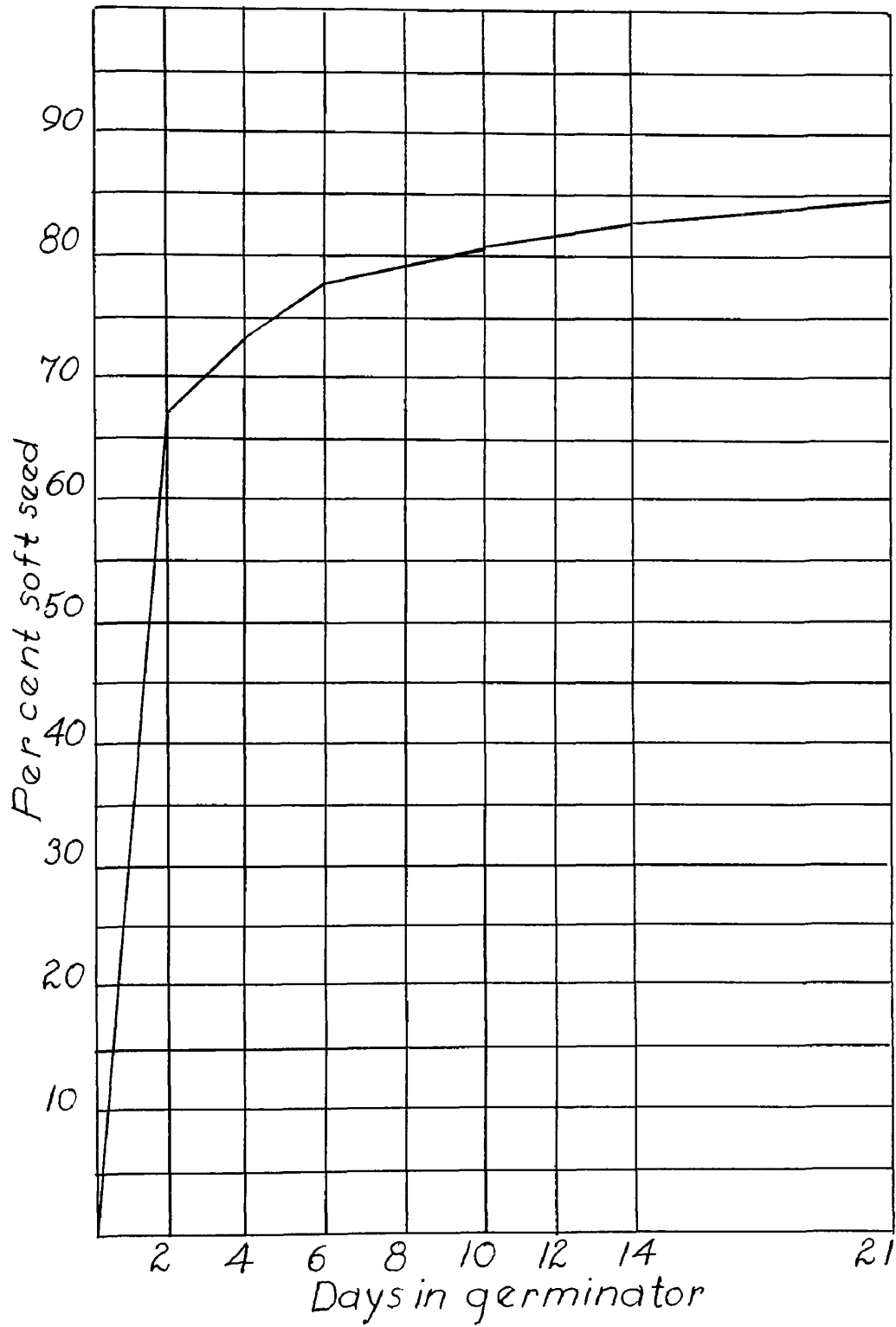


Fig. 11. Germination Rate of Soft-seeded S₃ Parents

TABLE XII

GERMINATION OF HARD SEEDED S_3 PLANTS SELECTED AS PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
117-1-1	14	21	21	21	21	21	21	25
119-3-1	24	26	28	28	28	31	31	35
175-2-2	16	29	37	42	52	57	57	70
175-2-3	37	39	40	41	44	47	49	53
175-2-4	16	19	20	20	20	22	23	29
175-2-5	17	18	18	18	18	18	18	19
175-2-6	10	11	12	14	15	16	17	27
175-2-7	12	12	13	14	15	16	16	20
183-1-1	12	24	28	33	34	36	37	44
183-3-1	26	28	30	31	33	35	35	39
219-3-1	30	30	32	35	39	42	45	72
221-1-1	24	26	31	31	31	33	36	36
266-1-2	6	6	6	6	6	6	6	9
Mean	18.8	22.1	24.3	25.7	27.4	29.1	30.0	36.7

A summary of the three parental and three progeny generations is given in Table XV and a visual comparison in Figure 15. The tendency, as shown in this figure, for both progeny groups to be approximately equal in all three generations would appear to indicate that crimson clover, in respect to permeability, is in fact a pure line and that any variations appearing within plants may be attributed to

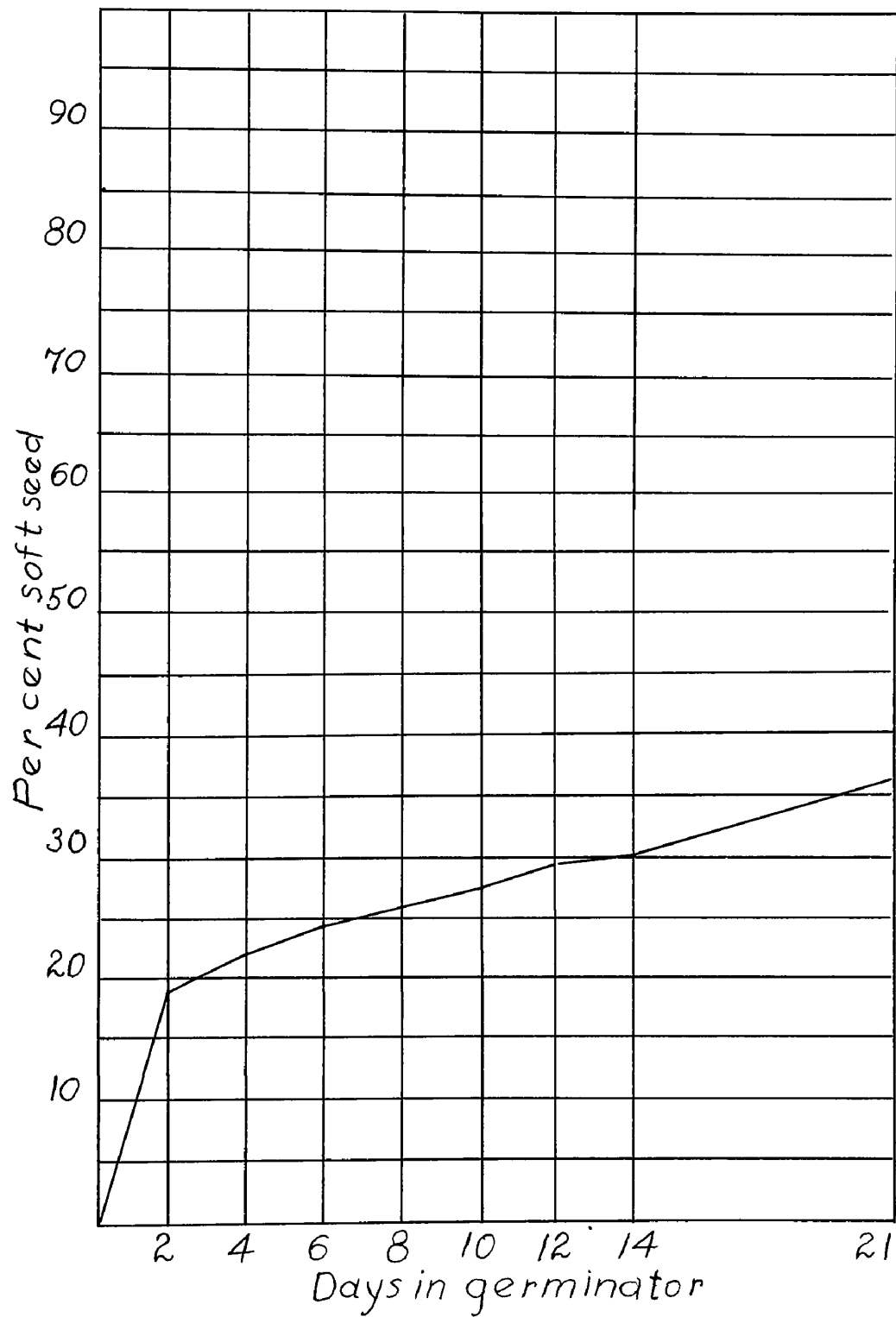


Fig. 12. Germination Rate of Hard-seeded S₃ Parents

external factors. If the flowers were self pollinated, this premise might be accepted but according to Pieters and Hollowell (23) the flowers are not self tripping even though highly self-fertile. The poor set of seed under greenhouse conditions serves to verify their observations.

The percentage of outcrossing also leads to the rejection of this premise. Through selfing white flowered plants in the field it was found that white flowers breed true as a recessives. Counts of progeny of selfed whites gave one-hundred and thirty four whites, and ten red blooming plants, whereas from open pollinated white plants 135 out of a total of 197 plants were red. The ten plants showing up in the selfed lines may be considered evidence of insect visitation before the heads were covered, but the large number of reds from the open pollinated plants indicates that outcrossing took place to the extent of 68.4 per cent.

This high percentage of cross-pollination practically eliminates the possibility of impermeability or the reverse being inherited as a pure line. Excepting that this characteristic of legumes is of a very heterozygous nature, it would appear that three years of inbreeding should have produced some evidence of inheritance. Therefore the conclusion that permeability is not inherited would appear to be justified. If this is the case any differences in plants must be due to a differential response to external factors.

TABLE XIII

GERMINATION OF PROGENY OF SOFT-SEEDED S_3 PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
35-1-3-1	28	80	81	81	82	82	82	82
35-1-3-2	48	95	95	95	95	95	95	95
35-1-3-3	6	78	78	80	80	80	80	80
35-1-3-4	26	95	96	97	97	97	97	97
35-1-3-5	19	97	98	99	99	99	100	100
35-1-3-6	30	100	100	100	100	100	100	100
35-1-3-7	22	100	100	100	100	100	100	100
35-1-3-8	24	96	99	99	99	99	99	99
35-1-3-9	41	98	98	98	98	98	98	98
35-1-3-10	31	95	95	95	95	95	95	95
35-1-3-11	94	96	96	96	96	96	96	96
Mean	33.5	93.6	94.1	94.5	94.6	94.6	94.6	94.6
35-1-7-1	28	97	97	98	98	99	99	99
35-1-7-2	91	91	93	100	100	100	100	100
35-1-7-3	20	80	80	80	80	80	80	80
35-1-7-4	20	65	65	65	65	65	65	65
Mean	19.2	83.2	83.7	85.7	85.7	86.0	86.0	86.0
44-1-3-1	35	55	60	60	60	60	60	60
44-1-3-2	30	50	55	55	60	60	60	70
44-1-3-3	47	57	57	60	60	63	63	70
44-1-3-4	60	70	70	70	70	75	75	80
44-1-3-5	58	73	73	75	75	75	75	80
Mean	46.0	61.0	63.0	64.0	65.0	66.6	66.6	72.0
44-1-4-1	60	96	96	96	96	96	100	100
44-1-4-2	80	97	97	97	97	97	97	97
44-1-4-3	40	84	88	96	96	100	100	100
44-1-4-4	70	80	85	85	85	90	90	95
44-1-4-5	70	75	75	75	80	85	85	85
44-1-4-6	80	90	90	95	95	95	95	95
Mean	66.6	87.0	88.5	90.6	91.5	93.8	94.5	95.3
44-1-9-1	64	73	75	75	78	78	78	78
44-1-9-2	45	80	80	80	80	80	80	85
44-1-9-3	67	79	79	79	85	87	87	90
44-1-9-4	77	92	93	96	98	98	98	98
44-1-9-5	70	90	93	95	95	95	95	98
44-1-9-6	52	80	80	80	80	80	80	80

