

IMPERMEABLE SEED OF ALFALFA

By ANNA M. LUTE



COLORADO EXPERIMENT STATION
AGRICULTURAL SECTION
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IMPERMEABLE SEED OF ALFALFA

By ANNA M. LUTE

Impermeable or so-called hard seeds occur commonly in alfalfa. Such seeds fail to take up water and germinate in a reasonable length of time. The causes of impermeable seeds and methods for increasing their planting value are of growing interest. This is especially true in our northern states and Canada where annually greater use is being made of hardy varieties which exhibit a higher percentage of impermeable seeds than do the less hardy ones.

It is desirable to market alfalfa seed free from, or with very few, impermeable seeds. So little has been known as to the causes which result in high hard-seed content that no method for prevention could be devised. Various methods of treatment for reducing the percentage of impermeable seeds have been found to be not entirely satisfactory.

It is the purpose of this paper to consider such factors as climate, altitude, maturity, drying, seed-coat injury and individual plant differences as affecting the impermeable-seed content of alfalfa.

A study of the structure of the seed coats is given as a suggested explanation of impermeability and the studies on the effect of moderate heat offer a satisfactory method for reducing the percentage of impermeable seed.

LITERATURE

The presence in alfalfa of impermeable seed has long been known and the percentage of such impermeable seeds has been noted by several workers. In 1916 Harrington⁶ in a summary of 1,737 commercial lots of alfalfa seed, gave the highest impermeable seed content as 72 percent, the lowest as 0 percent and the average as 13.8 percent. In some hand-threshed samples he found 70 percent of impermeable seed. In 1912 Love and Leighty⁸ and in 1926 Schmidt¹³ reported that there is not only a variable percentage of impermeable seeds in alfalfa but the degree of impermeability is equally variable and that the two are directly associated. They found that those samples having the highest percentage of impermeable seeds have also the greatest degree of impermeability.

The writer wishes to express to L. W. Durrell appreciation for the illustrations, the study of the seed coats, and for assistance with the manuscript; to acknowledge the cooperation of D. W. Robertson in growing many of the plants used in this work, and P. K. Blinn for the work in connection with the plantings at the Rocky Ford Experiment Farm.

Annual summaries made at the Colorado Seed Laboratory² show that the average percentage of impermeable seed for all commercial lots of Colorado-grown seed varies only about 1 percent from year to year, remaining always approximately 22 percent. Hojesky⁷ reports that in some years a high percentage of impermeable seeds, even 40 to 50 percent, appears.

Since impermeability prevents immediate germination by the usual seed-laboratory methods it has been assumed that something similar happens when such seeds are planted in the field. Various methods of treatment aiming to increase the planting value of such seeds have therefore been devised by many workers. In 1904 Thornber¹⁷ reported that treating seeds of alfalfa with water at 85 degrees to 88 degrees C. for two to six minutes increased the germination by reducing the percentage of impermeable seed; also that clipping, abrasion on a grindstone, or treatment with concentrated sulfuric acid for a short time had the same effect. In 1912 Love and Leighty⁸ worked out a method for sulfuric-acid treatment. They found that 15 minutes in concentrated sulfuric acid usually gave maximum results, while one hour frequently caused serious injury to seed coats. Seed must be thoroly washed after acid treatment. Hojesky⁷ reported that he established the fact that the percentage of impermeable seeds in alfalfa in dry soil is greatly reduced by summer warmth up to 50 degrees C. even for a few days. Schmidt¹¹ in 1924 said that treatment of alfalfa seed with live steam for one minute causes a notable decrease in impermeability and increases germination.

Eddy³, discussing treatment by the Eddy huller and scarifier, said that a number of Grimm alfalfa samples, with an average of 63 percent impermeable seeds before scarification, had an average of 1.58 percent after scarification. Schmidt¹¹ found that impermeability is increased when seeds are subjected to dry heat. He did not specify the degree of heat nor the length of exposure.

Staker¹⁴, in connection with an experiment to determine whether weed seeds could be killed by heat without killing the alfalfa seed in which they occur, found that heat stimulated germination of the alfalfa sample with which he was working. The sample of seed treated had an initial content of 15 percent hard seeds. After heating, the germination percentage was higher and the impermeable-seed percentage lower, varying from zero to 4 percent in various samples of the lot treated for periods of one half hour to four hours at 60 degrees to 75 degrees C. Bresaola¹, in experiments to kill *Cuscuta* seeds by heating them, remarked that *Cuscuta* was more easily killed than alfalfa and that in fact seeds of alfalfa sometimes germinated a little better

after heating. He used 65 degrees, 70 degrees and 75 degrees C., for one to one and one-half hours.

Stewart¹⁰ showed that dry heat increases germination by reducing the number of impermeable seeds in mature bright lots, while it markedly reduces the germination of badly discolored seeds.

Time alone appears to change many impermeable seeds to permeable. Harrington⁹ found that seeds stored in manilla envelopes under laboratory conditions became permeable as follows: Fresh hand-threshed seed containing 32 percent impermeable seeds had only 7 percent when one year old. Fresh hand-threshed seed having 80 percent had 60 percent when one year old and 24 percent when two years old. He also reported on seeds between moist blotters showing that in hand-gathered and machine-threshed seeds practically all hard seeds had softened in one year. He also found that storing for six months at 45 degrees C. slightly increased germination and that alternate freezing and thawing in greenhouse flats increased permeability but that seeds left out of doors on the plants were mostly dead in the spring. Goss⁴, in his examination of buried seeds added weight to the idea that alfalfa seeds do not long remain impermeable in the soil, for after having been buried one year there was only 5.5 percent live seed, none of which were impermeable. Schmidt¹² said that in alfalfa seed sown in the fall, mid-winter or early spring, but few hard seeds survive and that hard seeds are of most agricultural value when planted after settled weather in the spring. Lute⁹ found that impermeable seeds planted in early April when germination conditions were ideal had less than half the agricultural value of permeable seeds.

MATERIALS AND METHODS

Care has been exercised to select and handle all material used in such a way that all results might be strictly comparable. Methods have been carefully checked so that no errors could creep in due to varying degrees of maturity, different methods of manipulation of the seed in hulling or to different conditions in blotter tests.

While it has been impossible to control conditions for soil testing as accurately as chamber conditions, careful records have been made of all temperatures so that differences in behavior may be correlated with differences in growing conditions.

Since some scarification is incidental to all machine threshing the major portion of this work has been carried on with hand-threshed seed. The first of such seeds used were freed from the

Pods by rubbing in a sieve. The high germination of these seeds suggested that a small amount of friction might break the impermeable layer in some seeds. Therefore methods of hand threshing were carefully studied in order to make sure that there should be no effects of scarification on the seeds used. The method finally adopted was as follows: The pods were carefully dried on the plants; then the seeds were freed by carefully rubbing between the fingers, followed by a gentle air blast. The seeds were then separated from all remaining foreign material with the aid of fine tweezers and a good magnifying glass. Unless otherwise specified all seeds used have been uniformly obtained from the pods in this manner.

In order to avoid all possible discrepancies due to use of seeds not equally mature, all seeds have been critically examined with a hand lens. Seeds containing larvae were discarded. Except in the study of immature seeds, only plump yellow seeds were used. As nearly as could be judged by the color they were all of the same degree of maturity.

In making germination tests the method approved by the Association of Official Seed Analysts has been used. This consists of placing seeds between folds of moist blotter in chambers at a temperature of 20 degrees C. The blotters are those especially made for germination work. Constant temperatures were maintained by a combination of water for cooling and electricity for heating. All seeds which had taken up no water in six days were considered as impermeable.

All alfalfa plants grown at the experimental farm for this work were started in the greenhouse. The seeds of the first planting were put into flats of mountain soil in the greenhouse. This soil had been brought down from the mountains to insure freedom from alfalfa seed. Due to the absence of bacteria these plants developed very slowly, few of them producing seeds the first year. The following year the soil used was sterilized in an autoclave to kill all seeds which it might contain. A drop of bacteria culture was put in with each seed planted. All plants grew rapidly and produced abundant seed that year.

