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The Effect of Alternate Freezing and Thawing on Impermeable Alfalfa and Dodder Seeds

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THE EFFECT OF ALTERNATE FREEZING AND THAWING ON IMPERMEABLE ALFALFA AND DODDER SEEDS

A THESIS
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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS

BY
A. R. MIDGLEY
APRIL 1926
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EFFECT OF ALTERNATE FREEZING AND THAWING ON IMPERMEABLE ALFALFA AND DODDER SEED.

By A. R. Midgley.

It is surprising to know the small amount of work that has been done on alternate freezing and thawing of seeds. Many experiments, however, have been conducted to see the effect of very low temperatures on seeds and to notice the effect it had on their vitality. Some experimenters subjected seeds to temperatures as low as liquid hydrogen with very interesting results but in very few cases, if any, was the effect of alternate freezing and thawing studied.

The work that follows deals directly with the effects of alternate freezing and thawing on impermeable seeds of alfalfa and of dodder. It is known that this seed does not germinate readily; therefore it often lays over winter in the soil. Does this natural freezing and thawing aid or hinder this impermeable seed in its later germination? This has been the main question kept in mind throughout this experiment.

Review of Literature.

Before discussing the literature a clear definition for impermeable seed should be understood. Numerous investigators have applied such terms as "hard seeds", hard seed coats and impermeable seeds. Harrington(20) states that "impermeable seeds are seeds whose coats are impermeable to water at temperature favorable for germination". This term together with the definition here given, is more appropriate than "hard coated" seeds as it implies the fact that they are capable of becoming permeable.
When the seeds do become permeable the readily absorb water, swell and become soft. Until this condition comes about, they cannot germinate no matter how favorable the conditions may be.

What is the cause of this impermeability?

Many suggestions have been given. Crocker(S) was one of the first to show that impermeability was not due to the embryo, but to the seed coat itself. Rees(32) states that a cuticle is secreted outside of the palisade cells. She also believes it is formed by the laying down of a waxy or fatty substance in the cell wall and that the cells are permeated through and through with these particles of wax. Druvell(17) says it is due to the presence of a cuticle developed while ripening and that it's presence or absence will determine its age or the length of time it will remain in the soil.

Harrington(26) in his work with impermeable clover seed, gives some very interesting statements as to the factors which influence this impermeability. A summary of his works show that:

I. It is not due to soil. He used soils ranging from heavy clay to light sand and obtained similar results.

II. The season in which they are grown had little or no effect. The late or early grown seeds gave the same results.

III. The effect of climate made very little difference. He used seeds from different states having different climates.

IV. The color of seed had very little influence, provided they were all mature and received the same treatment. He concludes that the exact cause is yet unknown.
Can Impermeable Seeds be Readily Distinguished?

This is another important and interesting question for farmer and seedsmen. According to Harrington(27) it is impossible to distinguish the impermeable seeds except by testing their ability to absorb water at germination temperatures. Furthermore he claims that "it is impossible even approximately to estimate in advance, the proportion of impermeable seeds in any given lot which will germinate in any given time."

Rest Period of Impermeable Seeds—Crocker(8') did some interesting work in 1925 on the physiology of germination with seeds. He studied their rest period very closely and concluded that all seeds, at some stage of development, underwent a rest period. In some cases this rest period was only transient, being overcome when moisture, oxygen and proper temperature were supplied, but in other cases certain seeds did not readily respond to these conditions. He states that their embryos were capable of germination, but the structure surrounding them forced them into a rest period, that is their coats would inhibit or restrict moisture and gases until this structure is broken they will remain in the rest period. Many examples can be given to illustrate this long rest period. Harrington(20) gives examples of impermeable seeds that retain their vitality for many years, some for at least eighty years. Beadden(22) in his studies with alfalfa showed that sound seeds under fair conditions maintain their vitality for 23 years. Kuntze(26) states that hawthorn will grow only after being in the soil from 1 to 3
years. These few examples are mainly to show the relation between the impermeability of seeds and their rest periods.

Relation of Color in Alfalfa Seed to It's Vitality--- Most samples of alfalfa seed show a great variation in color. Very little work has been done in determining which color has the greatest vitality or the largest number of hard-coated seeds. Kajamus(2/) found that the yellow and light green seed had a greater vitality than the brown. Staker(3/) worked with seven different color samples and found that the nearer the seed approached the natural color the more hard coated seeds were found and the greater its vitality.