TABLE XIII (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
44-1-9-7	55	87	87	89	89	89	89	89
Mean	61.4	83.0	83.8	84.8	86.4	86.7	86.7	88.2
49-1-1-1	55	95	95	95	98	98	98	98
49-1-1-2	32	96	100	100	100	100	100	100
49-1-1-3	40	92	96	96	96	96	96	98
49-1-1-4	41	86	90	91	91	94	94	96
Mean	42.0	92.2	95.2	95.5	96.2	97.0	97.0	98.0
49-1-2-1	20	50	50	55	60	65	65	65
49-1-2-2	7	50	50	50	50	50	50	57
49-1-2-3	10	80	85	90	95	100	100	100
49-1-2-4	9	64	64	68	70	70	70	72
49-1-2-5	12	62	68	70	70	74	74	76
Mean	11.6	61.2	63.4	66.6	69.0	71.8	71.8	74.0
49-1-3-1	3	13	40	43	43	43	49	62
49-1-3-2	60	84	100	100	100	100	100	100
49-1-3-3	48	68	73	73	75	80	80	85
49-1-3-4	55	94	100	100	100	100	100	100
Mean	41.5	64.7	78.2	79.0	79.5	80.7	82.2	86.7
49-1-4-1	26	92	95	98	98	98	98	98
49-1-4-2	24	92	100	100	100	100	100	100
49-1-4-3	66	94	97	97	97	97	97	97
49-1-4-4	16	72	96	96	100	100	100	100
49-1-4-5	47	90	97	97	97	97	97	97
Mean	35.8	88.0	97.0	97.6	98.4	98.4	98.4	98.4
49-2-3-1	46	85	90	94	98	98	98	100
49-2-3-2	25	70	70	80	100	100	100	100
49-2-3-3	3	33	60	70	90	97	97	97
49-2-3-4	40	79	91	95	99	100	100	100
Mean	28.5	66.7	77.7	84.7	96.7	98.7	98.7	99.2
49-2-5-1	58	96	98	100	100	100	100	100
49-2-5-2	45	85	90	100	100	100	100	100
49-2-5-3	80	88	96	100	100	100	100	100
49-2-5-4	48	84	96	100	100	100	100	100
49-2-5-5	85	95	95	95	95	100	100	100
Mean	63.2	89.6	96.6	99.0	99.0	100.0	100.0	100.0
49-2-7-1	40	80	100	100	100	100	100	100

TABLE XIII (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
49-2-7-2	53	86	93	97	97	97	97	97
49-2-7-3	40	76	84	100	100	100	100	100
49-2-7-4	76	92	100	100	100	100	100	100
49-2-7-5	50	82	86	94	96	96	96	98
Mean	51.8	83.2	92.6	98.2	98.6	98.6	98.6	99.0
49-2-8-1	56	74	75	77	77	77	77	80
49-2-8-2	98	98	98	98	98	98	99	99
49-2-8-3	85	93	93	95	95	95	95	95
49-2-8-4	88	94	94	94	94	94	96	96
49-2-8-5	54	60	62	62	62	62	62	72
49-2-8-6	85	90	90	90	90	90	90	95
49-2-8-7	70	70	70	70	70	70	70	70
Mean	76.5	82.7	83.1	83.7	83.7	83.7	84.1	86.7
84-1-4-1	3	6	9	19	19	19	19	19
84-1-4-2	7	15	23	30	32	37	37	42
84-1-4-3	20	40	44	48	48	48	48	48
84-1-4-4	14	31	34	37	37	40	43	46
84-1-4-5	0	11	61	73	73	75	75	79
84-1-4-6	4	20	44	48	52	72	72	84
Mean	8.0	20.5	35.8	42.5	43.5	48.5	49.0	53.0
84-1-5-1	10	15	15	20	20	20	20	20
84-1-5-2	60	84	88	92	92	92	92	96
84-1-5-3	35	42	44	44	44	46	48	58
84-1-5-4	30	38	38	39	42	44	46	62
Mean	33.7	44.7	46.2	48.7	49.5	50.5	51.5	59.0
266-1-1-1	55	80	80	80	80	85	85	95
266-1-1-2	5	10	15	25	55	55	55	65
266-1-1-3	0	4	8	12	20	25	29	88
266-1-1-4	0	30	40	70	85	95	95	100
266-1-1-5	0	10	30	45	45	45	45	80
266-1-1-6	5	40	55	75	85	85	90	100
Mean	10.8	29.0	38.0	51.1	61.6	65.0	66.5	88.0
GENERAL								
Mean	40.3	71.9	76.6	80.4	81.5	82.8	83.1	86.3

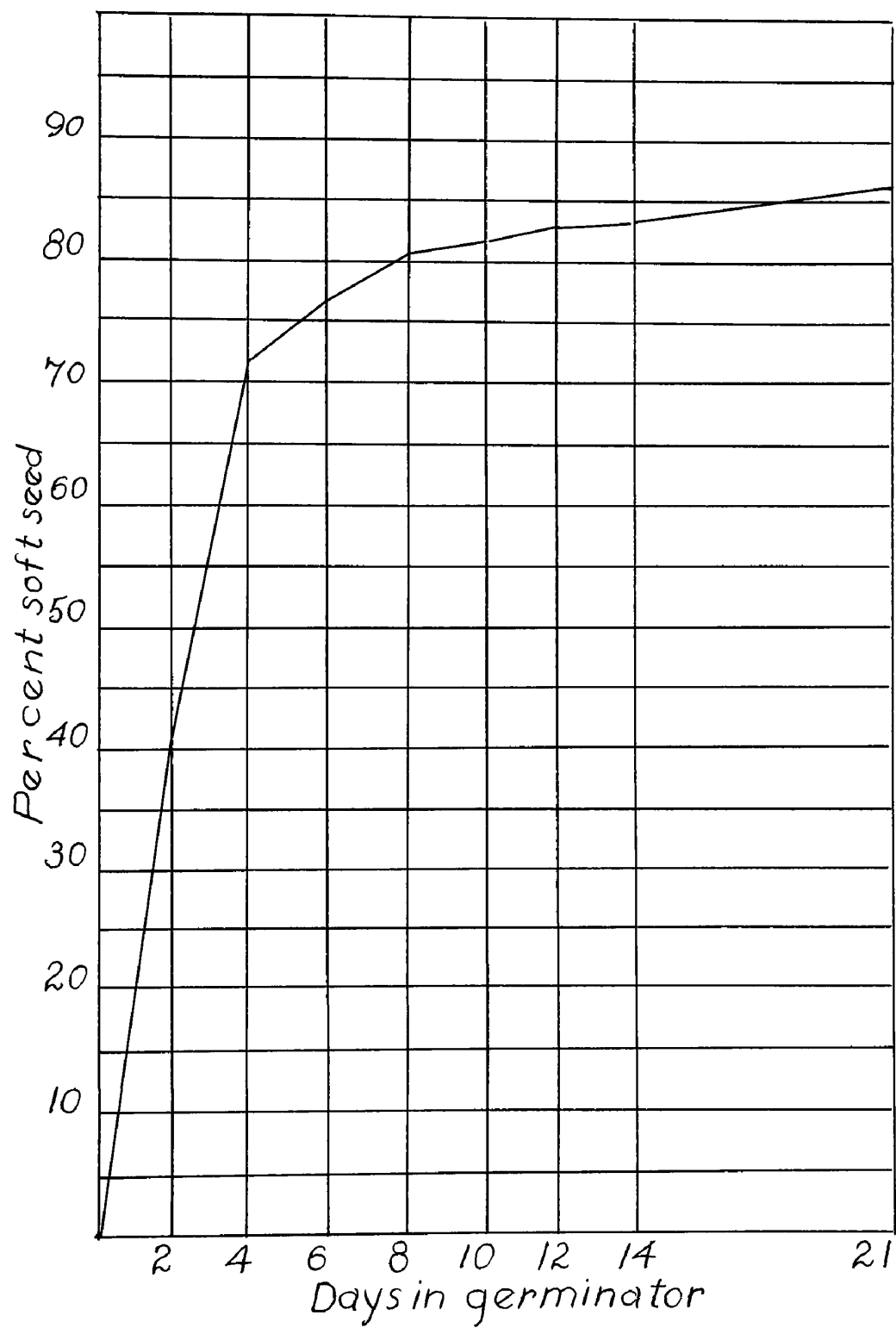


Fig. 13. Germination Rate of the Progeny of Soft-seeded S_3 Parents

TABLE XIV

GERMINATION OF PROGENY OF HARD-SEEDED S_3 PARENTS

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
117-1-1-1	12	12	12	15	15	15	20	25
117-1-1-2	6	18	18	18	18	18	20	38
117-1-1-3	9	14	14	16	20	25	28	38
117-1-1-4	8	16	17	17	22	24	32	41
117-1-1-5	6	20	20	20	20	28	40	48
Mean	8.2	16.0	16.2	17.2	19.0	22.0	28.0	38.0
119-3-1-1	85	93	96	96	96	97	97	99
119-3-1-2	60	85	90	100	100	100	100	100
119-3-1-3	27	83	90	97	100	100	100	100
Mean	57.3	87.0	92.0	97.6	98.6	99.0	99.0	99.6
175-2-2-1	68	72	72	72	76	84	84	96
175-2-2-2	60	60	67	77	80	80	80	90
175-2-2-3	50	70	78	78	80	83	86	89
175-2-2-4	52	60	60	60	60	60	60	72
175-2-2-5	48	60	68	72	73	75	76	81
Mean	55.6	64.4	69.0	71.8	73.8	76.4	77.2	85.6
175-2-3-1	68	73	78	85	85	87	87	92
175-2-3-2	85	90	95	95	95	95	95	95
175-2-3-3	62	66	70	74	76	76	76	80
175-2-3-4	84	84	84	84	84	84	88	96
175-2-3-5	60	67	70	77	80	80	80	86
Mean	71.8	76.0	79.4	83.0	84.0	84.4	85.2	89.8
175-2-4-1	63	76	76	80	83	83	83	86
175-2-4-2	44	44	46	50	50	50	50	58
175-2-4-3	80	87	87	90	90	90	90	94
Mean	62.3	69.0	69.0	73.3	74.3	74.3	74.3	79.3
175-2-5-1	30	30	30	30	30	30	30	40
175-2-5-2	52	60	68	70	70	70	70	74
175-2-5-3	50	50	55	55	60	60	60	60
175-2-5-4	50	60	60	65	65	65	65	70
175-2-5-5	55	60	65	70	80	85	85	95
Mean	47.4	52.0	55.6	58.0	61.0	62.0	62.0	67.8
175-2-6-1	80	84	86	86	87	87	89	93
175-2-6-2	25	47	49	50	50	51	51	58

TABLE XIV (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
175-2-6-3	57	67	80	83	83	83	86	94
175-2-6-4	95	95	95	95	95	95	95	95
175-2-6-5	65	85	90	90	90	90	90	95
175-2-6-6	23	89	93	93	97	97	97	97
Mean	57.5	77.8	82.1	82.8	83.6	83.8	84.6	88.6
175-2-7-1	90	90	95	100	100	100	100	100
175-2-7-2	76	76	80	80	84	84	84	84
175-2-7-3	95	95	95	95	95	95	95	95
175-2-7-4	90	95	100	100	100	100	100	100
175-2-7-5	80	92	93	93	95	95	97	97
175-2-7-6	87	87	93	93	93	93	93	93
Mean	86.3	89.1	92.6	93.5	94.5	94.5	94.8	94.8
183-1-1-1	100	100	100	100	100	100	100	100
183-1-1-2	100	100	100	100	100	100	100	100
183-1-1-3	96	100	100	100	100	100	100	100
183-1-1-4	95	98	100	100	100	100	100	100
Mean	97.7	99.5	100.0	100.0	100.0	100.0	100.0	100.0
183-3-1-1	100	100	100	100	100	100	100	100
183-3-1-2	92	94	94	94	94	96	96	98
183-3-1-3	75	77	80	82	84	84	84	90
183-3-1-4	83	93	93	97	97	97	97	97
183-3-1-5	89	98	98	98	98	98	98	100
183-3-1-6	100	100	100	100	100	100	100	100
183-3-1-7	85	95	95	95	95	95	95	95
183-3-1-8	92	92	92	92	94	94	96	96
Mean	89.5	93.6	94.0	94.7	95.2	95.5	95.7	97.0
219-3-1-1	90	95	95	95	95	95	95	95
219-3-1-2	43	46	46	46	46	46	49	49
219-3-1-3	85	90	92	92	92	92	92	96
219-3-1-4	48	49	49	50	50	50	52	54
219-3-1-5	60	64	66	66	67	67	68	72
Mean	65.2	68.8	69.6	69.8	70.0	70.0	71.2	73.2
221-1-1-1	55	55	55	55	60	60	60	70
221-1-1-2	40	60	60	60	60	60	60	60