Daily records of temperatures in the greenhouse were recorded from a maximum-minimum thermometer. Data for the period of the field tests at Rocky Ford were secured from the records of the Experiment Station at that place.

EXPERIMENTAL DATA

For the past fifty years impermeable seeds of various legumes have been studied from the two standpoints, of their commercial value and the cause of impermeability. Little attention, how-

ever, has been given until recently to the study of the hard seeds of alfalfa. The work reported in this paper was carried on from 1919 to 1927 entirely with seeds of alfalfa and for the most part with a view to determining what conditions produce impermeable alfalfa seeds. A large amount of work was done to determine whether, under Colorado conditions, the various published and unpublished ideas as to the behavior and cause of such seeds would hold true. It has been said that the impermeable seeds of alfalfa are merely a little slower to take up moisture than are the permeable ones; second that the percentage found in the usual blotter test is very inconstant even for the same sample and therefore not to be taken very seriously; third that such seeds behave differently in soil than they do in blotters; fourth that impermeability is a device for securing longevity, especially in soil; fifth that a dry climate is conducive to production of impermeable seeds; and sixth that mechanical scarification does away with any undesirability resulting from impermeability. Each of the foregoing points is taken up in the discussion of experiments which follows, together with studies of the seed-coat structure as affecting permeability.

BEHAVIOR OF IMPERMEABLE ALFALFA SEED KEPT CONTINUOUSLY UNDER CONDITIONS SUITABLE FOR GERMINATION

In 1919, 96 samples of alfalfa seed were placed in moist blotters in germination chambers to determine the time required for all impermeable seeds to germinate or decay. At the end of eight days of constant germination conditions the seeds remaining impermeable in these samples varied from 11 to 60 percent. A study of these data shows that in many cases one-half of these impermeable seeds germinated in two months as illustrated in Table 1.

Table 1.—* Behavior of Impermeable Alfalfa Seed Kept Under Conditions Suitable for Germination.

No. of days in blotters	Lot 1		Lot 2		Lot 3	
	Percent germina- tion	Percent imper- meable	Percent germina- tion	Percent imper- meable	Percent germina- tion	Percent imper- meable
4	39	58	85	14	62	41
8	44	54	86	13	66	37
16	47	51	87	12	68	29
32	56	41	88	11	70	27
60	35	12	93	6	74	23
120	90	7	98	1	85	12
160	96	1			85	12
192					86	11
366					88	9
402					89	8

* From records of G. E. Eggington formerly in charge of State Seed Laboratory.

In the above table are given the germination records of three samples of alfalfa seed representative of the 96 samples tested. The rate of decrease in the percentage of impermeable seed was extremely variable, suggesting that not all such seeds are equally impermeable.

CONSTANCY IN IMPERMEABLE SEED CONTENT IN A SAMPLE AS SHOWN BY BLOTTER TESTS

Twelve pairs of tests were made from each of many lots of commercial seed to ascertain whether individual samples when subjected to standard conditions for germination of alfalfa seed would exhibit a variable percentage of impermeable seed.

Table 2 gives the results for three representative lots of alfalfa seed used in these tests.

Table 2.—Variability in Impermeable Seed Content in Alfalfa Samples.

Lot 1		Lot 2		Lot 3	
Percent germination	Percent impermeable	Percent germination	Percent impermeable	Percent germination	Percent impermeable
82	11	53	38	53	42
83	14	53	37	45	46
83	12	54	36	50	41
83	13	55	38	45	43
93	10	55	37	52	41
86	11	57	38	48	44
86	8	57	35	49	45
87	10	57	36	54	40
87	11	58	34	52	39
89	9	59	33	50	43
89	7	60	32	52	42
89	7	61	34	52	43
85	11	56	35	50	42
Average					

The above table illustrates the close correspondence between the percentage of impermeable seed in repeated tests from the same sample. It will be noted that in one set of 12 tests from one lot of seed the impermeable seed at the time of the final count varied from 7 to 14 percent; for a second lot it varied from 32 to 38 percent; and for a third lot it varied from 39 to 46 percent. Since all of these figures are well within the limits of tolerance recognized in seed testing, it may be said that the percentage determined by blotter tests when made under uniform conditions is reasonably constant.

BEHAVIOR OF IMPERMEABLE SEED IN SOIL

In order to determine if the blotter test for impermeable seed is a fair index of what the germination would be in the soil, seed was tested in the laboratory and the greenhouse. Fif-

teen lots of machine-threshed seed and eight lots of hand-threshed seed were planted in greenhouse flats. The seedlings in these flats were counted at intervals of 21, 28 and 60 days. Laboratory blotter tests were begun the day seeds were planted in the soil. In Table 3 are given the results of this experiment.

Table 3.—Germination of Machine-threshed Alfalfa Seed in Blotters as Compared with that in Greenhouse Soil Flats.

Lot Number	Blotter Test Germination	Percent Impermeable	Percent Plants in Greenhouse		
			21 das.	28 das.	60 das.
24395a	87	5	80	80	81
b			74	80	81
c			75	76	76
24345	84	12	78	80	80
24107	79	9	76	78	78
24458	77	22	78	82	82
24346	66	32	73	84	92
24348	64	32	76	87	87
24321a	65	25	50	59	60
b			62	61	61
c			56	59	60
d			52	60	65
24366	59	36	77	82	82
24102	57	28	61	73	74
24287	51	42	56	64	71

It can be seen that several lots from the same sample when under uniform conditions behave very similarly since those designated by letters are from the same lot. Table 3 shows that all samples having more than 25 percent of impermeable seeds in the blotter tests produced more plants in the greenhouse than the germination percentage. It also shows that the impermeable seeds which germinate do so more slowly than the permeable ones.

A similar test was made of hand-threshed seed at the same time, under the same greenhouse conditions. Table 4 is a summary of the behavior of typical lots of seed, each taken from an individual plant.

Table 4.—Germination of Hand-threshed Alfalfa Seed in Blotters as Compared with that in Greenhouse Soil Flats.

Lot Number	Hand-Threshed Seed		Percent Plants in Greenhouse		
	Percent in Blotter Test Germination	Percent Impermeable	21 das.	28 das.	60 das.
11-20	23	75	76	91	91
1-4	21	78	43	73	84
12-24	19	79	52	70	71
17-8	13	83	53	79	79
11-32	12	79	57	83	85
17-29	11	88	37	69	69
15-30	11	86	38	68	68
17-36	4	96	38	68	72

A comparison of Tables 3 and 4 emphasizes the fact that impermeable seeds have a considerable agricultural value when there is a very high impermeable seed content. It is also shown that the scarification incidental to machine threshing affects seeds having the lowest degree of impermeability since a smaller percentage of the impermeable seeds in machine-threshed lots produces plants in the greenhouse than is true of hand-threshed seeds.

Table 5 is a summary of the data in tables 3 and 4. It shows definitely that under the conditions of this experiment some impermeable seeds produce plants, that they germinate more slowly than other seeds and that they do not increase the number of plants when only a few are present.

Table 5.—Summary of Tables 3 and 4.

	Blotter Test		Plants in Greenhouse	
	Germina- tion percent	Imper- meable percent	21 das.	28 das.
Machine threshed:				
25 per cent or less impermeable seed	78	11	68	71
More than 25 percent impermeable seed	59	34	69	78
Hand threshed:				
All lots	14	83	49	76

The greenhouse conditions during this experiment were recorded from a maximum-minimum thermometer. There was a daily difference between the extremes of more than 25 degrees C. The usual maximum was 45 degrees C. while the usual minimum was 10 degrees C. The extremes for the period were 9 degrees C. and 50 degrees C. The combination of this daily variation together with the high daily maximum seems to have been extremely favorable for the germination of previously impermeable seeds. Greenhouse plantings made earlier in the season when maximum temperatures were lower resulted in germination of fewer impermeable seeds. It appears likely therefore, that the high daily maximum and the occasional maximum of 50 degrees C. were the factors which caused impermeable seeds to grow.

In order to test the actual behavior of seeds under field conditions, plantings were made at the State Experiment Station, Rocky Ford, Colorado. Five lots of seed were planted of which four were commercial seeds of different varieties while the other was composed of seeds which two months previously had been taken from blotter germination tests on the sixth day as impermeable. Moisture conditions for germination were ideal at the time these seeds were planted and for two months thereafter,

as indicated by weather records of the experimental farm. During April the temperature fell slightly below freezing nine nights, and reached a maximum of 29 degrees C. once.