Effect of Sulfuric Acid on Impermeable Seed--- It has long been known that concentrated sulfuric acid is valuable in destroying the hard seed coat so that they may become permeable and germinate. It is a very valuable method in getting a quick germination test for the impermeable seed. The writer found, with his sample of hard alfalfa seed, that twenty minutes in concentrated sulfuric acid gave nearly one hundred per-cent germination. The dodder gave seventy-five per-cent when the seeds were treated for fifteen minutes, and later given an extra five minutes.

Effect of Scarification on Impermeable Seed--- Graber(18) worked out an interesting table on the germination of seeds before and after scarification. The results showed a very marked decrease in the number of impermeable seed and a greater increase in the germination of the scarified sample. He also claims that when any lot of alfalfa seed contains over 10% hard seed it should first be scarified before planting. Many seedmen scarify their
seed but fail to sell or plant it the same year. In this case, much of the scarified seed loses its ability to germinate. He therefore stresses the importance of delaying the process until a few months before planting.

**Effect of Heat on Impermeable Seed**—There has been considerable work and speculation as to the effect of dry heat on alfalfa seed. It has long been noted that when this seed was submitted to a slight amount of heat and then submitted to higher temperatures, the seed remained viable. This may suggest that the first heat decreased the water content by drying, thus making them more resistant to the higher temperatures. Harrington and Fodari has shown that high temperature with slight fluctuations gave a lower per-cent of hard seed. He suggests that it may be due to the melting of the wax in the seed coat. These results show that dry heat can be used to advantage in reducing the number of impermeable seeds.

**Effect of Extremely Low Temperature on Seeds**—As early as 1887 De Condolle subjected seeds to the low temperature of liquid hydrogen -250° to -255°C. for six hours and when they were tested many of the seeds gave an almost perfect and complete germination. Seeds containing much moisture, however, were killed. Becquerel found a close relationship between the moisture present in seeds and their ability to withstand the cold. He further states that whenever seeds contain over 12% moisture these low temperatures were fatal. The cause of this, according to Adams, is the withdrawal of the moisture from the cells, and "if there is sufficient gas and water present the protoplasm and nucleus becomes disorganized."
Effect of Alternate Freezing and Thawing. - Harrington (20) has done the only work, of which the writer is aware, regarding the effect of alternate freezing and thawing on the germination of alfalfa seed. He found that freezing caused many more seeds to germinate, but many of these seeds which had previously softened or began to germinate were later killed by the next freezing. Impermeable seed often remain in the ground in a freezing condition without injury and a high percentage will germinate the following spring unless some of them began to germinate during the warm weather in winter. If such occurs "the seedlings produced during the winter is liable to be killed by subsequent freezing."

EXPERIMENTAL WORK

Purpose -

It has long been known that the average sample of commercial alfalfa and clover seeds contain many impermeable seeds. Much experimental work has been done in trying to reduce this impermeability and cause a higher percentage of these seeds to germinate. It has been generally accepted, without much experimental data, that alternate freezing and thawing will reduce very greatly this impermeability. The main purpose of this experiment is to find out just what effect alternate freezing and thawing has on impermeable alfalfa and dodder seeds. It was also conducted to find out, as near as possible, what effect natural freezing and thawing has on the impermeable seed that remains in the ground during the winter and early spring in a freezing and thawing condition.

Seed used - Alfalfa.

The alfalfa seed used was a composite sample obtained from various growers in Duchesne County. A representative proportion sufficiently large for the entire experiment was placed in a separate package. The
impermeable seed from this sample was used throughout the experiment.

The colored samples used, were taken from different sources and were carefully separated out, by the writer, into six fractions. The colors used in the tests were: light green, dark green, shrivelled green, light brown, dark brown and shrivelled brown.

Dodder used

Dodder seed was also used in the experiment as it has long been known to remain in the ground for many years and germinate very slowly. In order to obtain the dodder seed, samples of alfalfa with heavy traces of dodder, were obtained from several seed houses. The dodder seeds were then carefully picked out by hand. The large seeded dodder, was the only kind used in the experiment as it is found more frequently in commercial alfalfa seed. Its size, due to the fact that, the small seeded varieties can be separated out with proper screens, also makes it easier to study the effect of freezing and thawing on its impermeability.

