D. N. Seward

Effect of Sulphuric Acid upon the Seeds of Peas
EFFECT OF SULPHURIC ACID UPON
THE SEEDS OF PEAS

BY

DORIS NAOMI SEWARD

THESIS

FOR THE

DEGREE OF BACHELOR OF ARTS

IN

BOTANY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Doris Naomi Seward

ENTITLED

Effect of Sulphuric Acid Upon the Seeds of Peas

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Arts

Chas. F. Hottes
Instructor in Charge

APPROVED:

HEAD OF DEPARTMENT OF Botany
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The Effect of Sulphuric Acid Upon The Seeds of Peas

I. Introduction

Large numbers of seeds, especially of the legumes, fail to germinate because of impermeable seed coats. When such a phenomenon exists, the period of dormancy is greatly prolonged. Crocker (2) in his article on "The Mechanics of Dormancy in Seeds", states that dormancy is due either to an immature embryo or to so-called hard seed coats, which prevent the absorption of water or of oxygen. It is a question whether the impermeability is due to the cuticle or to the "light zone" of the modified cellulose of the palisade layer.

Seeds have been treated in different ways to increase permeability and thus to increase the per cent of germination. Sulphuric acid has been used and strongly recommended for certain seeds. In 1910 and 1911 Love and Leighty (5) treated several varieties of clover seed and cotton seed with concentrated sulphuric acid, varying the time of immersion. They found that an increased germination was secured treating red, sweet, alsike, Japan and white clover with concentrated sulphuric acid. (184 SpG) They also learned that many varieties of weed seed commonly found in samples of clover or alfalfa seed were killed by the acid treatment. The effect of the treatment was the same in seeds germinated as in seeds dried previous to germination. In the case of the cotton seed, the lint was easily removed by the acid, the per cent germinating was increased, and the rate of germination hastened by the treatment. The fact that different lots of red clover seed varied greatly in permeability and consequently in per cent of germination, was brought out.

V. Arcichovsky (1) has extended our knowledge of the effect of sulphuric acid. He has shown that the weaker and the stronger concentrations of the acid cause less injury than do the intermediate concentrations.

When comparing organic and inorganic acids, C. E. Davenport (3) tells
us that strong inorganic acids act in general more powerfully than organic acids. Poisons may vary in their action, the effect being proportional to the strength of the solution. The strength of action in poisons is governed to a certain limit by the molecular composition.

In 1917, a study of the relation between the swelling of plant tissue and various dissolved substances was made by Stiles and Jorgensen (3). They used distilled water, sucrose, sodium chloride, alcohols, and sulphuric acid. They found that tubers of potatoes and roots of carrots absorb water or swell for some days before equilibrium is reached. In solutions of sodium chloride and sucrose the swelling of potato is reduced with the increase of the concentrations of the solutions. In the case of certain substances which enter the cell such as primary alcohols preliminary swelling takes place in solutions of much higher concentrations than in isotonic solutions as determined by the experiments with sodium chloride and sucrose. Subsequently shrinkage or depressed swelling occurs in all concentrations, which is correlated with the alteration of the permeability of the protoplasm and the death of the tissue. This shrinkage is due to toxic action and not to plasmolysis. They further bring out the fact that in the lower strengths of sulphuric acid the swelling is at first greater than in distilled water, and that acids behave similarly to the primary alcohols, producing the preliminary swelling and subsequent shrinkage due to toxic action.

W. J. V. Asterhout (6) discovered that the effect of acid on permeability varies greatly, not only with the concentration but also with the duration of the exposure. He says, "We may regard the permeability as equal to the net conductance. We may therefore put the permeability at the start as equal $1 - 100 = 0.01$ and at the end of five minutes it was $1 - 119 = 0.0084$. The decrease in permeability is, therefore, $0.01 - 0.0084 = 0.0016$ or 16 per cent increase. It will also be seen that in higher concentrations (0.02m and 0.03m) the maximum was reached earlier than in 0.01m, while in the lower concentrations (0.01m and
0.001m) it occurred later than in 0.015m. It is evident as the concentration increases the rise in resistance is more rapid and that the maximum point is passed more quickly. If the concentration be sufficiently increased, the period of increased resistance becomes shorter and shorter until it becomes difficult to detect it. Acid produces a rapid decrease of permeability followed at once by a rapid increase, which continues until the death point is reached." He used living tissue of Laminaria Saccharina in sea water containing various amounts of hydrochloric acid.