During May the minimum was 3 degrees C. while the maximum was 34 degrees C. In Table 6 is given a summary of the results of the field plantings.

Table 6.—Comparison of Germination of Alfalfa Seed under Field and Laboratory Conditions.

Seed Planted	Blotter Tests		Field Plantings		
	Percent germination	Percent impermeable	Number of seeds planted	Number of plants in two months	Percent in plants in two months
Impermeable	8	91	5000	898	18
Grimm	92	4	5000	2056	40
Turkestan	91	0	5000	2056	40
Common	40	34	5000	669	13
Baltic	78	17	5000	1055	21

Continued observations of the field were made thruout the summer but no additional plants were observed after the first two months.

Hard seed proved of less planting value in these field tests than in the greenhouse tests discussed in Tables 3, 4 and 5. A comparison of the temperatures to which they were subjected offers an explanation. The seeds in the greenhouse were exposed to a maximum of 50 degrees C. while the temperature in the field did not exceed 34 degrees C. In the field plantings at Rocky Ford the extremes of temperature were 34 degrees C. and —7 degrees C. The variation between these extremes was greater than the variation in the greenhouse. The percentage of germination of impermeable seed in the field was much lower than in the greenhouse. A comparison of these two results suggests that high temperatures are favorable for germination of alfalfa seeds.

PERSISTENCE OF IMPERMEABILITY

In speculating upon the occurrence of impermeable seeds, Guppy⁵ suggested that nature has thus provided a distribution of germination over a period of time and that impermeability is merely a device to increase the longevity of the seed. The work of Harrington⁶, Goss⁴ and Schmidt¹¹, as cited under "Literature," shows that few or no impermeable seeds of alfalfa survive even one year in the ground. They are, however, somewhat longer lived when stored in manila envelopes under laboratory conditions. In order to further test the persistence of the impermeable condition of alfalfa seeds, lots of commercial alfalfa were tested and kept for a period of years to determine the effect time has on the impermeable seeds and also on the total live seed content.

They were stored in shell vials under laboratory conditions, the temperature of which varied from 15 to 28 degrees C. The interval between the first and second tests was nearly three and a half years. In that time more than one-half of the impermeable seeds had become permeable in practically all samples. In many cases three-fourths of them were no longer impermeable. In this period the total live seed had, however, become somewhat less. The average live seed when the seeds were put into storage was 94 percent, three and a half years later it was 80 percent. In the next two years there was a decrease of impermeable seeds at a somewhat slower rate, and a considerable decrease in live seed. By December 1923, 12 years after the first test, most of the impermeable seeds had softened while the total live seed had decreased materially. Table 7 illustrates the behavior of 20 such lots.

Table 7.—Viability and Impermeability of Stored Alfalfa Seed.

January 1912		June 1915		June 1917		December 1923	
Percent germination	Percent impermeable	Percent germination	Percent impermeable	Percent germination	Percent impermeable	Percent germination	Percent impermeable
98	1	69	0	64	0	58	0
97	0	83	1	80	0	69	1
97	0	91	0	67	0	65	0
96	1	78	0	78	0	60	0
96	1	78	0	78	0	60	0
96	2	86	1	79	0	69	0
90	7	81	1	73	2	71	1
87	1	89	1	76	1	68	0
82	0	68	1	56	1	56	0
78	9	68	2	60	2	45	0
65	33	51	19	59	6	47	5
63	36	59	16	66	12	63	3
61	35	66	16	64	12	70	8
61	38	58	19	64	13	66	6
59	17	72	5	68	8	39	3
58	41	66	13	71	8	59	5
58	41	71	12	70	5	69	7
50	48	60	26	58	20	65	8
45	48	67	14	64	19	61	0
41	31	38	31	48	21	51	16
38	54	72	14	65	13	64	9
Average							
71	21	70	9	66	7	60	4
Live seed 92							
		79		73		74	

The above data indicate the rate of change from impermeable to permeable while alfalfa seeds are stored under laboratory conditions.

An additional study was made of impermeable seeds which had been taken out of commercial lots. These seeds were obtained by removing from germination blotters the seed which had

not taken up water at the end of six days when kept in a chamber at 20 degrees C. After removal from the blotters they were dried in air ten days and then stored in the laboratory. Their behavior is illustrated by Table 8.

Table 8.—Change in Permeability of Alfalfa Seed.

Number of sample	Dec. 1920	December 1923		December 1926	
	Percent impermeable	Percent germination	Percent impermeable	Percent germination	Percent impermeable
1	100	92	6	88	6
2	100	83	16	79	16
3	100	75	24	78	16
4	100	81	17	81	13
5	100	74	23	76	16
6	100	86	14	74	18
7	100	80	19	75	12
8	100	79	20	78	16
9	100	72	27	73	24
10	100	77	21	84	11
11	100	82	16	86	9
12	100	77	18	79	20
Average	100	80	18	79	15

Table 8 brings out the fact that during the first three years of storage there is a notable decrease in percentage of impermeable seed. In December 1920, all seeds were impermeable; by December 1923, the impermeable seed content had been reduced to 18 percent. During the next three years there was little change in impermeability, while there was a small decrease in live seed. The impermeable seed used in the above may be looked upon as having been especially selected since they had escaped scarification by threshing and had also been kept in conditions suitable for germination for six days. In contrast to this, work was carried on with hand-threshed seed of individual plants.

Twenty-three samples of seed each from an individual plant were gathered in July, 1925. These seeds were threshed by hand and tested for germination the following October. The impermeable seeds in blotter tests varied from 11 to 76 percent, the average for the whole set being 52 percent. When again tested in October, 1926, the impermeable seeds varied from 5 to 60 percent, the average being 33 percent. Thus in one year, more than one-third of the impermeable seeds had become permeable. There was practically no loss in vitality. The average live seed in October, 1925, was 99 percent while in October, 1926, it was 97 percent.

In Table 9 and Fig. 1 are given the results of this experiment.

Table 9.—Decrease in Impermeable Seed in Hand-threshed Lots of Alfalfa.

Plant Number	October 1925		October 1926	
	Percent germina- tion	Percent imper- meable	Percent germina- tion	Percent imper- meable
51	38	62	55	43
51	70	30	87	8
52	63	29	74	23
53	35	65	56	41
54	56	44	72	25
55	70	30	68	28
56	51	49	58	40
57	80	20	89	10
58	52	48	77	21
59	89	11	87	11
60	87	14	93	5
61	38	62	44	52
62	60	34	73	25
63	43	57	53	45
64	47	53	56	41
67	37	63	57	42
68	84	16	93	6
69	43	57	59	31
70	35	65	48	42
71	21	76	39	60
72	49	51	57	43
73	26	74	40	56
78	33	67	44	54
Average	47	52	64	33
Live Seed Average	99		97	

It may be noted in Table 9 that there is a marked difference in the behavior of impermeable seeds from individual plants. Two samples show practically no decrease in this one-year period, while in one sample practically three-fourths of them had become permeable. The average decrease is one-third.

RELATION OF SOURCE OF SEED TO PERCENTAGE OF GERMINATION

In the testing of alfalfa seed it is a general knowledge that commercial samples from different localities show different percentages of impermeable seed.

Greater use is being made each succeeding year of seeds of hardy strains of alfalfa. These varieties exhibit a higher percentage of impermeable seeds than the less hardy varieties which they are superseding. During the period when numerous lots of imported Turkestan alfalfa seed were on the market it was a conspicuous characteristic that there were few or no impermeable seeds present in commercial lots of that variety. More recently it has been noted that there are fewer impermeable seeds present in commercial alfalfa seed from the southwestern than from the northern alfalfa-seed-producing regions in the United States.