TABLE XIV (Cont'd)

Plant number	Germination by days							
	2	4	6	8	10	12	14	21
221-1-1-3	48	49	54	56	59	60	63	68
221-1-1-4	46	48	56	56	58	58	58	64
Mean	47.2	53.0	56.2	56.7	59.2	59.5	60.2	65.5
266-1-2-1	95	100	100	100	100	100	100	100
266-1-2-2	97	97	100	100	100	100	100	100
266-1-2-3	85	95	97	97	97	100	100	100
266-1-2-4	78	84	90	90	94	94	96	98
266-1-2-5	86	90	94	94	94	96	98	98
266-1-2-6	79	84	88	89	89	89	91	96
266-1-2-7	76	80	84	88	88	88	92	100
266-1-2-8	95	100	100	100	100	100	100	100
266-1-2-9	97	98	98	98	98	98	100	100
266-1-2-10	100	100	100	100	100	100	100	100
266-1-2-11	100	100	100	100	100	100	100	100
Mean	89.8	93.4	95.5	96.0	96.3	96.8	97.9	99.2
General Mean	67.8	74.9	77.3	78.6	79.6	80.3	81.2	84.7

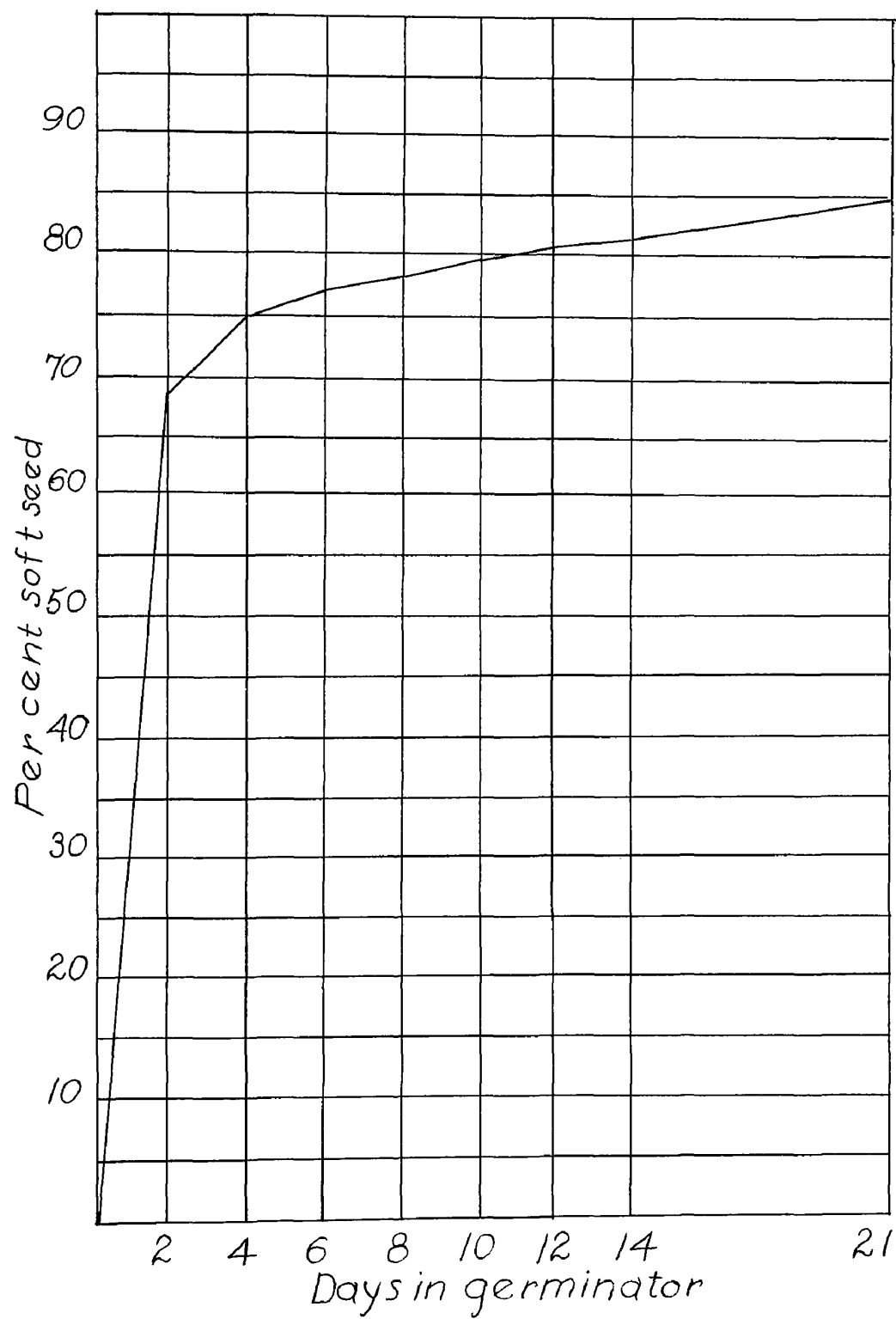


Fig. 14. Germination Rate of the Progeny of Hard-seeded S_3 Parents

TABLE XV

MEAN GERMINATION OF SOFT, AND
HARD-SEEDED PARENTS AND
THEIR PROGENY

Generation	Germination by days								
	2	4	6	8	10	12	14	21	63
Soft									
S1 parents	44.8	58.4	64.0	66.5	69.6	71.4	72.1	76.8	85.7
S1 progeny	24.4	36.0	39.1	41.3	42.8	46.4	48.4	53.5	
S2 parents	39.9	58.4	62.1	64.2	66.6	68.3	70.6	77.1	
S2 progeny	50.3	60.1	63.1	66.2	68.2	70.0	71.4	76.5	
S3 parents	66.6	73.4	77.2	78.6	80.3	81.1	82.4	84.2	
S3 progeny	40.3	71.9	76.6	80.4	81.5	82.8	83.1	86.3	
Hard									
S1 parents	9.0	11.9	13.4	14.1	14.8	15.9	16.3	19.1	25.4
S1 progeny	30.4	40.9	44.1	45.1	46.6	47.9	49.4	53.2	
S2 parents	19.6	25.0	26.5	26.8	27.5	28.5	29.9	32.3	
S2 progeny	40.6	57.3	59.6	62.4	64.6	67.5	68.4	72.8	
S3 parents	18.8	22.1	24.3	25.7	27.4	29.1	30.0	33.7	
S3 progeny	67.8	74.9	77.3	78.6	79.6	80.3	81.2	84.7	

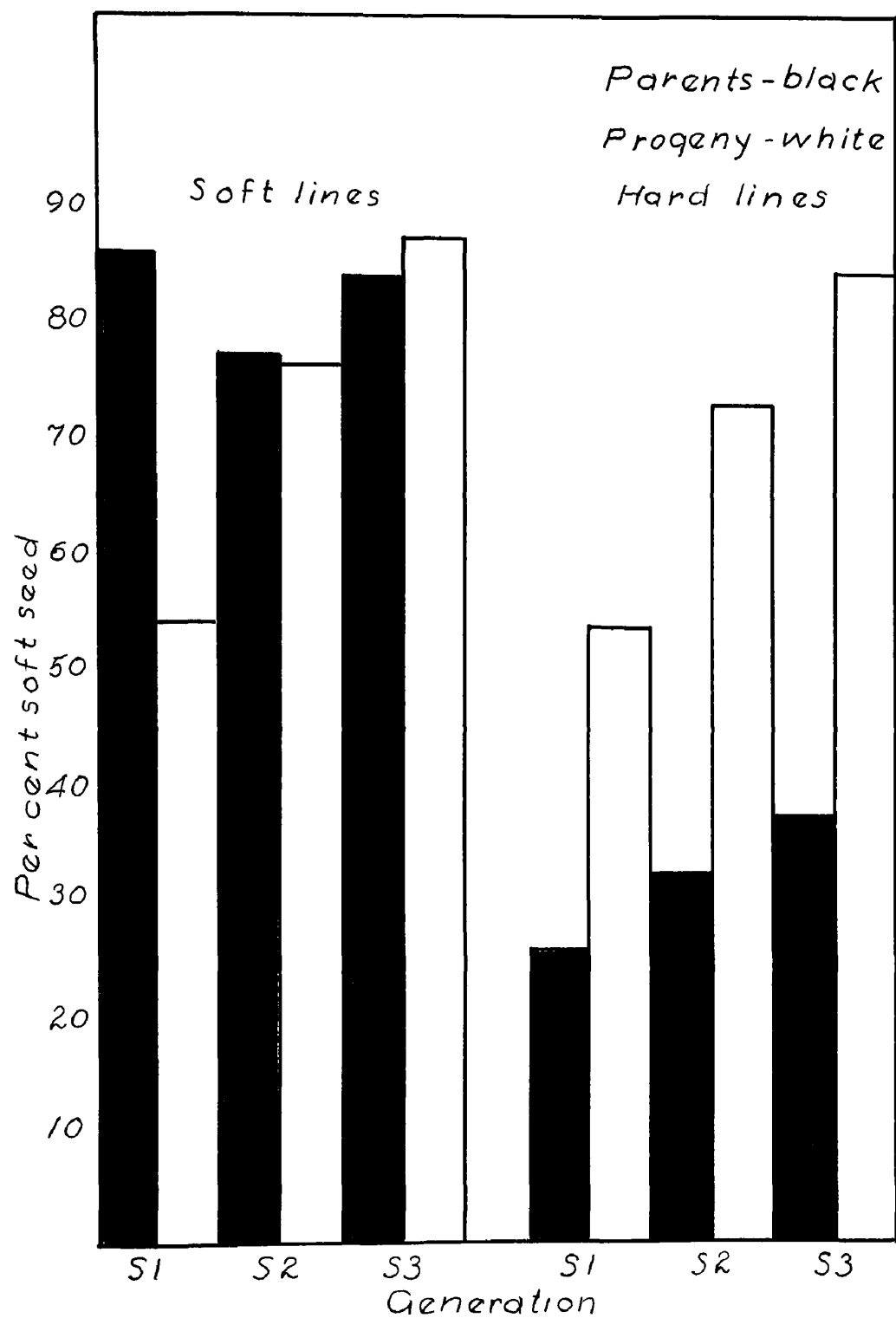


Fig. 15. Germination of Three Parental Generations and Their Progeny

III. PHYSIOLOGICAL FACTORS

Investigations of the effect of physiological factors affecting permeability of the seed of crimson clover were begun when it became evident that it would not be possible to isolate strains with different rates of germination. Seven phases of this problem were investigated, namely: seed size, moisture supply, humidity, light intensity, flowering sequence, nutrient levels, and moisture contents of seed. Each will be considered in the above order.

SEED SIZE

Methods and Materials

When conducting germination tests on crimson clover it often appeared that seeds which were impermeable were smaller than seeds which germinated readily. To determine whether this was a true observation or an illusory one resulting from the swelling of soft seed, tests were run on the effect of seed size on germination.

Hand hulled seed from three different years were gently screened through two sieves with perforations of 1.6 and 1.9 millimeters in diameter. Seed which passed the smaller size were classified as small and those retained as medium. Seed retained by the larger screen were graded large.

Duplicate germination tests were of 21 days duration

with counts made every two days for two weeks, and the final count made on the twenty-first day.

Results and Discussion

Results in Table XVI, which present only the final counts, show that in every instance small and medium-sized seed were less permeable than large. With two exceptions small seed were also less permeable than medium.

TABLE XVI
GERMINATION OF CRIMSON CLOVER AS AFFECTED BY
SIZE OF SEED

Year	Rep.	Germination by seed size		
		Large	Medium	Small
1944	1	81	80	60
	2	84	71	71
1945	1	75	66	67
	2	76	59	70
1946	1	89	70	67
	2	96	83	60
Means		83.5	71.5	65.8

The analysis of variance in Table XVII discloses that a highly significant difference prevailed between the germination of the different sized seed.