Method of Obtaining Impermeable Alfalfa Seed

In order to expedite the process in obtaining impermeable seed the following arrangements were made. A large wooden box six feet long, sixteen inches wide and six inches deep was used. This wooden box was then filled about three-fourths full of sand. Large strips of blotting paper was then placed on the sand. In order to keep the sand and paper wet, a few holes were bored in the bottom of the box. It was then placed inside a zinc box which was slightly larger and water-tight. The metal box was kept full of water which in turn kept the soil and paper wet. Part of the original sample of alfalfa seed was sprinkled over the wet filter paper. It was then covered with cardboard and the seeds allowed to germinate for ten days. At the end of ten days, all the seeds that did not germinate were scraped off, placed in a package and labeled Impermeable
alfalfa seed." This process was repeated until many thousands of impermeable seeds were obtained. The impermeable seed used in the experiments were always taken from this package.

**Methods of Freezing and Germinating**

**Freezing.** Two refrigerating rooms of the Dairy Department were used in freezing the seeds. The first room had an outside entrance and the door was opened often during the day, therefore its temperature varied greatly. It was found, however, to fluctuate between 0° C. to -10° C. (32° F. to 14° F.). The second room opened into the first, therefore its temperature remained more constant and colder. Thermometer readings showed that it varied between -15° to -20° C. (5° F. to -4° F.).

**Germinating.** In order to germinate the seeds before or after freezing they were placed on moistened filter paper laid over wet sand. Tin plates about six inches in diameter were used to hold the sand. Throughout the experiment the sand and paper was kept moist with water to facilitate germination. The germinating room was kept about 23° C. or 70° F. Unless otherwise stated, impermeable seed was always used and one hundred seeds set on each pan.

**EXPERIMENTAL RESULTS**

**Effect of Alternate Freezing and Thawing on Impermeable Alfalfa Seed with Different Temperatures of Freezing.**

Does the degree of freezing have any influence in reducing the number of impermeable seeds in alfalfa? To find out what influence this had, the two refrigerating rooms were used for freezing the seeds. Ten plates containing one hundred seeds on each, were used in the experiment. The plates were carefully labeled and half of them, or five plates, were used for each room. At the close of each week they were placed in their respective freezing rooms and frozen for about thirty six hours (Saturday
night to Monday morning). They were removed Monday morning of each week and placed in the germinating room where they were allowed to germinate for six days. Saturday of each week, the number that had germinated in each pan was counted and recorded. The remaining seeds were then returned to their respective freezing rooms and the above process again repeated each week. The experiment was started January 6, 1926. The seeds were always kept moist or wet.

Tables I and II show the number germinating after each freezing.

Table I shows the number that germinated after each freezing in the first room with an average freezing temperature of \(-5^\circ\) C. Table II shows the number that germinated after each freezing in the second room with an average freezing temperature of \(-15^\circ\) C.

Table I. Effect of alternate freezing and thawing on impermeable seed with an average freezing temperature of \(-5^\circ\) C.

<table>
<thead>
<tr>
<th>Plate</th>
<th>1/12</th>
<th>1/20</th>
<th>1/26</th>
<th>2/1</th>
<th>2/6</th>
<th>2/12</th>
<th>2/19</th>
<th>2/27</th>
<th>3/5</th>
<th>3/13</th>
<th>3/20</th>
<th>3/27</th>
<th>4/3</th>
<th>4/10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>52</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>D</td>
<td>21</td>
<td>15</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>Average</td>
<td>22.2</td>
<td>7.4</td>
<td>4.0</td>
<td>3.9</td>
<td>2.6</td>
<td>.6</td>
<td>2.2</td>
<td>3.2</td>
<td>.6</td>
<td>1.4</td>
<td>1.6</td>
<td>3.8</td>
<td>3</td>
<td>1</td>
<td>57.2</td>
</tr>
</tbody>
</table>

Table II. Effect of alternate freezing and thawing on impermeable alfalfa seed. (Freezing temperature \(-15^\circ\) C.).
Table II.