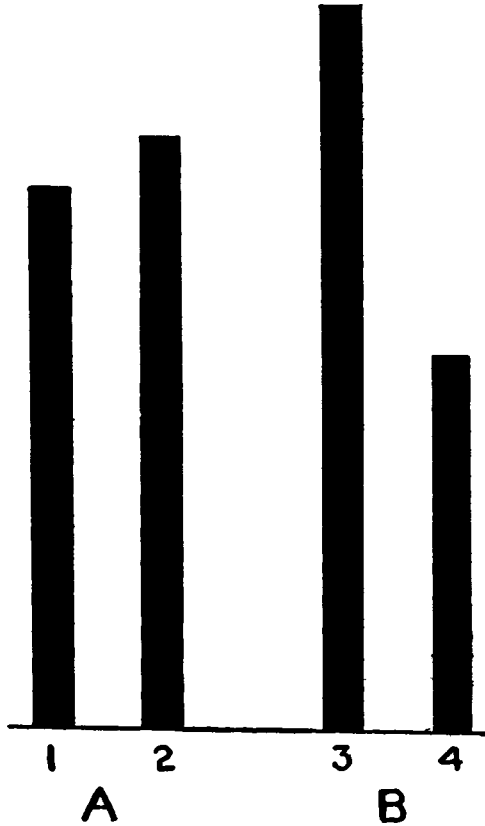


Fig. 1. Decrease in impermeable seed in hand-threshed lots of alfalfa.
 A.—October 1925. B.—October 1926.
 1 and 3—percentage germination.
 2 and 4—percentage impermeable.

In order to determine if this difference was due to varietal difference or to geographic effects a study was made of the germination of alfalfa seed grown in different parts of Colorado.

Records of samples tested by the Colorado Seed Laboratory over a number of years show that the average impermeable seed content each year has been approximately 22 percent. The records were studied from the point of view of the various producing sections. Practically all lots were grown under irrigation and at altitudes from approximately 4200 feet to 7000 feet. Since all lots studied were machine-threshed seed, it has been impossible to separate the effect of scarification incidental to threshing from originally impermeable seeds. Whatever the contributing factors are, there is a marked difference for various alfalfa-seed-

producing sections of the state. Table 10 shows the variation for the year 1922 and is typical for all years studied.

Table 10.—The Percentage of Impermeable Seed When Grown at Various Altitudes

Location	Approximate altitude	Percent impermeable seed
Meeker	6200	18
Ft. Collins	5100	21
Arkansas Valley	4200	21
Western Slope	4500	23
Allison	6500	43
Norwood	7000	43
Average		22

Since the seed-producing areas represented in the above table are widely separated, the seeds in each vicinity were without doubt threshed by a different machine. The uniformity in impermeable-seed content, therefore, can not be said to be due to uniform treatment by a single thresher.

A sample of unthreshed seed grown in Arizona was harvested in October. It was threshed by hand and tested for germination the last of October. The germination test showed that 65 percent of the seed germinated and 33 percent remained impermeable. That is a much lower percentage of impermeable seed than was found for hand-harvested seed grown in Colorado.

Supplementing these data, seeds from several fields were secured for successive years and their behavior in blotters studied. In Table 11 are given the results of these tests.

Table 11.—Tests for Successive Years of Seed from Different Localities.

Field	Approximate altitude	1921 Crop		1922 Crop		1923 Crop	
		Percent germination	Percent impermeable	Percent germination	Percent impermeable	Percent germination	Percent impermeable
1	6100	58	29	71	21	55	37
2	6100	49	38	62	29	53	32
3	6100	51	22			56	39
4	6200			51	38	57	31
5	6200			57	39	59	39
6	6200			51	17	55	41
7	6200			69	26	64	26
8	6200			84	12	67	26

The first six fields are in the northwestern part of the state; the last two are in the southwestern part

In one case the percentage of impermeable seed in the samples from a field varied little for two successive years; however,

in most cases the variation was more than 10 percent. A study of Table 11 which contains the details of this work shows that as far as may be judged from commercial samples a given field does not produce a similar percentage of hard seed in successive years.

EFFECT OF DRYING AND MATURITY ON IMPERMEABLE SEED CONTENT

Among the possible factors influencing the percentage of impermeable seed in alfalfa, drying and maturity are suggested. Whether impermeable seeds are the result of drying after harvesting, drying on the plant after ripening or whether they exist at all stages of development are questions that may throw light on impermeability.

Studies were therefore made of the germination of alfalfa seed of different degrees of development. The first tests were made of samples of commercial seed which had been separated into two lots, immature, that is plump green seeds, and mature plump yellow seeds.

Care was taken to select seed as nearly as possible identical in size, the seeds differing only in color. From 10 to 20 lots of 200 seeds each were selected from numerous commercial samples. The average germination of such selections for four different samples is given in Table 12. These are typical of all the lots tested.

Table 12.—Germination of Mature and Immature Alfalfa Seed from Machine-threshed Lots.

Laboratory Number	Yellow Seeds		Green Seeds	
	Percent germina- tion	Percent imper- meable	Percent germina- tion	Percent imper- meable
15522	67	30	41	25
15324	77	18	63	26
15325	69	24	62	26
15774	80	17	58	13
Average	73	22	55	22

It is evident from the above tests that little difference in impermeable-seed content exists between mature and slightly immature seed.

There appear to be more dead seeds among the slightly immature than the mature seeds. Subsequent studies of hand-hulled seed take up more in detail the degrees of maturity. The green seeds here used were more immature than those in the preceding study. Care was taken in selecting green seed to use no seed so immature as to be thin or wrinkled. The details of this study are given in Table 13.

Table 13.—Germination Tests of Hand-threshed Mature and Green Alfalfa Seed.

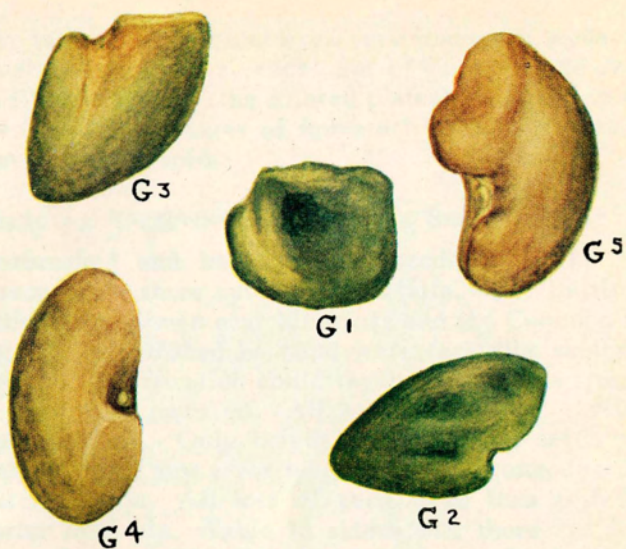
Plant Number	Yellow Seed			Green Seed		
	Percent germination	Percent impermeable	Percent dead	Percent germination	Percent impermeable	Percent dead
4- 5	3	97		17	68	15
11-26	6	94		41	42	17
13-30	4	96		41	37	22
14- 1	8	92		25	59	16
14-10	9	91		33	45	22
14-15	2	98		38	49	13
18- 8	1	99		23	48	29
18-11	6	40		18	59	25
18-17	4	96		16	82	2
19-24	23	77		25	61	14
19- 6	7	93		14	67	20
13-32	8	87	5	11	22	57
13-36	6	92	2	64	2	34
16-19	6	93	1	20	30	50
19-13	7	93		38	52	10
Average	7	92		30	45	23

The preceding table indicates that impermeability increases with maturity; also that in all lots having some seed sufficiently developed to germinate under the usual chamber conditions, some impermeable seeds are present. The average dead seed among plump green seeds was 23 percent. The average dead seed among mature seed was only one-half of one percent.

A further study of samples of hand-hulled seed was made to ascertain the percentage of germination of impermeable and of dead seeds at definite stages of development. Seeds were carefully graded into four shades of green and one of yellow. These have been designated respectively as G1, G2, G3, G4, G5 and Y. Each separation was then graded as to size by the use of sieves with circular openings. The seeds were then weighed in order that a complete comparison might be made as to correlation between color, size and weight and the living characteristics of the seed. Table 14 gives the details for one such lot.

Table 14.—Percentage of Impermeable Seeds Increases with Maturity.