TABLE XVII
ANALYSIS OF VARIANCE OF DATA IN TABLE XVI

Source	D.F.	S.S.	Var.	F.
Total	17.	1806.28		
Years	2.	232.45	116.22	3.9
Sizes	2	976.45	488.22	16.4 **
Years X sizes	4	328.88	82.22	2.8
Error	9	268.50	29.83	

The difference at the 1 per cent level applies only to the comparison of small and large seeds. The difference between medium and large, however is significant at the 5 per cent level.

These results would appear to indicate that, if growing conditions were such that small sized seed were produced, the crop would be relatively more impermeable than those produced under conditions favoring the development of large seed. It has been noted that seed size varies within individual heads but it is not felt that this nullifies the over-all effect of environmental factors on the crop as a whole.

MOISTURE SUPPLY

Methods and Materials

With the absence of pure lines, and owing to the difficulty of regulating moisture supply to plants in the field, other methods had to be employed which would give results analogous to those which might have been obtained had pure lines been available. Greenhouse space limited the number of plants that could be studied, hence clonal separation was employed.

Crimson clover being an annual does not adapt itself to plant cuttings as do alfalfa and other perennials. By the time the stems at the crown have elongated sufficiently to be cut and be placed in a rooting medium the transition to the reproductive stage has already begun. This stage appears to inhibit root initiation. All attempts to propagate crimson clover by cuttings following stem elongation ended in failure. It was found that plant separation could be made with semi-dormant crowns in the late fall or early spring.

Plants with a well developed crown were obtained from the field and all soil washed from the roots. All shoots except two or four opposing ones were pruned off, and the tap root was then carefully sliced down through the center with a knife or a scalpel. Most of the lateral roots were pruned back to about one inch in length and the two halves

bound together. The halves thus prepared were then placed in sharp sand. In 2 to 3 weeks the individual halves developed enough new roots to permit transplanting.

The rooted halves were transferred to pots which were filled with well mixed soil so as to eliminate fertility differences as a factor. The two halves of each plant used received opposite treatments. Any differences appearing were then capable of being compared on a plant basis. This design also had the advantage over the bulk planting in that it served to show variable responses by individual plants.

Twenty pairs of plants were prepared and through the whole growing period one half were supplied with 150 ccs. of water daily which was enough to keep the soil thoroughly moist at all times. The other half received 150 ccs. of water only when the plants showed signs of water deficiency.

Eighteen plants produced enough seed for germination tests and were stripped only when fully mature. The seed were graded on a weight basis of milligrams per hundred seeds and germinated for 21 days. All seeds softening within this period were classified as permeable.

Results and Discussion

Table XVIII shows that moisture supply had a small but highly significant effect on seed size but not on permeability. Although the larger seed with a slightly higher percentage of permeability resulted from a liberal supply of moisture these

differences become meaningless when a correlation of -0.23 ± 0.12 between seed size and permeability is taken into consideration.

TABLE XVIII
EFFECT OF MOISTURE SUPPLY ON SEED
SIZE AND PERMEABILITY

Plant number:	Mgs. per 100 seeds			:	Per cent permeable		
	Moist soil:	Dry soil:	M-D		Moist soil:	Dry soil:	M-D
1	373	271	102		94	85	9
2	372	474	-102		92	64	28
3	408	355	53		98	86	12
4	438	408	30		99	56	43
5	262	282	- 20		90	81	9
6	359	339	20		100	100	0
7	471	310	161		93	84	9
8	391	342	49		81	82	- 1
9	387	308	79		100	93	7
10	319	304	15		83	92	- 9
11	440	370	70		91	56	35
12	410	355	55		64	87	- 23
13	339	310	29		67	93	- 26
14	361	319	42		96	100	- 4
15	425	331	94		74	80	- 6
16	424	358	66		73	92	- 19
17	370	368	2		84	54	30
18	439	329	110		65	73	- 8
Mean	388.2	340.7	47.5 $\pm$ 13.4		85.8	81.0	4.8 $\pm$ 4.6

The response of small seeds under the conditions of this experiment is not in agreement with that of small seed produced under field conditions. The small seed which developed under

the dry conditions maintained perform in a manner similar to immature alfalfa seeds. Lute (15) has shown that green immature alfalfa seeds are much more permeable than seeds which are fully mature. Martin and Wyatt (17) found that impermeability in sweet clover develops in the late stages of cell differentiation. It is apparent that the extreme moisture deficiency arrested the development of impermeability in these small seeds. With one exception all seed harvested were apparently mature. Under magnification, however, 70 per cent of the seed not used for germination showed a slight tinge of green at one end, a feature generally accepted as an indication of immaturity.

The practical application of the foregoing results deserve consideration. Plants selected for impermeable seed in a dry growing season may produce, in a moderately wet season, plants with a higher degree of permeable seeds. It is evident also that the reverse may take place. It would appear that a uniform moisture supply from year to year would be necessary to eliminate these effects on seed permeability.

HUMIDITY

Methods and Materials

The slight variation in the humidity (12) during the period of the breeding study reported above makes it improbable that this factor would have any effect on the development of impermeability in the seed of crimson clover. With no definite information at hand relative to its effects the following experiment was devised.

Twenty-four plants were located in a thin volunteer stand of clover in the spring of 1947 and two heads of each plant subjected to a high, and two heads to a low relative humidity. To maintain heads at a high humidity each pair was inclosed in a water-proof cellophane bag in which a piece of sponge was suspended by a string which led out through a small perforation in the sealed end of the bag. Each sponge was saturated daily with water by directing a small stream through the perforation onto the sponge. To aid in maintaining a high humidity the uppermost leaves on each head were also inclosed.

To maintain heads at a low relative humidity, small cheesecloth bags containing 15 grams of activated alumina were suspended in the cellophane bags in the same manner as the sponges, and the heads only inclosed. The activated alumina was replaced every other day with another lot which had been in an oven at 105 degrees Centigrade for 24 hours. The strings

leading from cellophane bags on each plant were tied to a stake for support.

When selecting heads for this study plants were used which had at least four heads in about the same stage of development. In order to remove the effects of dominance of earlier heads, all others were removed and later heads removed as they developed. The treatments continued until all heads were mature, at which time they were harvested only when they stripped with ease. Hulling, grading and germination tests were the same as for the previous study.

The design of this experiment did not permit the determination of the different relative humidities obtained. To obtain an estimation, tests were run with two relative humidity chambers originally designed for this study, but which were not used because of unforeseen technical difficulties. Figure 16 is a diagrammatic drawing of the chambers devised. The small blower used turned at 390 r.p.m. generating an air flow of 4.9 cubic feet per minute. The air stream was evenly split and directed up through the cylindrical towers which measured $4\frac{1}{2}$ x 18 inches. One tower was filled with dry activated alumina and the other with wet corn cobs cut to approximate the size of the alumina. The corn cobs were kept moist with a constant drip and were replaced when needed with a new lot which had been soaked in water over night. Flexible

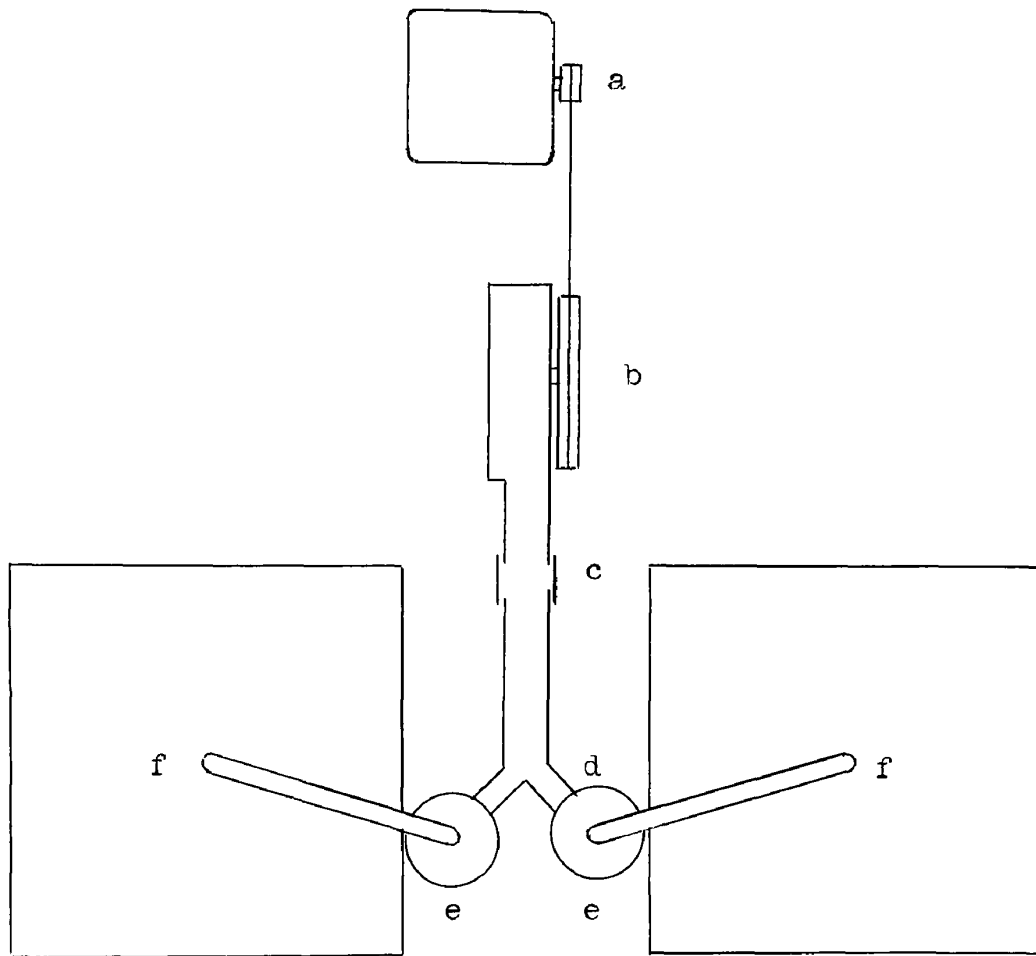


Fig.16. Diagram of Humidity Chambers

a - Motor, b - Blower, c - Rubber
coupler, d - Airstream splitter,
e - Drying and wetting towers,
f - Flexible pipes, g - 8 cu. ft.
chambers.

tubing $1\frac{1}{2}$ inches in diameter led from the top of the towers into the top center of the chambers. Humidity readings were made by directing the air stream from each tower onto a sling psychrometer. Determinations over a temperature range of 56 to 86 degrees Fahrenheit showed an average difference in relative humidity of 35.7 per cent. The amount of condensation inside the envelopes containing the sponges leads to the possibility that the differential was even greater than that obtained above.

Results and Discussion

The results from eighteen plants were almost as inconsistent as those obtained in the previous experiment. Table XIX shows that there was only a mean difference of 2.83 per cent permeability between the two treatments. Although no significant differences were found in germination, it may be noted that in two-thirds of the replications high humidity produced softer seed than low humidity. Further analysis however reveals that the differences in treatments of these twelve plants have a mean of 8.6 per cent, which may be considered identical with the mean difference, 8.7 per cent, of the plants with opposite effect. Though lacking statistical significance the differences obtained are of some practical value. On the basis of these results, the odds are two to one that heads surrounded by a humid atmosphere will bring about an increase in permeability of 8 per cent over those subject to a dry atmosphere.

TABLE XIX

EFFECTS OF HUMIDITY ON SEED SIZE AND GERMINATION

Plant number	Milligrams per 100 seed			Percent permeability		
	Humid	Dry	H - D	Humid	Dry	H - D
1	280	292	-12	93	80	13
2	370	353	17	99	98	2
3	312	226	86	92	66	26
4	350	270	80	92	95	7
5	245	240	5	85	93	- 8
6	340	333	7	100	92	8
7	292	250	42	77	70	7
8	330	307	23	97	89	8
9	370	375	- 5	97	90	7
10	253	260	- 7	73	86	-13
11	267	280	-13	83	82	1
12	274	255	19	68	70	- 2
13	342	280	62	100	90	10
14	380	360	20	38	84	4
15	370	329	41	90	96	- 6
16	400	355	45	92	95	- 3
17	316	317	- 1	67	88	-21
18	320	257	63	92	80	12
Mean	322.8	296.6	26.2± 8.6	88.0	85.2	2.83± 7.9

The extreme differences, 26 and -21 per cent are of some interest when considered in terms of the inbreeding study reported above. These almost conform to the differences established for dividing parents into permeable and impermeable classes.