<table>
<thead>
<tr>
<th>Plate No.</th>
<th>1/12</th>
<th>1/20</th>
<th>1/26</th>
<th>2/1</th>
<th>2/6</th>
<th>2/12</th>
<th>2/19</th>
<th>2/27</th>
<th>3/5</th>
<th>3/13</th>
<th>3/20</th>
<th>3/27</th>
<th>4/3</th>
<th>4/10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>23</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>C</td>
<td>24</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>23</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>54</td>
</tr>
<tr>
<td>E</td>
<td>25</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>56</td>
</tr>
</tbody>
</table>

Average: 25.0 4.6 4.8 4.6 1.8 0.8 1.0 1.2 2.0 1.2 2.6 2.6 3.4 1.8 58.6

An examination of the data given in tables I and II seems to indicate that the degree of freezing has no effect on the number germinating or on the rate of germination. The average number germinating in each case was about the same for both temperatures. The nature and equipment of this experiment was such that only two different temperatures could be used, but the difference in temperatures was great enough to indicate that greater extremes (as long as the seeds were completely frozen) would have little if any influence.

Probably the most striking result in this experiment is the small effect that alternate freezing and thawing has on impermeable alfalfa seed. A large number (Average 23%) was found to germinate after the first freezing, but the number germinating for each subsequent freezing was very small, yielding an average germination of 2.5 for each week.

The hardness of alfalfa seed to freezing and thawing temperatures is also very noticeable. During the sixteen weeks of alternate freezing and germinating, none of the seeds were found to be dead. All seeds that became permeable readily germinated. It would have been interesting to note how many more freezings would have been necessary to get the remainder of the sample to germinate, but this experiment had to be completed by April 15.
Effect of Time on Alfalfa Seed while in a dry Condition, with and without Dry Freezing.

It has long been known that alfalfa seed will become permeable with age, if kept long enough, even in a dry condition. The main question confronting the writer in his experiments was just how rapid these seeds became permeable with age. This factor may influence the results of later experiments as some of the later experiments were carried on several months after the first. To find out what effect time had in reducing the impermeability of alfalfa seed, the experiment which follows was performed. In this experiment, the original sample, from which the impermeable seeds were taken, was also used. It will be labeled "Sample Seed" in order to distinguish it from the "hard-Coated" or impermeable seed.

A small portion of "Sample Seed" and impermeable seeds were placed in separate dry sacks, carefully labeled and subjected to two or three dry freezing. Three hundred seeds of each were then set out to germinate for ten days. A similar portion of "Sample" and impermeable seed were treated in like manner except that they were kept under room temperature and not subjected to any cold.

The effect of age on alfalfa seed while in a dry condition, with and without dry freezing is shown in the table IV. Only two dates of germination are given. The date when the experiment began and when it was concluded.

Table IV. Effect of time on alfalfa seed, while in a dry condition with and without dry freezing.

<table>
<thead>
<tr>
<th>Description of Sample</th>
<th>Oct. 15</th>
<th>Apr. 15</th>
<th>No. Increase over non-frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample seed (no freeze)</td>
<td>16</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>&quot;&quot; (dry freeze)</td>
<td>41</td>
<td>49</td>
<td>8</td>
</tr>
<tr>
<td>Impermeable seed (no freeze)</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>&quot;&quot; (dry freeze)</td>
<td>22</td>
<td>30</td>
<td>8</td>
</tr>
</tbody>
</table>
An examination of Table IV shows that alfalfa seed does become permeable with age. It is very interesting to note, that the number becoming permeable during the six months (Oct. 15 to Apr. 15) was nearly the same in each case, producing about 8 permeable seed for that period of time.

Dry freezing is shown to have some value in reducing the number of impermeable seeds. This increase in number which dry freezing causes is found to be practically the same in the "sample" as well as the impermeable seed. From the above it would indicate that the seeds which were kept in the store houses where they are subjected to the natural changes in temperature, would have their impermeability reduced.

Different Durations of Wet Freezing.

Does the length of time a seed is frozen influence its impermeability? In some of the experiments described in this paper, the seeds were not frozen for the same length of time and the question may be asked "would not the length of time at which the seeds were frozen influence the results?" To find out what effect different duration of freezing have on impermeable alfalfa seed, five samples were frozen for: one hour; two hours; four hours; eight hours; and two months. A check sample was also used to note the effect of an unfrozen sample. No less than one hour could be used as it required that length of time to freeze the seeds completely. The temperature of the freezing room was -15° C. One hundred seeds in each of three pans were used in each case. They were prepared in the ordinary way for germination with the seeds thoroughly wet. They were placed in the freezing room for the desired length of time, frozen only once and removed to the germinating room. After six days the number germinated were counted and recorded.