Separation Number	Weight of One Seed in Grams				Chamber Test, 6 Days		
	Size of circular opening in screen thru which seeds did not pass				Percent impermeable	Percent dead	Percent germination
	1.016	1.207	1.448	1.651	17	11	80
G1	.077				57	18	0
G3			.140		22	20	2
G4			.198		5	85	5
G5		.188			0	10	
G5			.209		3	20	
G5				.225	86	25	tion
Y		.191			86	58	Percent
Y			.213	.220	74	meable	germina-
Y					14	24	78



Above—Green, Immature Alfalfa Seed.
Below—Mature Alfalfa Seed.

The above table indicates that even very immature seeds contain a high percentage of live seeds, and of impermeable seeds. Reference to figures 2 and 3 (the colored plates) will give an adequate idea of the various stages of immaturity. The largest mature seeds have no dead seed.

THRESHING AS AFFECTING IMPERMEABLE SEED CONTENT

Machine-threshed and hand-threshed seeds from the same fields were secured of three varieties of alfalfa. The Baltis and Grimm varieties were grown near Montrose and the Common near Fort Collins. Seeds threshed by hand were carefully shelled in such a way that no scarification could result. The seeds from all three fields were well matured. All seeds used were carefully examined under a lens. Only bright yellow plump seeds were selected so as to avoid any error which might be introduced by difference in maturity. All lots of seed were then tested by standard blotter methods. Table 15 shows that there are fewer impermeable seeds in machine-threshed lots than in hand-threshed seed from the same field.

Table 15.—Scarification Incidental to Threshing.

Variety	Laboratory Germination		Germination	
	Machine-threshed Percent germina- tion	Percent imper- meable	Hand-threshed Percent germina- tion	Percent imper- meable
Baltic	66	30	35	62
Grimm	63	34	48	45
Common	86	8	43	51

The above table brings out the varying amount of scarification which may be expected to result from threshing.

RELATION BETWEEN IMPERMEABILITY IN SEED PLANTED AND IMPERMEABLE SEED CONTENT OF THE RESULTING CROP

To determine if seed would be produced having the same content of impermeable seed as those planted, a series of experiments was planned. The first results obtained were from the 1921 planting at Rocky Ford. These seeds were threshed by rubbing in a sieve which probably resulted in some degree of scarification. Germination tests of these lots showed that the seeds produced resembled each other much more than they resembled the seeds which were planted. Table 16 shows the details of this experiment.

Table 16.—Comparison as to Impermeable-Seed Content of Seed Planted and Seed Produced.

Lot Number	Seed Planted, 1921		Seed Harvested, 1921	
	Percent germination	Percent impermeable	Percent germination	Percent impermeable
1	8	91	72	12
2	92	4	66	12
3	91	0	59	17
4	40	34	70	11
5	78	17	57	14

It may be noted from the above figures that there was a wide variation in the percentage of impermeability of the original seed planted. The extremes between the percentage of hard seed in the resulting crop is slight, being only six percent. All seeds planted were machine threshed, except lot 1, which had been taken out of blotters as impermeable three months earlier.

Since it was apparent in the preceding work that incidental scarification might have taken place, care was exercised in the following experiment to avoid such a possibility. Seedlings from seeds planted in the greenhouse in soil in 1924 were set out in the field at the experimental farm at Fort Collins. Each lot was set out in a separate row, plants two feet apart in the row and the rows two feet apart. Seeds from these plants were harvested in November, 1924. The seeds were stripped by hand, thoroly dried in the laboratory and then carefully threshed and cleaned by hand in such a manner as to avoid any possible scarification. They were then tested between moist blotters at 20 degrees C. Lot 1 was hand threshed before planting in 1924; all other lots planted were commercial, machine-threshed seed. Lots 1 and 2 were from the same field. All seeds were hand threshed for harvests of 1924 and 1925.

In 1924 and 1925 the plants were cut off in October, hung in a shed to dry and then brought into the laboratory, threshed and tested in January and February. In Table 17 are shown the results of these tests.

Table 17.—Results from Planting Impermeable Seed.

Lot Number	Planted in 1924	Harvested in 1924	Harvested in 1925
	Percent impermeable	Percent impermeable	Percent impermeable
1	51	39	84
2	6	57	88
3	29	51	88
4	21	no seed	90
5	10	no seed	81
6	23	no seed	80
7	24	no seed	83
8	45	66	84
9	11	no seed	87
10	36	no seed	88

Table 17 is a comparison between seeds planted and the two harvests from the plants produced. Lot 1 was a hand-threshed lot when planted; its progeny had fewer impermeable seeds in 1924 than were in the lot planted, while the same plants in 1925 produced more than twice as many impermeable seeds as in 1924.

All other lots were machine threshed. They showed a wide variation between the two harvests.

PRODUCTION OF IMPERMEABLE SEED BY INDIVIDUAL PLANTS

Preliminary studies on the occurrence of impermeable seed pointed toward the fact that there are individual plant differences and also that the various portions of a plant do not produce seeds which are uniform in respect to impermeability.

Seeds from individual stems of a bunch of alfalfa taken from a stack before threshing were hand threshed and tested separately. Twelve stems from a lot which was cut and stacked in October, 1923, hulled and threshed in May, 1924, had a variation in impermeable seeds from 14 to 91 percent. Twelve stems from the same sheaf were hulled by hand in May, 1924, and tested for germination in November, 1925. These showed a variation from 18 to 74 percent. A further step was the testing of individual stems from a single plant. A large plant bearing seed was found in a garden in Fort Collins in September, 1925. No other alfalfa plant was present in this garden. Seeds were hulled separately from its 35 individual stems. Germination tests were made the following October. There was a variation in impermeable seed from 54 to 95 percent.

The details for the study of single stems will be found in Table 18.

Table 18.—Impermeable Seeds in Hand-picked Seeds from Individual Stems of Plants.

Harvested July, 1925 Germinated Oct., 1925 Plant 85. Impermeable seed		Harvested Oct., 1923 Germinated May, 1924 Impermeable seed		Harvested Oct., 1923 Germinated Nov., 1926 Impermeable seed	
Stem 1	93	Stem M	91	Stem A	51
2	92	N	79	B	60
3	85	O	64	C	60
4	82	P	81	D	48
5	97	Q	86	E	66
6	84	R	77	F	56
7	60	S	90	G	18
8	86	T	88	H	62
9	97	U	67	I	61
10	68	V	14	J	53
11	94	W	86	K	74
12	78	X	46	L	51
13	58				
14	95				
15	81				
16	87				
17	65				
18	85				
19	85				
20	82				
21	80				
22	54				
23	81				
24	68				
25	64				
26	60				
27	58				
28	94				
29	75				
30	78				
31	87				
32	87				
33	98				
34	79				
35	64				

In the above table stems 1 to 35 are from one plant, growing in a yard in Fort Collins. Stems A to X are from Mr. Martin's field, cut for seed and stacked. It may be noted that there is wide variation of hard-seed content in seed from different stems from a single plant. The extremes of percentages of impermeable seed from individual stems from one plant range from 54 to 98 while the impermeable seeds in a bunch of stems from a stack, which were probably not all from the same plant vary from 14 to 91 percent. The foregoing data seem to show that there is less variation between the stems of an individual isolated plant than there is between the stems from various plants growing near together, at least in the same field.

A study was also made of individual plants grown from each of 20 lots of seed. A total of 433 plants were tested in this way. These plants were the survivors, after transplanting to the field, of seeds planted in soil in a greenhouse in 1924 and 1925. The details for 433 plants tested will be found in Table 19. In this test, rows 1 to 10 inclusive were planted in April, 1924. Rows 11 to 20 inclusive were planted in April, 1925. All seeds tested were from the crop harvested in October, 1925. All seeds were hand threshed.

Table 19.—Comparison of Percentage of Impermeable Seed Planted and Produced.