The difference of 26.2± 8.6 in the weight of seed is significant but not as large as the differences brought about by varying moisture supply. It may be seen that the largest differences are in favor the seed resulting from high humidity. A few exceptions are evident but in general high permeability is associated with large seed. The extent of this association is indicated by a correlation coefficient of 0.50± 0.15.

The variable response in eighteen replications is indicative of the difficulty that would occur even though selection for impermeability were made under controlled conditions with the expectation that all plants would react in the same manner.

LIGHT INTENSITY

Materials and Methods

Mature seed were harvested in three replications from a portion of a field shaded with muslin to simulate cloudy conditions and from the same field in full sunlight. The muslin was found to reduce the light intensity by 8,000 foot candles. The seed were hulled with the rotary huller previously described and graded into three sizes with 1.9 and 1.6 millimeter sieves. Germination tests were run for 21 days and all seeds softening were considered as germinating or permeable.

Results and Discussion

The results of this experiment are given in Table XX. It is evident from an examination of the data presented that the treatments had no material effect on either seed size or permeability.

Light intensity ranges have not been determined for crimson clover, but it has been shown (18, 19) that for various plants the optimum light intensity ranges from 2,000 to 5,000 foot candles. The reduction in light in this experiment exposed the plants to 20 per cent of full sunlight which is within range given above. It is apparent from the results of this experiment that partly cloudy weather and overcast

skies in the spring have no effect on the permeability of the crimson clover seed crop.

TABLE XX
EFFECT OF LIGHT INTENSITY ON SEED
SIZE AND PERMEABILITY

Treatment	Rep.	Seed size in percent			Percent Germination
		Large	Medium	Small	
Shade	1	5.6	41.7	52.7	86
	2	4.4	49.3	46.3	84
	3	6.6	40.9	52.5	86
	Means	5.6	43.7	50.7	85.3
Light	1	9.4	33.6	57.0	91
	2	10.0	38.9	51.1	82
	3	6.7	45.5	47.8	74
	Means	8.4	40.2	51.3	82.3

FLOWERING SEQUENCE

Materials and Methods

Reference to Lute's (15) observation on the difference in permeability of seed from different branches of an alfalfa plant has already been made. To test the possibility of flower dominance in relation to permeability, the following experiment was conducted. Seventy-five well branched clover plants were located in the spring of 1946, and 1947. The plants were visited daily and the heads numbered as they emerged from the bud. Fifteen heads per plant were numbered in 1946. The opportunity offered by the profuse branching of the plants used in 1947 permitted the numbering of twenty heads per plant. In all cases the apical heads appeared first and were followed by the laterals. Seed were harvested when fully mature and identical head numbers bulked. Grading and germination tests were conducted as in the preceding experiment.

Results and Discussion

The results obtained in 1946 are presented in Table XXI, and some striking comparisons are evident. It may be noted that the amount of small seed increased with head sequence. The correlation coefficient of this relationship was found to be $+0.82 \pm 0.15$. Impermeability also increased with the sequence of flowering as indicated by the correlation coefficient of -0.68 ± 0.20 . Whereas no significant correlation between seed size and permeability could be established for

TABLE XXI

EFFECT OF HEAD SEQUENCE ON SEED SIZE AND
PERMEABILITY OF SEVENTY-FIVE PLANTS IN
1946

Head number :	Seed size in percent			Percent germination :
	Large :	Medium :	Small :	
1	27.0	40.0	33.0	83.2
2	24.0	40.7	35.3	79.0
3	19.9	43.6	36.5	76.2
4	15.5	36.9	47.6	77.2
5	20.1	28.4	51.5	78.0
6	22.3	31.1	46.6	68.7
7	32.7	17.2	50.1	71.0
8	24.1	33.5	42.4	68.0
9	18.7	23.9	57.4	70.0
10	18.3	26.3	55.4	70.0
11	20.2	30.5	49.3	64.7
12	18.5	34.8	46.7	60.0
13	14.0	18.5	67.5	59.5
14	4.1	13.4	82.5	62.0
15	7.0	17.6	75.4	62.0

the moisture and humidity studies a close relationship resulted in this experiment. The correlation coefficient between the percentage of small seed and permeability was found to be -0.72 ± 0.19 .

It may be observed that range of permeability produced a difference of 23.7 per cent. The differences also have a highly significant linear relationship. The regression coefficient of impermeability on head sequence was found to be $+1.575 \pm 0.46$ and the relationship is shown graphically in Figure 17.

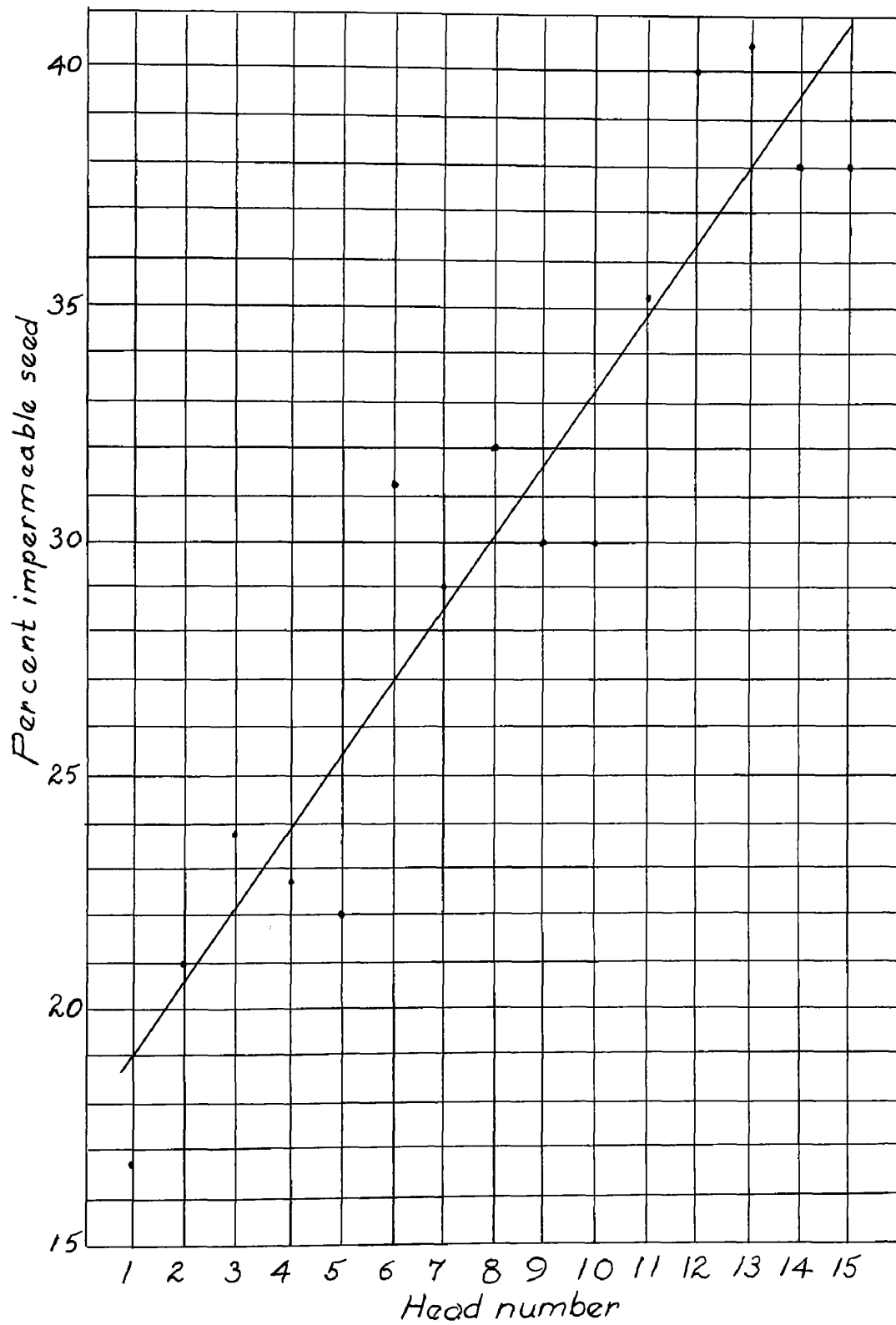


Fig. 17. Regression Line of Seed Impermeability on Head Sequence.

Ability to compete for moisture in descending effectiveness would appear to be the most logical reason for the decreases in permeability. It has been shown above that the moisture supply had a significant effect on seed size and a slight effect on permeability. It is conceivable also that competition for nutrients would also have some effect, but this possibility is obviated by a study to be presented later.

These results offer a reason for the ability of some clover stands to persist over a period of years. Branching of clover plants varies inversely with the density of stands. As stands volunteer from year to year one of the most noticeable effects is the gradual thinning out of the plant population. This leads to a comparatively high degree of branching and a relatively high amount of hard seed in the crop contributed by the laterals. The hard seed thus produced would be slow in germination and consequently prolong the stand. It also becomes obvious, on the basis of the breeding work reported herein, that the ability to volunteer could not be considered a persistent characteristic when seed developed by well branched plants produce a normal stand.

It may also be pointed out that other factors affect the manner in which flowering sequence performs. Table XXII shows that, in the spring of 1947, a significant association between head sequence and small seed resulted. This was correlated with the value of $+0.85 \pm 0.01$, but it is obvious that there is no relationship between size and permeability in this year's crop.

TABLE XXII
EFFECT OF HEAD SEQUENCE ON SEED SIZE
AND PERMEABILITY OF SEVENTY-FIVE
PLANTS IN 1947

Plant number	Seed size in percent			Percent germination
	Large	Medium	Small	
1	20.8	45.3	33.9	91
2	20.7	41.5	37.8	93
3	30.0	44.0	26.0	94
4	21.4	43.6	35.0	94
5	18.3	54.7	27.0	96
6	18.0	52.9	29.1	97
7	17.7	49.0	33.3	97
8	16.7	46.4	36.9	98
9	23.0	47.9	29.1	98
10	19.2	42.5	38.3	99
11	18.8	44.7	37.2	98
12	24.7	41.7	33.6	98
13	18.2	36.8	45.0	99
14	22.8	35.7	41.5	100
15	18.0	37.9	44.1	99
16	14.2	35.8	50.0	98
17	13.3	35.7	51.0	98
18	12.1	39.2	48.7	97
19	12.1	31.6	56.3	97
20	5.6	34.5	59.9	98

There is a slight tendency for higher percentage of permeability to be associated with small seed, but a correlation coefficient of 0.36 ± 0.22 between seed size and permeability shows that the relationship is not significant.

The disagreement of these results with those of 1946 would lead to the thought that weather conditions were responsible. Figures 18 and 19 show that the temperatures and rainfall for the two years when seed were being formed