In their search for a substitute for the porous pot in the construction of Pfeffer's cell for the demonstration of osmotic pressure, Paine and Saunders (7) thought pea testa might afford a non-extensible membrane in which a film of copper ferrocyanide might be deposited. However, instead of the expected uniform deposit, a definite irregular net work appeared, the spaces of which remained perfectly colourless. Wrinkled peas had been employed and the colloid had been deposited only at the positions of the wrinkles. When round peas were substituted the deposit was perfectly uniform. This clearly showed that there was a difference in permeability in different parts of the wrinkled testa, the ridges being freely permeable. The cause for such a phenomenon is due to the presence of a waxy bloom on the surface of the seed coat, which had not been rubbed off in the depressions of the wrinkled coat.

V. Arcichovsky (1) further found that subsequent washing after immersion in sulphuric acid was beneficial, i.e., the per cent of germination is increased.

From the investigations as reported by Kidd and West (4) serious injury occurs through soaking peas and beans in excess of water. The injury occurred at all temperatures, being more marked at low temperatures than at intermediate temperatures (15°C - 20°C). The injurious effect was evident in the per cent of germination. The curve of the number of plants produced from seeds soaked at
different temperatures rises and falls about an optimum in the region of 15°C - 20°C. It is likely that soaking in other fluids would not be injurious and at the same time hasten germination.

We have been able to learn that hard seeds do not germinate, thus affecting crop production. For this reason seeds have been treated in a number of ways to increase the number germinating.
II. Materials and Methods

In taking up this problem different varieties of peas were chosen for experimentation. The varieties chosen - Canada Field, Alaska, Black Eyed Marrow-fat and Extra Early Gradus - present differences in chemical composition and nature of the seed coat, more particularly toward the imbibition of water. The samples of seeds were taken at random, though the individual seeds were examined for defects in coat such as cracks and insect punctures. All defective seeds were discarded. The dry weight of fifty seeds was taken, the seeds placed in glass bottles and then in an incubator at 35°C along with the reagent for five hours. At the end of this period the acid was added to the peas and the bottles returned to the incubator and left for one hour. At the close of one hour the acid was poured off and the seeds thoroughly rinsed in running water. They were then dried between towels, weighed and immediately placed upon Plaster of Paris blocks in pans for germination. The pans contained water to within 1 cm of the top of the blocks. A daily record of germination was made.

III. Experimental Results

The experiments were done in triplicate for each concentration. The general method described in "Materials and Methods" was followed. For the final results the average of the three was taken. The daily record as made shows the rate and maximum germination. The increase in weight is obtained by taking the difference between two weights of each 50 peas treated, the first before the peas were placed in the incubator and the last just after they were thoroughly rinsed and dried between towels. The difference in weight is given in per cent increase in weight. The temperature at which the peas were grown remained constant at 21°C.

The weaker concentrations were employed in the first experiments, the concentrations being used in order of their strength. In the first, n/32 - n/16 and n/8 was used. The rate of germination differs for the different varieties.
The maximum germination for any single day is represented in the following table:

Table I  

<table>
<thead>
<tr>
<th></th>
<th>n/32</th>
<th>n/16</th>
<th>n/8</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada Field</td>
<td>2nd day</td>
<td>3rd day</td>
<td>3rd day</td>
<td></td>
</tr>
<tr>
<td>Alaska</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black Eyed Marrowfat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra Early Gradus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Canada Field germinated more rapidly, germination being completed one day earlier than in Black Eyed Marrowfat and Extra Early Gradus. The total germination for Alaska in three experiments varied as shown by the table,

Table II  

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/32</td>
<td>82%</td>
<td>100%</td>
<td>94%</td>
</tr>
<tr>
<td>n/16</td>
<td>56%</td>
<td>96%</td>
<td>90%</td>
</tr>
<tr>
<td>n/8</td>
<td>36%</td>
<td>90%</td>
<td>54%</td>
</tr>
</tbody>
</table>

The effect of treatment with n/8 showed the widest variation. The number of peas in a random sample with impermeable or only slightly permeable coats are responsible for such differences. A greater number of such seeds apparently were present in the first and third experiment.