Row Number	Number of plants	Average Impermeable Seed		
		High	Low	Averages
1	17	97	64	84
2	19	98	72	88
3	15	98	84	88
4	14	98	83	90
5	16	94	53	81
6	18	94	56	80
7	18	93	61	83
8	17	94	66	84
9	14	96	74	87
10	16	98	82	88
11	32	97	73	89
12	28	99	82	91
13	36	99	81	93
14	21	99	91	94
15	32	98	80	93
16	29	99	83	91
17	38	99	84	93
18	12	97	88	93
19	26	100	63	93
20	14	90	70	82

Another group of individual plants tested showed an even larger variation. These plants were the remnants of an old alfalfa field which had given way to lawns and buildings. There are no data as to the age of these plants. They set abundant seed in the summer of 1925. Seeds from 25 plants were harvested in July, threshed by hand and tested separately.

In Table 20 are the germination and permeability records of this lot of seed.

Table 20.—Records on Twenty-five Individual Plants.

Plant Number	Percent germination	Percent impermeable seed
50	38	62
51	70	30
52	63	37
53	35	65
54	56	44
55	77	23
56	51	49
57	80	20
58	82	48
59	89	11
60	89	11
61	38	62
62	66	34
63	43	57
64	47	53
65	34	66
66	77	23
67	37	63
68	91	9
69	43	57
70	35	65
71	28	72
72	49	51
73	26	74
74	33	67
Average	55	46

The extremes of impermeable seeds for these plants were 9 and 74 percent. The average for all plants was 46 percent which was distinctly below the average for seed harvested from other plants in October at Fort Collins.

Seeds from more than one thousand individual plants were hand threshed and given separate germination tests. Table 21 which follows, gives the records for two seasons of 18 of these individual plants. They are typical of the results obtained from the large number of plants studied. These seeds were all harvested in October.

Table 21.—Successive Crops from Individual Plants.

Plant	Crop of 1925		Crop of 1926	
	Germination	Impermeable	Germination	Impermeable
11-17	11	90	12	85
11-20	26	74	23	75
12- 1	18	83	17	81
12-25	17	83	17	77
14-22	8	97	2	94
17-36	4	96	4	96
11- 4	4	97	21	78
13-21	3	97	24	79
13-32	6	94	16	77
13-37	1	99	22	75
14- 4	5	95	20	80
16-18	6	94	16	78
15-33	20	80	7	88
19- 2	20	80	10	88
19-20	30	70	10	87
19-27	37	63	17	78
20- 2	37	63	12	88
20-15	30	70	13	85

It will be noted from Table 21 that, judged from the standpoint of the impermeable-seed content, the seeds produced fall into three groups, first those which produced approximately the same percentage in the two consecutive years, second those which produced a much higher percentage the second year and the third those which produced a much lower percentage the third year.

A comparison of tables 20 and 21 brings out the great variability in seeds produced by individual plants and suggests a possible difference due to time of harvest. Since the plants harvested in July were not the same ones harvested in October, a direct comparison can not be made at this time. The much lower percentage of impermeable seeds from individual plants in the summer harvest than the autumn one suggest a climatic influence, while the great difference between individual plants suggests a genetic influence. Alfalfa plants cross-pollinate readily so the factors, if genetic, are obscured.

STRUCTURE OF THE SEED COAT OF ALFALFA

Experiments with scarified seed indicate the seed coat as the controlling factor in impermeability of alfalfa seed and suggest study of the histology of this part of the seed.

Microscopic examination of alfalfa seed shows that a cuticle is present over the entire surface of the seed. However, it is not smooth as illustrated by other workers¹⁸ (Fig. 4) but follows the configuration of the rounded end of the palisade cells. It varies in thickness, frequently decreasing to a very thin layer.

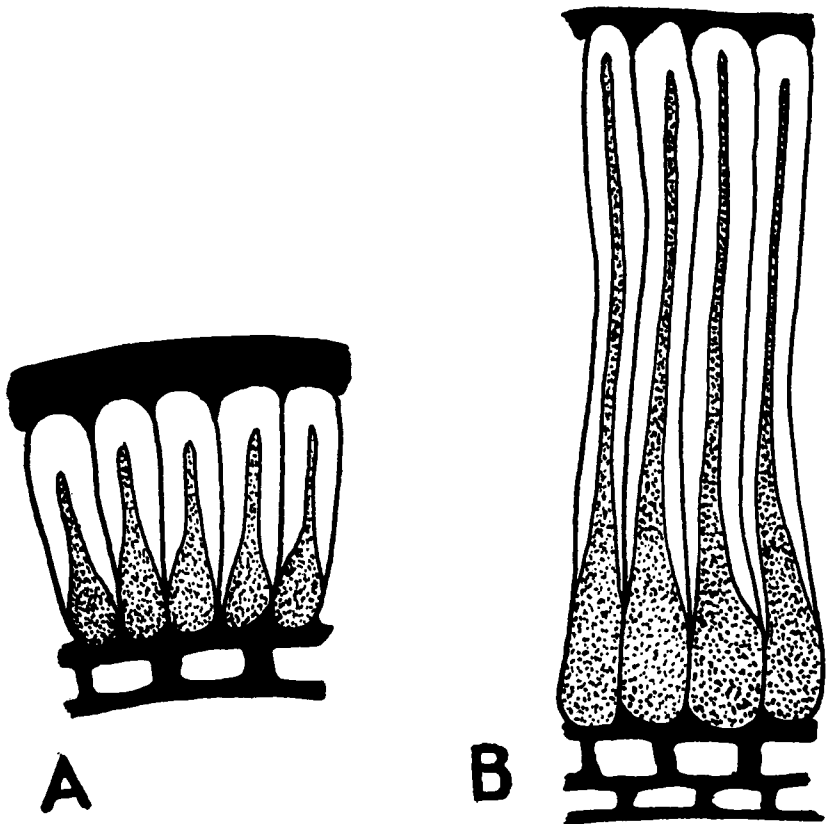


Fig. 4. Details of cellular structure of seed coats. (After White)
 A. Alfalfa.
 B. *Caesalpinia bonduc*.

Below the cuticle is a layer of palisade cells. These have rounded ends which vary from semi-circular in section to rather pointed, often truncated. This apparently depends upon from what part of the seed the section is taken. The palisade near the hilum is very deep. As illustrated in the drawings (Figs. 5 and 6) the lumen of these cells is much convoluted. At their base the lumen is large and the walls smooth; at the tip the walls are deeply pitted. This sometimes gives the appearance in section of fingers, often fan-shaped. The extreme ends of the apical pits in many cases extend to almost the outer surface of the cell wall and a slight scratch opens an avenue to the interior of the cell. Unless very careful focusing is done with the use of the oil immersion, it is almost impossible to tell whether these pits do not actually exist as openings thru the cell walls. Below the palisade

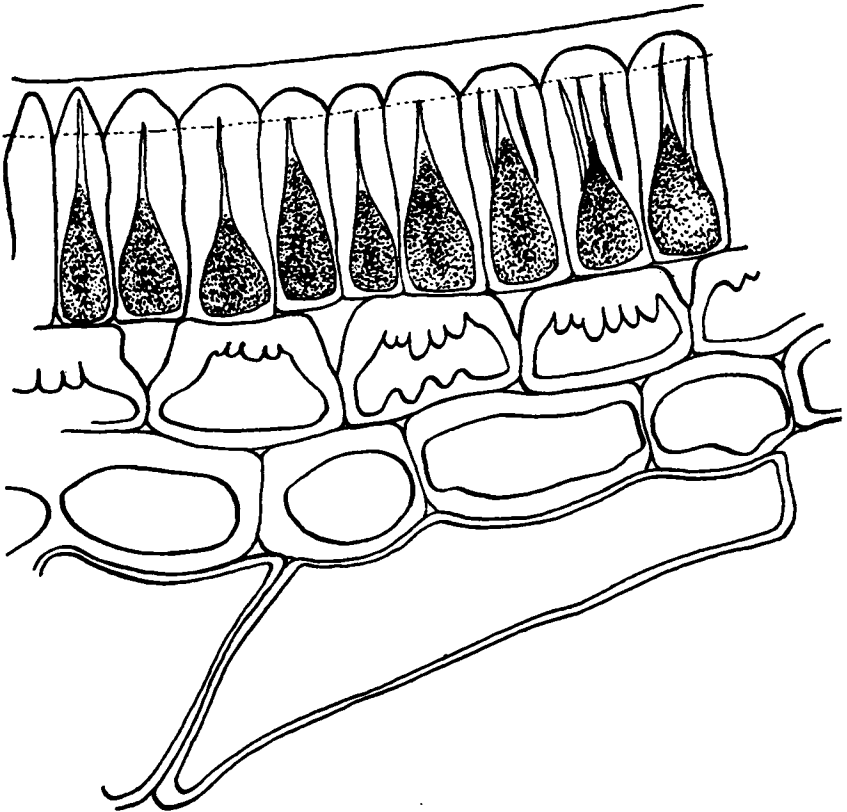


Fig. 5. Detail of seed coat of alfalfa showing palisade cells in longitudinal section.

is another layer of cells one to several cells thick. These cells are also thick-walled with deep corrugated pits. The lower cells are loosely joined together and are separated frequently by air spaces. In sectioning these often tear apart. The corrugations in these cells in cross section appear fan-like.