Table V gives the average germination on a one hundred seed basis.

Table V shows that there is practically no difference due to the length of time the seeds are frozen. The check shows that five seeds had become permeable during the process of the experiment. From this it seems
Table V. Effect of different duration of freezing.

<table>
<thead>
<tr>
<th>Duration of Freeze</th>
<th>Number Germinating</th>
</tr>
</thead>
<tbody>
<tr>
<td>One hour</td>
<td>21</td>
</tr>
<tr>
<td>Two hours</td>
<td>22</td>
</tr>
<tr>
<td>Four hours</td>
<td>20</td>
</tr>
<tr>
<td>Eight hours</td>
<td>25</td>
</tr>
<tr>
<td>60 days</td>
<td>24</td>
</tr>
<tr>
<td>Check (no freeze)</td>
<td>5</td>
</tr>
</tbody>
</table>

that the main point is to get the seeds completely frozen, otherwise no more seeds would germinate than the check indicated, at that particular time at least. It is also interesting to note that the average number germinating is practically the same as is shown in other experiments after the first freezing, whether the seeds were in a wet or dry condition.

**Effect of Different Lengths of Thawing with Subsequent Freezing.**

Does the length of time that impermeable seeds are allowed to thaw, between freezings, have any effect on germination? It is thought that if alfalfa seed is sown in early fall, the impermeable seed that did not germinate in the fall, would do so the next spring, after being subjected to the natural freezing and thawing that goes on in the soil during the winter and early spring. It is thought that this natural freezing and thawing breaks up this hard seed coat and causes the seed to become permeable. Crocker (8) at Yonkers, N. Y. claims that if impermeable seed is sown in the fall they will soften and a higher yield will be obtained. If freezing does cause a larger number to become permeable the question that arises is: will not the seed that becomes permeable and takes up water, be killed with the next freezing? During the warm winter months and early spring there are different lengths of thawing during the day with a heavy freeze the following night. To find out, as near as possible, what this effect has on impermeable seed, this experiment was conducted.
To insure greater accuracy in the results, triple tests were run and repeated on three different dates. Four periods of thawing were used as follows: 2 hours, 4 hours, 8 hours, and 15 hours. After each thaw they were frozen and this was repeated for five consecutive days in the one case and ten consecutive days in the other. With the exception of the 15 hour thaw the seeds were removed from the refrigerator each morning at eight o'clock. The 2 hour thaw samples were replaced at ten o'clock; the 4 hour samples were replaced at twelve o'clock, and the 8 hour thaw samples were replaced at four or five o'clock. The fifteen hour thaw samples were left in the germinating room over night from 5 P.M. to 8 A.M. and frozen during the day. After the five and ten day period of treatment the seeds were allowed to germinate for six days.

Table VI shows the number germinating and the number dead after the 5 day treatment. The results for the 10 day treatment are given in Table VII.

Table VI. Effect of Different Lengths of Thawing, each day for five consecutive days. (Impermeable seed used).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Germ.</th>
<th>No. Dead</th>
<th>Total No. Softened</th>
<th>No. Hard Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hour thaw</td>
<td>21</td>
<td>9</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>4 hour thaw</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>8 hour thaw</td>
<td>6</td>
<td>24</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>15 hour thaw</td>
<td>1</td>
<td>31</td>
<td>32</td>
<td>68</td>
</tr>
</tbody>
</table>
Table VII. Effect of Different Lengths of Thawing, each day for ten consecutive days. (Impermeable seed used.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Germ.</th>
<th>No. Dead</th>
<th>Total No. Softened</th>
<th>No. Hard Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hour thaw</td>
<td>8</td>
<td>23</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td>4 hour thaw</td>
<td>6</td>
<td>27</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>8 hour thaw</td>
<td>3</td>
<td>29</td>
<td>32</td>
<td>68</td>
</tr>
<tr>
<td>15 hour thaw</td>
<td>No sample</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

An examination of the above tables shows that:

1st. As the length of thaw increased the number of dead seeds increased, with a corresponding lower number germinating.

2nd. The total number that became permeable or softened was practically the same regardless of the length of thaw.