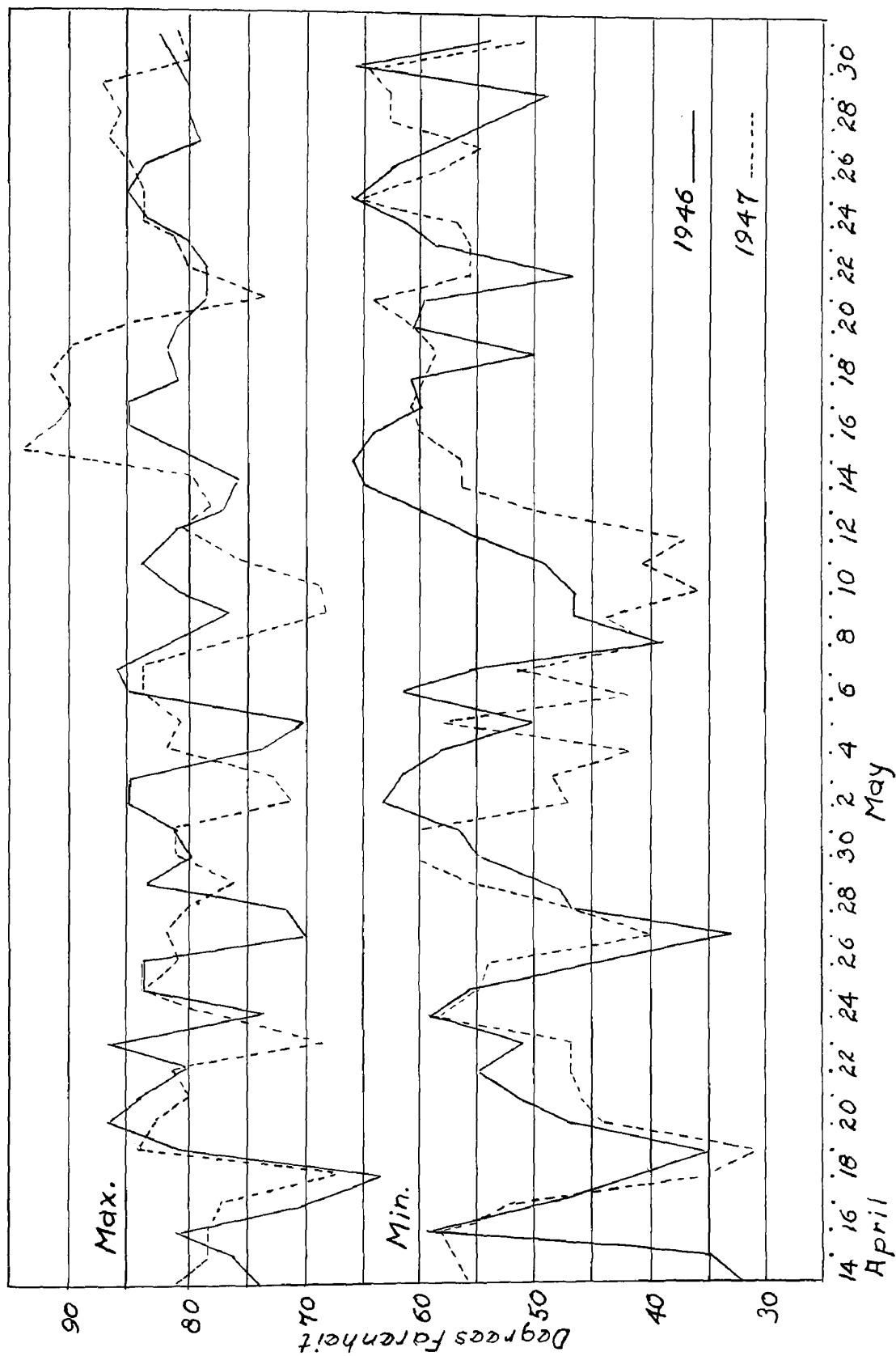


Fig. 18. Temperature Ranges for 1946, and 1947 During Seed Development of Crimson Clover

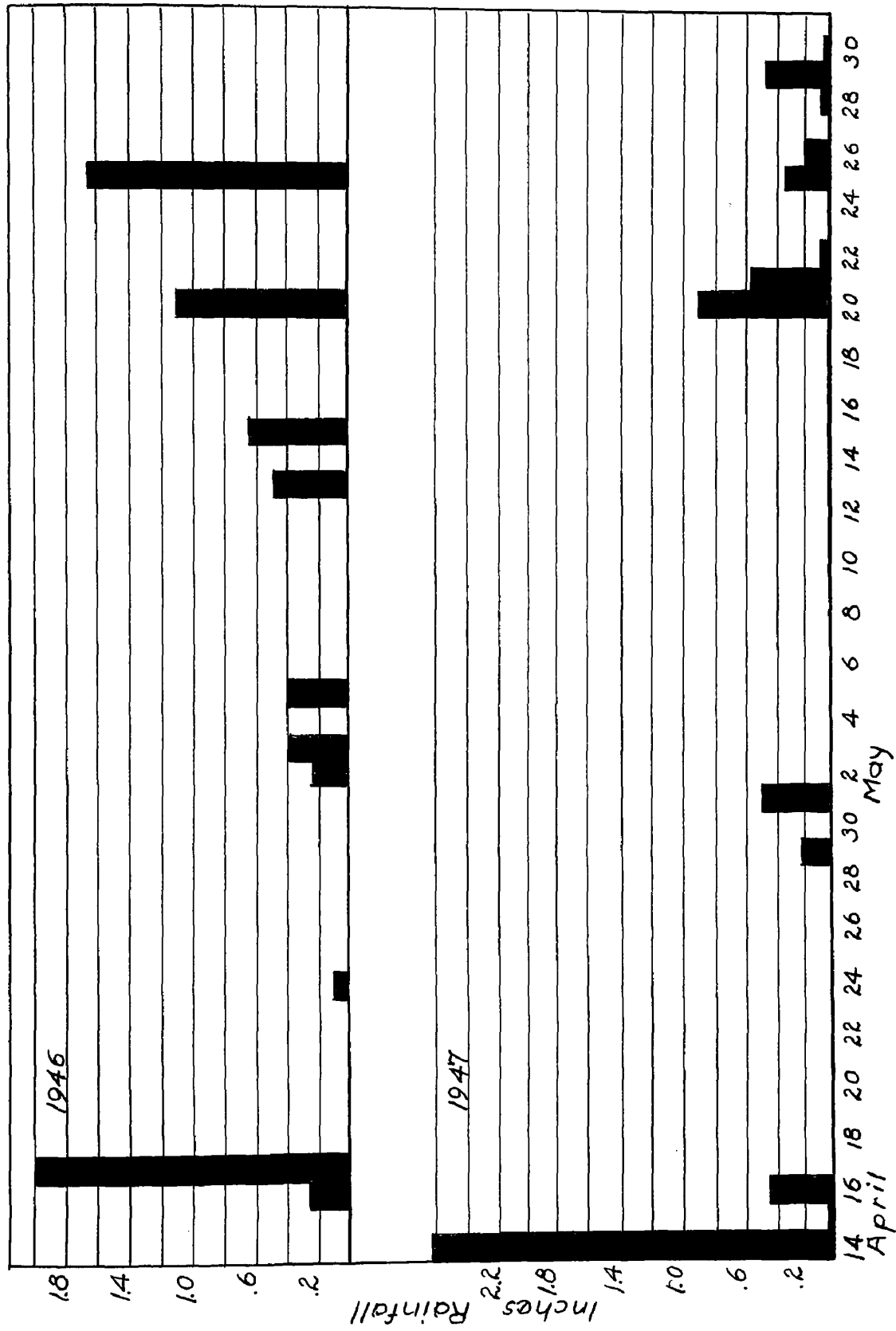


Fig. 19. Rainfall Distribution for 1946, and 1947 During Seed Development of Crimson Clover

were not radically different. The mean maximum temperatures for the period represented differed by only one degree. The longest period of any pronounced differences was of only 5 days duration, from May 15 to 19.

The season in 1947 actually had 1.15 inches less rain than that of 1946. When this is considered on the basis of the moisture study above, seed somewhat less permeable should have developed. There is a possibility that the two drought periods, April 17 to 29, and May 2 to 20 may have hindered the development of impermeability. Investigations by the writer (11), however, show that the duration of this drought would not develop a moisture problem in the soil where the plants were growing.

Attention has been called to the profuse branching of the 1947 plants, which averaged considerably above the twenty heads per plant. These plants which were in an extremely thin stand attained unusual stature and were subject to little or no competition by surrounding vegetation. Considering the environment and the type of plant it is probable that the earlier heads were far enough advanced in their development so that dominance of early heads was not a factor hindering the development of later heads.

NUTRIENT LEVELS

Methods and Materials

It has been found by the writer that when seed of identical origin was planted at Experiment, and at Tifton, Georgia, 150 miles farther south, that the seed crops at Tifton were less permeable than the ones from Experiment. Although Harrington (6) discounted soil conditions, the suspicion arose that it might be a factor influencing permeability. Since this experiment has been concluded, a study of the weather records at both stations make it seem likely that less precipitation at Tifton was the most effective factor bringing about the difference.

To check the influence of nutrient levels, half-plants as described above were subjected to two different levels of calcium, phosphorus, and potassium. A soil mixture consisting of one-fourth clay loam with peat moss, and three-fourths sand was prepared. To establish different fertility levels calcium carbonate was added at 500 to 1000 pounds per acre, 18 per cent superphosphate at 400 pounds to the high level, and 200 pounds of 50 per cent muriate of potash to the high level. Low levels of phosphorus and potassium received no additions of these elements. Sixty-four half-plants were used which would have permitted thirty-two comparisons based on differences resulting from any one of the element variables. A partial plan of the design is given in the following table.

TABLE XXIII

DESIGN OF NUTRIENT LEVEL EXPERIMENT

Treatment Comparisons							
$C_1P_1K_1$ vs $C_2P_2K_2$	$C_1P_1K_2$ vs $C_2P_2K_1$	$C_1P_2K_1$ vs $C_2P_1K_2$	$C_1P_2P_2$ vs $C_2P_1K_1$				
Plant	Plant	Plant	Plant	Plant	Plant	Plant	Plant
A*	A ₁ *	B	B ₁	C	C ₁	D	D ₁
E	E ₁	F	F ₁	G	G ₁	H	H ₁

*Paired letters represent identical plants

The design as presented offered a satisfactory substitute for a factorial which could not be used, and had an advantage over an incomplete block in that a loss of plants would not seriously affect the experiment unless too many plants of one comparison were lost.

All plants in the test were grown to maturity and treated in the usual manner except that the seed were not graded for size. The low yields of seed produced made it necessary to combine plants in the same comparison to obtain enough for germination purposes. When this was done each half contributed an equal amount for the tests. By so doing four complete replications were obtained. All plants, after seed harvest, were saved for mineral analysis provided any treatment effects were determined by germination tests.

Results and Discussion

The efficiency of the above design proved to be quite satisfactory for isolating single element effects. Table XXIV gives the results of the germination tests wherein the identity of individual plants is lost by combining them into replications.

TABLE XXIV
EFFECT OF NUTRIENT LEVELS ON PERMEABILITY

Rep.*	Per cent Germination					
	Comparison 1*			Comparison 2		
	C ₁ P ₁ K ₁	C ₂ P ₂ K ₂	Diff.	C ₁ P ₁ K ₂	C ₂ P ₂ K ₁	Diff.
1	73	63	10	97	92	5
2	76	67	9	93	80	13
3	94	75	19	59	30	29
4	76	87	-13	100	95	5
Means	79.7	73.0	+ 6.25	87.2	74.2	+ 13.0
Rep.*	Comparison 3			Comparison 4		
	C ₁ P ₂ K ₁	C ₂ P ₁ K ₂	Diff.	C ₁ P ₂ K ₂	C ₂ P ₁ K ₁	Diff.
1	92	77	15	76	92	-16
2	83	75	8	81	71	10
3	97	84	13	98	78	20
4	91	83	8	100	98	2
Means	90.1	79.7	+ 11.0	88.7	84.7	+ 4.0

*Two plants in each replication of each comparison

Excepting two instances the higher degree of impermeability is associated with the higher level of calcium. In three cases the differences are as large as those used to separate parents in the inbreeding study reported above. Had the higher phosphorus

level been the main factor contributing to seed impermeability, the $C_1P_2K_1$, and $C_1P_2K_2$ treatments should have resulted in the higher amount of impermeable seed. In only one instance, in treatment $C_1P_2K_2$, was this the case. If the higher level of potassium were the most influential factor in this respect, treatments $C_1P_1K_2$, and $C_1P_2K_2$ should have produced the higher amount of impermeable seed. The reverse was true except the one replication in treatment $C_1P_2K_2$.

It may be noted in the analysis of variance in Table XXV that there is a highly significant difference between the sixteen pairs of comparisons.

TABLE XXV
ANALYSIS OF VARIANCE OF DATA IN
TABLE XXIV

Source	D. F.	Sums of Squares	Variance	F
Total	31	6,726.47		
Groups	3	528.00	176.0	
Pairs	12	4,692.9	291.32	4.37**
Comparison 1	1	92.2	92.2	
" 2	1	338.0	338.0	5.07*
" 3	1	242.0	242.0	
" 4	1	32.0	32.0	
Error	12	799.37	66.61	

That the effect on permeability can be attributed to calcium is strengthened by the higher percentage of calcium in the plant tissue. Two replications of the material were combined

to obtain enough material for the analyses. Table XXVI gives the means of duplicate determinations for each of the analyses. The differences obtained are not large enough to establish statistical significance even though the higher calcium level plants contained one-third more calcium than the low level plants. The failure to establish significance can be attributed to the inconsistency of the results in the first comparison in replication one and the second in replication two.

The results of this experiment do not conform to the observations of Harrington (6) relative to the effect of soils on permeability. When the effect of calcium on cell permeability (18) is taken into consideration the higher degree of impermeability resulting from the high calcium treatment should have been anticipated. The lower calcium content of coastal plains soils as compared to the heavier piedmont types in the vicinity of Experiment, Georgia, strengthens the assumption that the higher degree of impermeability of south Georgia seed may be attributed to moisture and temperature differences.