Along the tip of the palisade cells appears what is commonly known as the light line. This is generally considered of different composition than the rest of the cell wall. Part of this optical illusion is no doubt due to the presence of the cuticle. The papillated nature of the surface of the palisade layers permits the waxy cuticle to extend deep into the crevices between the ends of the palisade cells. In cross section this gives a different refraction of light than the rest of the cell wall.

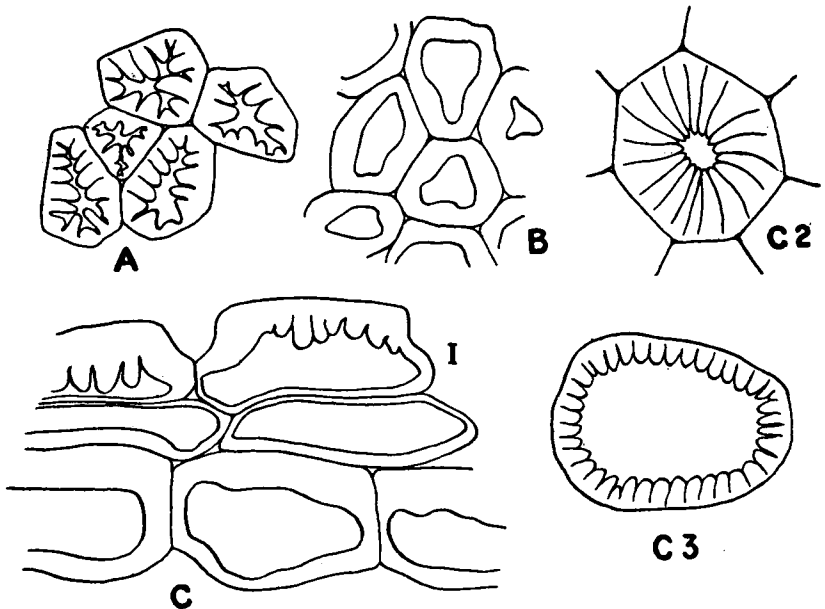


Fig. 6. Detail of cells of seed coat of alfalfa.

- A. A cross section of palisade cells at tip showing convolutions in wall caused by pits.
 B. Cross section of palisade cells at base.
 C. Section of cells below the palisade showing thickened walls and pits.
 C2 and C3. Transverse sections of pitted cells shown in C1, cut at different levels.

WATER INTAKE.—The water intake of the seed thru its seed coat is apparently not governed by the cuticle. Seeds were strongly illuminated from a micro arc lamp and scraped with a sharp knife needle while being observed thru the microscope, magnification 85X. In this way the cuticle could be carefully pared away without cutting into the cells below. Such treatment produced no effect on the rate of water intake. Where, however, this planing process was carried down thru the tips of the palisade cells, seeds took up water rapidly, swelling occurring in a few seconds. Where the palisade was punctured by a stab of a fine needle the same was true, local swelling being quickly manifest by a swollen bump at the point of puncture.

Various dyes were applied to impermeable, permeable, and treated seed, with a view to tracing the water route into the seed and determining the speed of entrance.

Gentian violet, acid fuchsin, methyl green, safranin, haematoxylin, methyl blue and eosin were used. All of these but safranin stained the seed readily.

The cuticle takes in the dye rapidly. However, short periods of soaking, that is ten or 15 minutes, produce no penetration with aqueous dyes beyond the depth of the cuticle. When such dyed seed were dried and examined with 85 magnification under strong illumination, the stained cuticle could readily be planed off, revealing the yellow palisade below. Further, seed stained with gentian violet, acid fuchsin, and eosin remained brilliant after the seed had been soaked 24 hours in clear water. Methyl green and haematoxylin, however, faded. The dyed seed frequently had a spotted or mottled appearance as tho certain sections of the cuticle were more permeable to the dye than others. On germination the cuticle which had been dyed, readily showed expansion cracks, much like a crumpled layer of wax. When the tips of the palisade cells were planed off even tho slightly, the entrance of the dye into the capillary pits of the lumen could be traced, not alone in section but it could be seen by external observation.

The permeable seed showed the passage of the dye into the palisade, as a slow infusion thru cuticle and palisade. The treated seed manifest this penetration.

No mechanical or structural difference can be observed in permeable and impermeable seed. That there is some change in the chemical composition of the palisade following heating is indicated by the ready penetration of dye in treated seed. The only positive reaction with any of the various reagents tried was with methylene blue. Altho this is not conclusive, it would indicate pectin-like structure as suggested by Martin in the case of sweet-clover seed. The behavior of the cuticle to dyes indicates a homogeneous film over the surface of the seed, and its reaction to Sudan III indicates its suberized nature.

THE EFFECT OF HEAT ON IMPERMEABILITY OF ALFALFA SEED

No method now in use for causing impermeable seeds to become permeable is entirely satisfactory. The use of scarification, acid or other wet treatments has decided disadvantages both in manipulation and in effect on the permeable seeds.

Dry heat as a means of increasing permeability has been given little attention. With the idea that such treatment might be effective, a series of tests was started, whereby alfalfa seeds were exposed to different degrees of dry heat. Temperatures of from 35 to 80 degrees C. were used and the seeds were heated for different periods of time.

In Table 22 the results of these tests are tabulated. All the seeds used were from one lot of commercial seed that before the treatment by heat germinated 69 percent with an impermeable seed content of 29 percent. The results uniformly indicate

that a large percentage of impermeable seeds will take up water and germinate immediately after being heated in a drying oven; that the degree of heat necessary to cause this change is not injurious to previously permeable seed; and that no deterioration in treated seed takes place within a year. This method has the advantage of being easily carried out and of causing no loss by broken or over-treated seed.

Table 22.—Effect of Dry Heat on Permeability of Alfalfa Seed.

Temp.	1 hr.		2 hrs.		3 hrs.		4 hrs.		5 hrs.		6 hrs.		7 hrs.		8 hrs.	
	G	I	G	I	G	I	G	I	G	I	G	I	G	I	G	I
35	70	25	67	32	67	25	71	23	68	22	67	25	62	35	61	33
45	67	27	74	23	67	25	72	16	70	20	65	28	61	26		
50	80	16	72	20	67	24	74	18	81	13	80	13	79	13	82	8
55	79	13	78	13	81	10	80	13	92	5	83	9	84	7	86	9
60	82	12	80	14	81	12	83	10	85	8	84	8	85	8	82	12
65	88	10	86	8	91	5	86	9	94	3	81	15	88	6	86	9
70	83	11	87	10	89	8	89	7	87	8	83	9	84	11	86	6
75	84	10	88	7	89	8	90	6	88	8	94	5	84	8	86	9
80	87	9	89	6	84	11	84	6	85	10	81	15	89	11	88	7

"G" has been used to designate percentage of germination.

"I" designates percentage of impermeable seed.

Temperatures below 50 degrees C. have little effect on impermeable seeds even when continued for eight hours. The best results with this lot of seeds was secured at 75 degrees C. for periods varying from 3 to 6½ hours.

Following the preliminary work on this one sample, thirty lots of seed, each of which had a high impermeable-seed content, were subjected to dry heat. As low a degree of heat as could be used for the shortest possible time seemed to be the most desirable combination.