By comparison of the two tables it is seen that a two hour thaw for ten days produced as many dead seed as the eight hour thaw for five days. In addition to this it is also noticed that the ten day treatment reduced the number of hard seed on an average of only two. This seems to indicate that the extra five days of alternate freezing and thawing had very little influence in reducing the impermeability. In general, the results of the above experiment, show that the seeds that became permeable by freezing and began to germinate, are later killed by the subsequent freezing.

The above results are also substantiated by a small experiment that was started on March 1st. Three pans containing 100 impermeable alfalfa seed in each, were moistened and the pans were set out in an orchard exposed to natural freezing and thawing. On March 31st. the seeds were examined. It was found that an average of thirty seeds in each pan had become permeable but they were dead as none of them germinated after being in the germinating room for five days.
Effect of Alternate Freezing and Thawing on Alfalfa Seeds of Different Colors.

It is generally supposed that the color of alfalfa seed is related to its vitality. If this is the case, which color has the greatest vitality and which color contains the greatest number of impermeable seed? In order to get the number of impermeable seed for each color fraction, the following experiment was carried out: Alfalfa seed which had previously been separated into six colors, were counted and set to germinate in the usual manner for six days. The number germinating and the number of hard seeds remaining, for each color are shown in columns 1 and 2 of Table VIII.

The number of impermeable seed, in each color fraction was so small that no attempt was made to separate them and use the impermeable seeds separately. In order to see the effect of freezing and thawing on the different color fractions samples of each color were run for five consecutive days. Each day they were allowed to thaw for the desired length of time and then returned to the freezing room. Group A was allowed to thaw for four hours each day and Group B for eight hours.

The results in percentage are recorded in Table VIII.

Table VIII. Effect of alternate freezing and thawing on alfalfa seeds of different colors. (Freeze and thaw for 5 consecutive days).

<table>
<thead>
<tr>
<th>Colors</th>
<th>Check no freeze</th>
<th>4 hour thaw</th>
<th>8 hour thaw</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. Brown</td>
<td>52 8 40</td>
<td>8 4 88</td>
<td>4 1 95</td>
</tr>
<tr>
<td>L. Green</td>
<td>40 57 3</td>
<td>14 54 32</td>
<td>6 34 60</td>
</tr>
<tr>
<td>D. Green</td>
<td>32 10 58</td>
<td>10 4 86</td>
<td>4 2 94</td>
</tr>
<tr>
<td>D. Brown</td>
<td>32 5 63</td>
<td>4 0 96</td>
<td>2 0 98</td>
</tr>
<tr>
<td>Sh. Green</td>
<td>27 20 53</td>
<td>4 10 86</td>
<td>4 8 88</td>
</tr>
<tr>
<td>Sh. Brown</td>
<td>11 4 85</td>
<td>0 2 98</td>
<td>0 0 100</td>
</tr>
</tbody>
</table>
An examination of the data given in Table VIII shows that the green contained more impermeable seeds than the brown. This is to be expected as the green color approaches more nearly the normal bright yellow color than does the brown. It is a noticeable fact that before freezing the light brown gave a higher germination than the light green. This is due to the large percentage of impermeable seed in the latter. The brown seeds appear to have less vitality than the green seeds, for in every case freezing and thawing produced a larger number of dead seed in the brown than in the respective green samples.

Effect of Continued Moisture on Impermeable alfalfa Seeds without Freezing.

If impermeable alfalfa seeds are kept in a moist condition will they not become permeable without freezing? If so at what rate do they become permeable? In the experiment on alternate freezing and thawing of alfalfa, seed it was noted that they became permeable very slowly. The effect of freezing in reducing the impermeability appeared to be very slight. The question then arose, would not the same seeds have germinated without freezing if they were kept in a moist condition for the same length of time? With this in view the following experiment was conducted: Four plates containing 100 seeds each were used. In half of these the seeds were placed in soil and the others on moist filter paper in the usual manner for germination. One of the reasons soil was used was to check it with the ordinary method of filter paper. In preparing the soil sample, two of the plates were partly filled with soil. The soil was then moistened and one hundred seeds were scattered over the moist soil in the two pans. Just enough soil was then sprinkled over the seeds to cover them. The seeds were always kept moist and at the end of each week they were examined to see if any had germinated. The only dates recorded are those on which some seeds germinated. The experiment was started Jan. 4, 1926. Tables IX and X give the results.
Table IX. Time required for alfalfa seed to germinate without freezing. (In moistened soil).