The agronomic implications of these results are worthy of note. Lime is not generally recommended for the crop unless the soil is distinctly acid (1, 13). It would appear, however, that a liberal application of lime would aid in maintaining a stand by virtue of the greater amount of hard seed produced. Also the infrequent use of this amendment may partially explain the short longevity of stands when once established.

TABLE XXVI

CALCIUM CONTENT OF PLANTS AS AFFECTED BY
LEVELS OF APPLICATION

Treatment	Per Cent Calcium Oxide			
	Replication			
	1	2	Mean	Dif.
C ₁ P ₁ K ₁	2.73*	1.87	2.30	
C ₂ P ₂ K ₂	2.18	2.99	2.58	0.12
C ₁ P ₁ K ₂	1.87	2.37	2.12	
C ₂ P ₂ K ₁	2.58	2.30	2.48	0.36
C ₁ P ₂ K ₁	1.72	1.86	1.79	
C ₂ P ₁ K ₂	2.22	2.21	2.22	0.43
C ₁ P ₂ K ₂	1.54	1.81	1.67	
C ₂ P ₁ K ₁	2.23	4.28	3.25	1.58

Means for Calcium Levels = C₁ - 1.97, C₂ - 2.64

L.S.D. = .75 @5%

*Each analysis on material from four plants.

MOISTURE CONTENT OF SEED

Methods and Materials

When the anatomical investigations reported above were in progress it was observed that almost invariably, an air space occurred between the seed coat and cotyledons. The possibility arose that delayed germination might be caused by the inability of the water imbibed by the seed coat to cross this air space and initiate germination. It was also deduced that lowered moisture in the seed would bring about a shrinkage of the cotyledons broadening the separation.

Seed of relatively high moisture content were dried in a desiccator and samples withdrawn at intervals for examination. Twenty seeds at each moisture content were halved by placing the seed in a specially constructed wooden holder and cutting across the hilum. One half of each seed was placed under a stereoscopic microscope, and a camera lucida drawing, at 90 diameters, made of the cut surface. Measurements were made at five uniformly selected portions of the drawings and the average width determined.

Germination tests were run on a hundred seed at each moisture level but the results are not reported owing to the possibility of becoming permeable in combining. Germination tests were later run on seed with different moisture contents, hulled as in the above experiments.

Results and Discussion

It appears obvious in Table XXVII that drying the seed had no material effect on the air space between the cotyledons and the seed coat. In view of the probability that impermeability is caused by other factors the data has not been subjected to critical examination.

TABLE XXVII
AIR SPACE BETWEEN COTYLEDONS AND SEED
COAT AS INFLUENCED BY MOISTURE
CONTENT

Per cent moisture	:	Space in microns
13.2		10.0
8.6		15.5
7.6		11.1
6.5		15.5
6.1		10.5

On hand hulled seed the moisture content did have a considerable effect as shown in Table XXVIII.

Several investigators (5,8,) have shown that drying out seed by the use of head makes sweet clover seeds more permeable. On the other hand, Lebedeff (14) reports that

TABLE XXVIII

EFFECT OF MOISTURE CONTENT ON PERMEABILITY

Rep.	:	Percent Moisture	:	Percent Permeable	:	Means
1		12.9		87		85.0
2		13.0		83		
1		8.1		78		77.5
2		8.5		77		
1		5.4		75		75.0
2		5.4		75		
L.S.D.	=	4.23% @ 5%		7.76% @ 1%		

drying of field beans slows up the rate of moisture absorption. The seed of crimson clover react to drying in the manner of field beans in this study with a maximum decrease in permeability of 10 per cent. This amount in itself is not sufficient to interfere materially with the selection of lines differing in permeability except where certain individuals may be on the borderline of limits selected for classifying seed as permeable or impermeable. The results presented stress the necessity of uniform storage conditions from year to year, if investigations are being conducted on permeability.

SUMMARY AND CONCLUSIONS

Anatomical investigations of the seed coats of Crimson clover show them to be structurally similar to the seeds of Red clover and the cell wall composition like that of Sweet clover. By using a disorganized seed coat as a membrane it was established that impermeability in Crimson clover is partially due to the external cuticle layer.

Having found marked variability in hard seededness among plants, breeding experiments were initiated and carried on over a 3-year period. Parental lines differing in permeability were selected from three hundred plants selected at random and selfed. Parents for the second and third generation were selected within lines from selfed progeny on the basis of germination tests. The first hard, and soft-seeded parents selected which had a mean difference of 60.3 percent in permeability with a germination of 25.4 and 85.7 percent, respectively, yielded progeny which differed in mean permeability by only 0.30 percent. Second generation parents with a mean difference of 44.8 per cent in permeability produced soft-seeded progeny with a mean permeability of 76.5 per cent, and hard seeded progeny with a mean of 72.8 per cent. Third generation hard-seeded parents with a mean germination of 36.7 per cent produced offspring with a mean permeability of 84.7 per cent, and soft-seeded parents showing 84.2 per cent permeable seeds

yielded progeny with a mean permeability of 86.3 per cent. Three years of selfing and selection for lines differing in permeability culminated with a mean difference of only 1.61 per cent.

Environmental and physiological factors studied, both in the field and by paired comparisons with clonal separations, showed that small seed from field harvests are more impermeable than large seeds produced under the same conditions. A high moisture supply, versus a low supply, produced larger seed with only a slightly higher degree of permeability. Severe drought conditions apparently restricted the late stages of cell differentiation which result in impermeability.

Seed produced on heads subject to a high relative humidity produced larger seed than heads surrounded by a low relative humidity. Plant response as to permeability was inconsistent. The odds were 2 to 1 that heads subject to a high relative humidity would produce seed 8 per cent more permeable than those produced by heads exposed to a low relative humidity.

Plants grown with 2000 foot-candles of illumination produced seed which did not differ in size or permeability from those produced in full sunlight.

A significant linear relationship was established for permeability as related to head sequence and highly

significant correlations were found between head sequence and seed size, and between seed size and permeability. When plants did not have to compete with adjacent vegetation, head sequence had no affect on permeability.

Clover grown at two levels of calcium, phosphorus, and potassium were found to be less permeable in the presence of high levels of calcium, but unaffected by different levels of phosphorus or potassium.

Seed with 12 to 13 per cent moisture were found to be 10 per cent more permeable than seeds dried to lower moisture contents without heat.

On the basis of the above investigations it appears that the following conclusions are justified:

1. Impermeability of the seeds of crimson clover is partially due to the well developed cuticle layer.
2. Wide variations in permeability exist among plants, from season to season.
3. Impermeability of seed coats does not appear to be an inherited characteristic.
4. Differences in soil moisture levels, and relative humidities have no consistent effect on permeability
5. Crimson clover plants develop normally under an illumination of 2000 foot candles.

6. A high level of calcium in the soil has the effect of increasing impermeability.

7. Seed become more impermeable as the moisture content of the seed is lowered.

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APPENDIX A

GERMINATION OF CRIMSON CLOVER PLANTS SELFED IN 1944

Plant Number	Germination by Days								
	2	4	6	8	10	12	14	21	63
1	11	44	64	65	68	68	69	72	82
2	12	20	32	33	33	34	34	36	43
3	11	15	16	17	17	17	20	35	79
4	24	29	35	39	40	43	45	47	56
5	17	17	17	17	17	17	19	19	23
6	18	29	29	30	34	34	35	38	56
7	46	62	67	71	72	72	73	75	80
8	17	27	32	33	35	36	36	38	48
9	4	8	12	15	18	18	22	26	44
10	2	3	3	4	5	6	6	9	16
11	38	56	67	69	72	72	73	74	80
12	16	19	27	28	28	28	29	30	36
13	29	50	60	71	72	74	77	81	90
14	6	8	8	8	9	9	9	9	17
15	42	62	73	82	86	87	87	91	93
16	2	4	4	4	5	5	6	7	16
17	26	42	49	55	62	68	75	77	91
18	9	15	16	18	21	22	25	32	43
19	40	87	89	93	94	96	98	98	98
20	91	94	94	94	94	94	94	97	100
21	4	5	7	7	7	9	11	29	50
22	9	19	24	29	34	39	43	48	57
23	1	6	8	10	11	12	13	17	32
24	1	11	19	31	37	39	42	52	62
25	18	38	44	47	51	54	57	67	77
26	5	13	13	17	21	23	26	34	48
27	10	18	21	28	32	35	36	40	47
28	4	5	9	10	12	13	13	26	48
29	21	23	26	26	27	27	29	39	37
30	54	59	59	60	63	64	66	69	80
31	6	8	9	9	9	9	9	11	25
32	20	26	29	32	35	35	35	37	51
33	3	18	20	26	26	32	33	42	59
34	61	90	98	99	99	99	99	99	99
35	24	37	47	54	57	59	59	76	90
36	24	33	38	51	58	62	66	70	83
37	9	25	30	33	37	39	39	47	53
38	4	9	13	14	17	18	20	32	42
39	14	17	19	25	27	28	29	43	63
40	18	23	25	26	28	28	29	47	54
41	8	11	14	16	18	18	21	22	34
42	10	12	13	13	16	17	18	23	34

APPENDIX A (Cont'd)

Plant Number	Germination by Days								
	2	4	6	8	10	12	14	21	63
43	18	28	40	49	56	56	59	60	63
44	20	36	45	64	71	72	74	79	88
45	17	23	27	38	44	46	51	60	72
46	36	38	40	43	44	46	47	54	65
47	21	24	28	31	32	33	35	42	49
48	43	52	61	69	74	77	80	89	96
49	50	62	64	65	66	66	67	69	77
50	13	17	18	18	19	20	21	24	33
51	67	83	87	88	92	95	95	97	99
52	28	30	32	33	38	39	40	49	57
53	10	11	16	17	22	23	27	39	51
54	7	8	12	14	22	22	24	38	57
55	5	6	11	11	14	14	14	21	29
56	15	16	21	24	26	27	28	32	61
57	42	54	59	62	65	65	65	72	84
58	4	5	7	8	9	9	9	14	23
59	13	18	20	21	21	21	28	35	47
60	8	12	14	15	18	18	29	50	81
61	23	32	41	46	59	59	61	75	89
62	22	23	26	31	36	36	38	52	74
63	25	47	66	78	91	92	92	94	95
64	5	11	16	19	27	28	34	38	62
65	7	12	18	26	36	38	42	64	82
66	4	5	7	7	13	13	14	17	22
67	3	4	6	6	7	7	9	18	36
68	20	31	46	49	57	58	61	64	76
69	43	62	68	78	79	79	80	84	86
70	47	55	56	65	69	69	69	78	89
71	11	14	19	21	25	25	27	79	46
72	20	35	42	48	54	54	54	61	84
73	9	13	16	19	22	22	23	28	43
74	15	25	31	35	38	38	43	47	52
75	1	1	1	1	1	1	1	6	21
76	6	8	12	17	18	18	23	28	51
77	5	8	12	12	13	13	14	17	43
78	5	11	23	26	32	36	38	47	61
79	1	2	7	10	17	17	18	27	51
80	11	22	25	30	32	32	38	44	54
81	7	40	52	61	63	65	66	70	74
82	2	13	25	30	34	36	39	45	63
83	5	20	31	41	47	53	60	74	92
84	9	14	23	28	35	36	40	54	77
85	4	5	5	5	5	5	5	6	6
86	5	6	21	23	29	31	33	37	65
87	6	12	24	31	37	41	45	61	70
88	12	13	14	15	16	17	18	26	62

APPENDIX A (Cont'd)