Heat is somewhat injurious to weak seeds. This, however, is not serious since it is not likely that such seeds would produce plants under field conditions. A temperature of 60 degrees C. for two hours gave good results. In most cases a small percentage of seeds remained impermeable. Table 23 illustrates the results of this trial for half of the samples treated.

Table 23.—Effect of Dry Heat on Impermeable Seed.

Untreated		2 Hours at 60°C. Dry Heat		
Germination	Impermeable	Germination	Impermeable	Live seed
42	54	91	5	96
41	53	85	7	93
35	63	95	4	99
49	48	95	4	99
59	26	91	1	92
76	18	83	1	84
69	29	94	2	96
47	46	94	0	94
46	38	81	14	95
72	24	94	2	96
72	21	86	5	91
60	29	87	11	98
68	27	88	8	96
73	23	84	10	94
69	21	90	6	96
56	36	85	6	91
68	28	90	6	96
55	38	82	5	87
74	15	89	3	92

It will be seen from the above table that 60 degrees C. for two hours has not decreased the percentage of live seed. Laboratory tests one year after treatment show that there has been no deterioration in the quality of the treated seed.

It would be easier to devise a practical method for treating large quantities of seed by heat if the time required could be made very short. In order to ascertain if a higher temperature for a shorter period is just as effective as 60 degrees C. for two hours, a series of tests was made at 70 degrees, 80 degrees, 90 degrees and 100 degrees C. The series at 80 degrees C. seemed to be the most successful. Table 24 shows that one hour at 80 degrees C. gives practically the same results as two hours at 60 degrees C.

Table 24.—Effect of 80°C. Dry Heat on Impermeable Seeds of Alfalfa.

Untreated		80°C.											
		1 hr.		2 hr.		3 hr.		4 hr.		5 hr.		6 hr.	
Ger.	Imp.	Ger.	Imp.	Ger.	Imp.	Ger.	Imp.	Ger.	Imp.	Ger.	Imp.	Ger.	Imp.
56	42	83	9	93	4	96	2	94	3	91	5	93	4
65	26	78	11	83	7	81	6	86	7	89	2	70	4
72	22	79	15	91	1	90	3	92	2	93	1	94	0
69	29	87	9	89	6	84	11	85	6	85	10	81	5
Average													
65	30	82	11	89	4	88	5	89	4	89	4	84	3

The table shows that there is a marked increase in germination resulting from exposing seeds with an average impermeable seed content of 30 percent to 80 degrees C. dry heat, for a period of one hour and that there is a small increase by continuing the heat-

ing process for an additional hour. After this there is little change until a decrease begins for a six-hour heating period. The graph, Fig. 7, illustrates what happens.

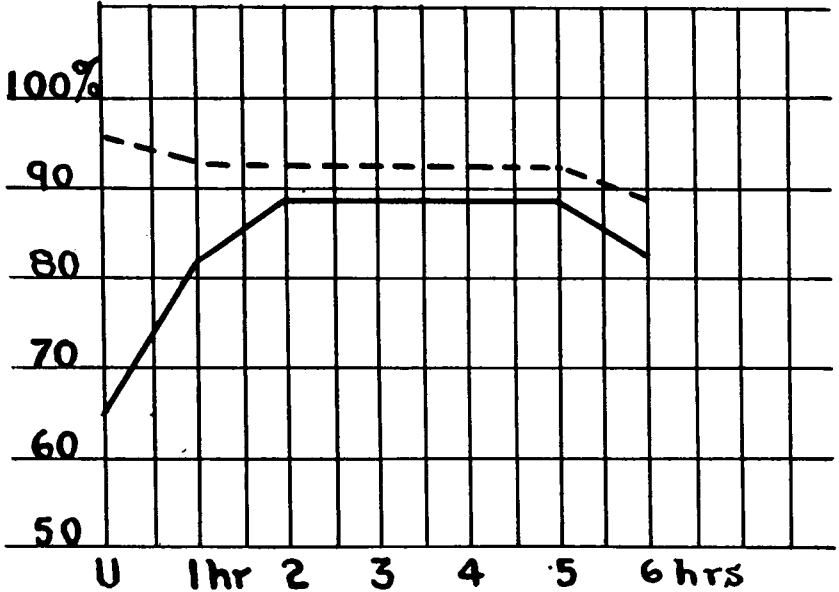


Fig. 7. Effect of dry heat, 80°C., on impermeability and germination of alfalfa seed.

.....-Percentage live seed.
 _____-Percentage germination.

SUMMARY

1. Impermeable seeds of alfalfa when kept between moist blotters at a temperature of 20 degrees C. become permeable in one year or less.
2. The percentage of impermeable seeds in a lot of alfalfa as shown by blotter tests is reasonably constant.
3. The germination of impermeable alfalfa seeds in the soil is increased by high temperatures.
4. A larger percentage of impermeable seed germinates in the soil at high temperatures than in blotters at 20 degrees C.
5. There are more impermeable seeds in hand-threshed than in machine-threshed seed.
6. Machine threshing scarifies seeds having the smallest degree of impermeability.
7. One-half of the impermeable alfalfa seeds when kept in storage become permeable in three and one-half years.
8. All impermeable seeds of alfalfa in storage become permeable in 11 years.
9. There appears to be no relation between altitude and production of impermeable seed.
10. The percentage of impermeable seed in machine-threshed lots varies from year to year for a given field.
11. The percentage of impermeable seeds increases with maturity.
12. The percentage of impermeable seeds varies for individual plants.
13. Individual plants produce seeds varying in permeability in successive years.
14. Individual stems of one plant produce seeds with a different percentage of impermeable seed than other stems of the same plant.
15. The impermeable layer in the seed coat is at the outer end of the palisade cells.
16. Dry heat changes the impermeable layer so that water will pass thru.

BIBLIOGRAPHY

1. Breasola, M.
Staz. Sper. Agr. Ital. 52:193-207.
Rev. in Bot. Abst. 5:112.
2. Annual Reports of the Colorado Seed Laboratory.
1920-27.
3. Eddy, Edgar. Scarification Experiments with Sweet Clover
1926. and Alfalfa. Seed World 20:14.
4. Goss, W. L. Vitality of Buried Seeds.
1924. Jour. Agr. Res. 29:349-362.
5. Guppy, H. B. Studies in Seeds and Fruits. 528 p. illus.
1912. London.
6. Harrington, Geo. T. Agricultural Value of Impermeable
1916. Seeds. Jour. Agr. Res. 6:761-796.
7. Hojesky, J. Hard Husk Clover Seeds.
1921. Jour. Aus. Exp. Sta. Quoted by Pammer, G. and
Schindler, J. 1924. Rep. Fourth International Seed
Testing Congress. 102. 7-12. VII.
8. Love, H. H. and Leighty, C. E. Germination of Seed as Af-
1912. fected by Sulfuric Acid Treatment. Cornell Agr.
Exp. Sta., Bul. 312:293-336.
9. Lute, Anna M. Some Notes on Hard Seed in Alfalfa.
1923. Proc. Assoc. Official Seed Analysts. 14:40.
10. ——— Alfalfa Seeds Made Permeable by Heat.
1927. Sci. n. s. 65:166.
11. Schmidt, David. Legume Seed Treatment to Decrease Hard
1924. Seed Content. Seed World. 15:22.
12. ——— Work on Hard Seed Problem to Date.
1926. Seed World 19:9.
13. ——— Hard Seed Question at Present.
1926. Seed World 20:8.
14. Staker, E. V. The Effect of Dry Heat on Alfalfa Seed and
1925. Its Adulterants. Jour. Am. Soc. Agr. 17:32-40.
15. Stewart, Geo. Color in Alfalfa Seed Is Important.
1926. Seed World. 19:12.
16. ——— Effect of Color of Seed, of Scarification, and
1926. of Dry Heat on the Germination of Alfalfa Seed and
of some of its Impurities. Jour. Am. Soc. Agr.
18:743-760.
17. Thornber, J. J. Some Practical Suggestions Concerning Seed
1904. Germination. Ann. Rept. Arizona Agr. Exp. Sta.
1904.
18. White, Jean. Proc. Royal Soc. of Victoria 21: pt. 1.
1908-1909.