<table>
<thead>
<tr>
<th>Dates when seeds were found to germinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate No.</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>Average</td>
</tr>
</tbody>
</table>

Table X. Time required for alfalfa seed to germinate without freezing. (On moistened filter paper).

<table>
<thead>
<tr>
<th>Dates when seeds were found to germinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate No.</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>Average</td>
</tr>
</tbody>
</table>

On examination of Tables IX and X it is surprising to note the large total number germinating. It is regrettable that more seeds were not used so that the data could be more accurate. However, the data presented seem to indicate that the results from alternate freezing and thawing, (as is given in Tables I and II), is no more effective in reducing the number of impermeable seed, than when a similar sample of seed is kept for the same length of time in a moist condition.

Further examination will show that a large number germinated about the middle of March. It appears that after remaining in a moist condition for that length of time (65 days in case of Table IX) many more of the seeds became permeable and germinated. It is also interesting to note that this greater number appeared about the same time in both tables and in consecutive order.
Practically no seed germinated until it had been moistened for 10 days. It is also noted that they germinated rather promiscuously, some weeks none germinated at all while at other times large numbers germinated.

Upon further investigation of the above tables it is seen that the seeds on the filter paper gave a higher total germination than those in the soil. The tin covering of soil may have hindered or stopped the growth of the very weakest. This seems to indicate that the actual number that germinate or make their way through even a small layer of soil, is slightly less than when they are germinated on filter paper.

In general, the results seem to be contrary to the general idea that freezing and thawing greatly reduce the impermeability of alfalfa seed. However, freezing with the aid of water seems to expedite germination and causes a more uniform number to germinate each week.

Effect of Alternate Freezing and Thawing on Dodder.

It has long been known that in dodder seed there is a very high percentage of impermeable seed and that the seeds will remain in the ground for many years and germinate a few each year. It was thought that by freezing and thawing, these seeds would become permeable and germinate.

The experiment was started on November 16, 1925. Seven pans having one hundred seeds in each were used in the experiment. They were first placed in the germinating room for six days in order to find out the number that would germinate without freezing. The germinated seed were picked out and counted, and the remaining seeds were frozen one day of each week until April 15, when the experiment was terminated. After freezing they were allowed to germinate for the remaining six days in each week. Table XI gives the results that were obtained from alternate freezing and thawing of dodder seeds once each week for 20 weeks. The only dates recorded are those on which some seeds germinated.
Table XI. Effect of alternate freezing and thawing on dodder seed.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>9</td>
<td>7</td>
<td>16</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>9</td>
<td>17</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>13</td>
<td>21</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>12</td>
<td>19</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>10</td>
<td>17</td>
<td>83</td>
</tr>
</tbody>
</table>

The data presented in Table XI indicate that freezing has very little if any influence in reducing the number of impermeable dodder seed. The greatest number germinating in any case being ten per-cent, and the largest total number softened was only twenty-one percent. This indicates a very high percentage of impermeable seed in dodder. The impermeable seeds are easily distinguished from the dead seed as the latter do not germinate but swell up with water and they are latter attacked by fungus growths if they are not removed.

**Effect of Long Freezings with Subsequent Thawing on Dodder.**

If dodder seed frozen for a long period will it not become more permeable than when frozen for a short period? To see what effect the length of freezing has in reducing the number of impermeable seeds and thereby causing a larger number to germinate, this experiment was conducted: Four plates of dodder seeds were given 5 freezings as follows: From Dec. 17 to Jan. 4; Jan 12 to Jan. 20; Jan. 25 to Feb. 23; Feb. 28 to Mar. 30; and from Apr. 5 to Apr. 10. They were allowed to germinate for five or six days between each freezing. The number germination after each period is given in
Table XII.

Table XII. Effect of Long periods of freezing on dodder seeds. (100 seeds used in each pan).