Plant Number	Germination by Days								
	2	4	6	8	10	12	14	21	63
89	9	24	34	38	48	52	54	59	70
90	10	15	17	22	23	23	24	28	38
91	16	21	38	41	43	45	45	47	59
92	5	8	14	20	20	22	26	32	41
93	4	4	4	7	7	7	7	7	17
94	13	14	14	17	17	17	17	21	27
95	6	8	9	10	10	10	11	13	25
96	7	12	17	18	18	18	18	18	18
97	4	4	4	4	4	4	4	6	8
98	3	8	9	12	13	14	14	29	53
99	5	9	9	9	9	10	10	13	22
100	3	11	15	19	25	25	25	29	33
101	8	18	28	33	36	37	37	52	62
102	26	39	42	43	44	44	44	47	59
103	7	9	12	14	14	14	14	16	24
104	31	48	54	59	60	60	60	71	86
105	20	24	26	29	30	31	31	33	49
106	14	16	17	17	18	18	18	22	32
107	12	17	20	23	25	27	27	31	52
108	18	46	69	79	86	84	86	91	72
109	22	54	59	62	64	65	65	80	91
110	24	49	64	72	75	75	75	84	91
111	12	22	29	30	33	34	34	45	57
112	16	29	37	40	44	44	44	55	61
113	13	17	20	24	26	26	26	31	43
114	29	36	39	43	44	44	44	50	63
115	11	18	23	30	38	40	40	49	56
116	0	2	2	3	3	3	3	7	22
117	3	4	5	5	5	5	5	7	15
118	11	16	18	18	19	19	19	26	38
119	18	24	26	27	27	27	27	29	38
120	9	11	12	12	15	16	16	21	30
121	9	11	16	18	18	18	18	25	47
122	28	46	60	69	75	76	76	89	94
123	14	26	40	46	53	54	54	74	91
124	7	18	24	29	31	31	31	38	44
125	20	28	32	35	39	41	41	46	54
126	13	18	26	31	34	36	36	50	77
127	15	19	22	24	27	27	27	39	47
128	7	11	14	25	15	16	16	22	42
129	7	13	19	25	25	25	25	37	57
130	15	22	27	32	34	34	34	50	70
131	20	22	23	27	28	28	28	31	34
132	4	13	16	19	23	24	24	41	55
133	12	16	29	42	47	49	49	57	68
134	6	15	17	18	21	23	23	55	95

APPENDIX A (Cont'd)

Plant Number	Germination by Days								
	2	4	6	8	10	12	14	21	63
135	6	11	13	14	15	15	15	23	42
136	19	22	23	24	25	27	27	27	39
137	2	4	7	7	7	7	7	9	14
138	0	0	1	3	3	3	3	14	47
139	0	1	1	1	1	1	1	1	24
140	4	15	20	22	24	27	27	41	47
141	29	50	55	57	59	60	60	63	66
142	10	12	16	21	21	23	23	40	60
143	8	10	11	13	16	18	18	34	52
144	6	8	17	18	19	20	20	31	36
145	8	13	20	27	32	34	34	59	78
146	1	6	7	10	11	11	11	16	31
147	66	77	79	79	79	79	79	81	87
148	19	24	28	29	30	31	31	37	40
149	10	14	17	21	23	25	25	36	52
150	4	8	12	14	16	17	17	31	41
151	40	69	83	85	92	93	93	95	95
152	13	20	22	22	23	24	24	29	40
153	3	6	9	11	17	22	22	37	47
154	4	7	11	11	11	11	11	27	47
155	1	7	13	18	23	28	28	54	72
156	6	12	20	23	25	26	26	35	60
157	4	12	21	27	28	32	32	46	65
158	25	38	43	45	46	47	47	53	64
159	32	69	78	79	82	84	84	92	100
160	4	16	27	37	42	46	46	89	97
161	12	15	17	18	21	22	22	36	43
162	27	56	70	80	80	84	84	93	99
163	6	7	9	10	11	14	14	29	51
164	8	12	17	19	20	22	22	37	52
165	27	35	39	45	49	49	49	53	62
166	46	47	49	50	51	51	51	54	56
167	8	11	12	12	12	12	12	15	18
168	4	11	12	12	14	15	16	19	21
169	9	18	20	21	25	25	25	28	39
170	17	28	36	37	41	42	45	58	73
171	8	14	16	16	16	16	16	18	22
172	8	11	11	12	12	12	12	15	15
173	7	8	9	9	9	9	9	11	13
174	8	12	12	13	13	16	18	29	42
175	9	15	19	19	24	24	24	25	27
176	20	23	23	24	27	27	27	27	34
177	35	54	56	61	64	64	66	76	82
178	11	15	22	27	31	33	35	47	69
179	2	3	4	5	5	5	5	7	17
180	32	39	42	42	43	43	44	45	47

APPENDIX A (Cont'd)

Plant Number	Germination by days								
	2	4	6	8	10	12	14	21	63
181	6	8	9	9	9	9	10	13	17
182	18	33	39	42	43	43	46	56	67
183	13	15	17	17	18	18	18	19	25
184	6	9	14	18	24	29	33	49	64
185	31	40	46	49	49	54	57	64	72
186	2	2	2	4	5	8	8	19	47
187	4	8	12	20	22	25	29	35	48
188	21	44	51	59	61	64	64	64	64
189	3	14	17	19	22	24	24	37	49
190	35	50	52	58	59	59	59	64	78
191	15	37	40	43	46	49	50	76	100
192	6	10	11	14	15	19	20	22	26
193	1	5	8	12	12	18	21	30	58
194	3	11	15	21	24	36	42	62	77
195	3	10	15	25	28	31	33	54	68
196	13	13	14	16	16	16	16	17	28
197	1	2	4	5	6	10	11	20	36
198	2	7	10	12	13	16	19	22	32
199	7	7	11	11	11	11	11	12	14
200	3	3	3	4	4	5	5	13	37
201	9	40	47	54	55	59	60	68	82
202	13	17	22	28	28	31	32	43	62
203	3	3	3	3	4	5	5	12	22
204	28	37	43	50	50	56	61	69	84
205	20	37	44	47	47	50	50	55	71
206	9	13	14	18	19	26	28	41	62
207	18	25	27	30	30	31	32	38	55
208	16	18	23	32	32	34	35	38	52
209	4	8	10	11	11	11	11	15	25
210	5	6	6	6	6	7	7	8	13
211	6	20	26	31	31	35	38	44	51
212	14	16	18	24	25	29	33	35	36
213	25	62	72	75	75	81	84	87	90
214	15	17	21	24	26	29	29	32	46
215	5	6	6	6	6	6	6	7	9
216	6	7	8	9	10	10	11	16	28
217	9	12	16	23	24	27	31	45	56
218	6	10	10	10	11	15	15	18	26
219	11	14	14	15	15	17	17	23	30
220	11	13	16	17	17	20	22	26	43
221	11	14	19	21	21	23	23	29	35
222	11	26	32	41	43	47	48	57	69
223	2	4	8	9	11	13	19	29	50
224	41	44	48	52	56	60	60	73	82
225	53	59	59	60	60	60	60	60	62
226	79	85	85	85	85	85	85	86	89
227	75	88	88	88	88	88	88	88	89

APPENDIX A (Cont'd)

Plant Number	Germination by Days								
	2	4	6	8	10	12	14	21	63
228	91	93	93	93	93	93	93	94	96
229	74	78	78	78	78	79	79	79	80
230	82	87	87	87	87	88	88	88	89
231	67	81	82	82	82	82	82	83	85
232	61	68	69	69	69	70	70	70	73
233	52	56	56	56	57	57	57	57	60
234	24	28	29	29	29	29	29	29	30
235	73	74	74	74	74	75	75	78	79
236	45	46	46	46	46	46	46	46	47
237	88	95	95	95	95	95	95	95	95
238	73	85	85	85	85	85	85	85	88
239	56	58	59	59	60	60	60	60	69
240	14	15	15	15	15	15	15	17	22
241	32	35	35	35	35	35	35	35	37
242	26	26	29	29	29	30	30	33	43
243	46	47	48	51	53	54	54	56	63
244	16	20	23	24	25	29	29	36	46
245	50	53	55	56	60	62	62	65	71
246	30	31	31	31	31	31	31	31	32
247	64	67	68	69	69	70	70	74	82
248	37	37	37	37	37	37	37	37	39
249	70	75	76	77	77	77	77	78	79
250	46	48	48	49	49	49	49	51	56
251	51	56	57	57	57	57	57	57	60
252	57	61	61	61	61	61	61	66	78
253	37	37	37	37	37	37	37	37	38
254	58	59	59	59	59	60	60	61	62
255	70	72	72	72	72	72	72	73	84
256	62	63	63	63	63	63	63	65	66
257	35	38	39	39	41	41	41	42	44
258	39	39	40	41	41	41	41	41	42
259	77	77	77	77	77	77	77	78	82
260	67	72	72	72	72	73	73	73	75
261	90	90	90	90	90	90	90	90	90
262	67	67	67	68	68	68	68	69	71
263	86	86	86	86	86	86	86	86	86
264	81	83	83	83	83	83	83	83	84
265	78	78	78	78	78	80	80	83	86
266	69	70	70	70	70	70	70	71	72
267	68	68	68	68	68	68	68	68	69
268	62	62	62	62	62	62	62	62	62
269	67	74	74	74	74	74	74	75	76
270	64	70	70	70	70	70	70	70	71
271	63	67	69	69	69	69	69	69	70
272	72	75	75	75	75	75	75	75	77

APPENDIX A (Cont'd)

Plant	Germination by Days								
Number	2	4	6	8	10	12	14	21	63
273	57	57	58	58	58	58	58	60	64
274	62	69	69	69	69	69	69	79	75
275	75	75	75	75	75	75	75	76	81
276	73	74	74	74	74	74	74	74	75
277	59	59	60	60	60	60	60	60	63
278	77	79	79	79	79	79	79	79	81
279	83	84	84	84	84	84	84	84	84
280	70	70	70	70	70	70	70	71	71
281	76	76	76	76	76	76	76	76	77
282	59	59	59	59	59	59	59	59	59
283	54	55	55	55	55	55	55	56	56
284	72	72	72	72	72	72	72	72	76
285	70	84	84	84	84	84	84	85	86
286	42	43	43	44	44	44	44	44	45
287	55	56	56	56	56	56	56	56	57
288	32	32	33	34	34	35	35	37	43
289	84	90	90	90	90	90	90	90	91
290	93	93	93	93	93	93	93	93	93
291	87	92	92	93	93	93	93	93	93
292	98	100	100	100	100	100	100	100	100
293	87	87	87	87	87	87	87	87	88
294	33	33	34	34	34	34	34	34	36
295	50	52	52	52	52	52	52	52	52
296	79	82	83	83	83	83	83	83	84
297	89	90	90	90	90	90	90	91	94
298	84	86	86	87	87	87	87	87	87
299	97	97	97	97	97	97	97	97	97
300	79	82	82	82	82	82	82	82	82
301	97	97	97	97	97	97	97	97	97
302	69	69	69	69	69	69	69	69	70
303	74	75	75	75	75	75	75	75	76
Mean	27.4	34.0	37.1	39.5	41.4	42.4	43.5	49.1	57.8

APPENDIX B

RELATIVE HUMIDITIES AT ATHENS, GEORGIA DURING
PERIODS OF SEED DEVELOPMENT OF CRIMSON CLOVER

Period	Year	Time			
		1:30 A.M.:	7:30 A.M.:	1:30 P.M.:	7:30 P.M.
Apr. 14-30	1945	82.2 %	89.7 %	60.4 %	75.3 %
	1946	79.3	83.0	44.3	58.7
	1947	82.7	84.5	47.8	59.6
May 1-31	1945	76.2	84.9	52.0	87.4
	1946	86.4	87.1	55.3	68.8
	1947	80.4	82.0	48.0	58.8

APPENDIX C

RELATIVE HUMIDITIES IN CHAMBERS DEvised
FOR HUMIDITY STUDY

Air temp.	Rel. humidity		Air temp.	Rel. humidity	
	Dry	Moist		Dry	Moist
71	56	73	65	49	74
74	36	76	75	45	82
75	37	72	69	43	80
75	43	71	80	17	67
87	20	63	75	63	78
79	39	78	82	37	66
84	34	72	78	38	82
82	37	76	77	32	82
79	29	72	72	36	75
85	58	83	73	58	72
75	46	85	76	53	86

Strain	1946		1947	
	Experiment	Tifton	Experiment	Tifton
Allen	100	80		
Thornton	93	89		
Hardy	85	68		
Commercial	67	93		
Dixie			61.5	62.5

MEAN TEMPERATURES AND RAINFALL AT EXPERIMENT AND
TIFTON, GEORGIA DURING PERIODS OF SEED
DEVELOPMENT IN CRIMSON CLOVER

Period	Temperatures				Inches	
	Mean	Maximum	Mean	Minimum	Rainfall	
	E ₁	T ₂	E	T	E	T
Apr. 14-30, 1946	75	79	53	54	1.74	2.51
May 1-31	79	84	59	63	5.79	3.82
Totals					7.53	6.33
Apr. 14-30, 1947	74	79	52	54	3.42	4.16
May 1-31	80	85	58	63	5.02	2.57
Totals					8.44	6.73
1. Experiment. 2. Tifton						