<table>
<thead>
<tr>
<th>No. Germ. before Freeze</th>
<th>1/10</th>
<th>1/25</th>
<th>2/28</th>
<th>4/5</th>
<th>4/15</th>
<th>Total Germinating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan No. A</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Pan No. B</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Pan No. C</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Pan No. D</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Table XII. shows that the length of time the dodder seeds are frozen has no marked effect in reducing their impermeability. It is interesting to note, however, that the total number germinating in each case is as high as the total number in Table XI, where they were frozen each week, having four times as many freezings. The length of time they are kept moist seems to be the main factor.

Effect of Scarification and Concentrated Sulfuric Acid on Alfalfa and Dodder Seeds.

Due to the large number of seeds that failed to germinate in the samples of seed used in the foregoing experiments, the question arises in regards to the viability of the sample used. To be sure that this lack of germination was due to the impermeability of the seed coats and also to test the viability of the seeds, scarification and the treatment with concentrated sulfuric acid was used.

When the sample of impermeable alfalfa seed was treated for ten minutes in concentrated sulphuric acid, 85 per cent readily germinated. The remaining seed later germinated when they were subjected to the same treatment for an additional 5 minutes. Usually 100 per cent was found to germinate if they were treated for twenty minutes.
When the original sample of alfalfa seed was scarified once (2600 rev. per minute), 95 per cent germinated.

Samples of dodder were scarified once and four times (2800 rev. per minute). With one scarification 16 per cent germinated. Four scarifications caused 25 per cent to germinate. With this result for dodder it indicates either that the seed coat of dodder is not easily scarified, or made permeable, or else the seed is not viable. However, when a similar sample was treated with sulphuric acid, 70 per cent germinated. The dodder seeds were treated with concentrated sulphuric acid for fifteen minutes and then thoroughly washed and allowed to germinate for six days. The seeds that did not germinate were again treated for seven minutes, washed, and again set to germinate. This method always resulted in the highest germination.

**SUMMARY**

I. Alfalfa seed contains a high percentage of impermeable seed.

II. After the first freezing, subsequent freezings and thawings seem to have very little influence in reducing the number of impermeable seed.

III. The first freezing is by far the most effective in this respect. It reduced the number of impermeable seed on an average of 23 per cent.

IV. The degree of freezing has no influence in reducing the impermeability. A temperature of 0° C. is fully as effective as -20° C.

V. When kept in a moist condition for several months as many seeds will germinate without freezing as a similar sample if frozen each week during the same length of time. This would indicate that freezing only expedites the process. The length of time the seeds are kept moist seems to be the more important.
VI. Alfalfa seed becomes permeable with age even when left in a dry condition. However, the rate is very slow when they are stored at room temperature with little or no variation in temperature.

VII. Freezing the seeds in a dry condition seems to be as effective as when the seeds are wet and frozen. This is especially true for the first freezing. It would seem from this, that the warehouse that allows for seasonal changes in temperature would be beneficial in causing alfalfa seed to become permeable.

VIII. The duration of freezing seems to have no influence; as many seeds become permeable and germinated after one hour freezing as when a similar sample was frozen for sixty days.

IX. The duration of thawing with subsequent freezing has very little influence in producing permeable seed, but it has a marked effect on the number of seeds that are killed. The longer the thaw the greater the number of seeds that were found dead with subsequent freezing. This strongly indicates that seeds which become permeable with one freezing are later killed with subsequent freezings.

X. The number of impermeable seeds in alfalfa varies according to the color of the seeds. The nearer the seeds approach the true color of bright yellow the more impermeable seeds were found. The green seed has a higher percentage than the brown.

XI. Alternate freezing and thawing has very little or no influence in reducing the number of impermeable dodder seeds. An average of only 8 per cent germinated after twenty freezings and of this number one-half, or 4 per cent, germinated before freezing.

XII. The alfalfa seed used in the above experiments proved to be 100 percent viable when a test was made with sulphuric acid or scarification.

The dodder seed used was found to be at least 70 percent viable with the sulphuric acid treatment.
Graph I. Showing the number of alfalfa seeds germinating each week, with three different treatments. (Impermeable alfalfa seeds were used).

Legend:

- Number of alfalfa seeds germinating when frozen each week.
- When kept moist without freezing.
- When kept in dry condition without freezing.

Dates when seeds germinated:

- 1/6
- 1/10
- 1/20
- 1/26
- 1/31
- 2/6
- 2/12
- 2/19
- 2/27
- 3/4
- 3/12
- 3/20
- 3/27
- 4/3
- 4/10
- 4/15
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