Mold appeared a day earlier on Black Eyed Marrowfat and Extra Early Gradus and was more abundant upon them than upon the two remaining varieties. The mold was evident first on those seeds in which the coats underwent the most striking change in color, this change in color being an index to the degree of permeability of the coat.

In the second group of experiments four concentrations were used; n/4 - n/2 - n and 2n. In this group the increase in absorption, as shown by an increase in weight, is followed by a decrease in the number of seeds germinating. This is especially the case in the concentrations n/4 - n/2 and n; germination for Extra Early Gradus occurred on the fourth day for the four concentrations n/8 - n/4 - n/2 and n; on the third day for Alaska and Canada Field; and on the fourth day for Black Eyed Marrowfat.

Mold appeared on the seeds treated with n/4 and n/2 earlier than in concentrations n and 2n. It developed most plentifully upon Black Eyed Marrow-
The variety Alaska germinated most readily in each experiment.

Solutions 4n and 8n were used in the next group of three experiments. The number of seeds, with coats impermeable to these higher concentrations, increased. The mold did not appear so rapidly in these concentrations and was first present on those seeds with the more permeable coats, being most luxuriant upon Black Eyed Marrowfat and Extra Early Gradus. The maximum germination for concentrations 4n and 8n, took place on the third day in every variety except in Extra Early Gradus, in which it occurred on the fourth day.

In the last group of experiments the three strongest solutions, 16n - 32n and concentrated were used. The action of 16n was not noticeably different from the action of 8n. The decrease in absorption, however, continued throughout these concentrations. The action of 32n and concentrated is so different from the action of the remaining concentrations that it deserves a more detailed description. When the concentrations 32n and concentrated were added to the peas a deep brown solution formed immediately in the bottle containing concentrated sulphuric acid. A solution lighter brown in color resulted from the action of 32n. On all varieties in concentrated acid minute brown dots appeared spreading rapidly and finally covering the seed coat. In 32n pits were formed on Canada Field and Black Eyed Marrowfat, while grooves were found on Alaska and Extra Early Gradus. The grooves were irregular and followed the ridges in the seed coat. Upon removing the acid it was found that a great part of the seed coat had been eaten away, and many of the cotyledons softened. The softened seeds failed to germinate and furnished excellent conditions for mold growth. A decrease in weight resulted from the action.

In Alaska the concentration in the stronger concentrations for maximum germination is 16n. The per cent of germination decreased in the concentrations 32n and Concentrated. In the other varieties the decrease begins at 8n and continues
through the concentrations, 16n - 32n and concentrated. On the second day the peas treated with the concentrations 16n - 32n and concentrated supported by a luxuriant growth of mold.

Seeds when treated with water for an interval equivalent to the immersion in the sulphuric acid solutions showed the maximum germination on the third day. Germination was completed by the seventh day, the period necessary for those treated with the acid solutions. The untreated peas required a slightly longer period for germination. The rate was somewhat slower with maximum germination on the fourth day in each variety. The decreased rate is due to the lack of water of imbibition which those treated had taken from the solution.

Mold was slowest to develop and slightest in growth in the untreated control seeds. It increased quite perceptibly in those immersed in water for a period equal to that in the various acid solutions. It was most abundant in those treated with acid solutions.

In table III are brought together the results as obtained by averaging the individual records of the triplicate experiments in each concentration. The curves in figures I, II, III, and IV were plotted from table III, the abscissae representing the concentrations in each and the ordinates the per cent increase in weight and the per cent of germination.

When comparing the permeability of the coats of all varieties it is evident that those of Extra Early Gradus are the most permeable. Permeability decreases in Alaska, Black Eyed Marrowfat and Canada Field respectively.

The concentrations for maximum germination in the weaker, intermediate and stronger solutions for the various varieties are shown in table IV -

<table>
<thead>
<tr>
<th>Variety</th>
<th>Weaker</th>
<th>Intermediate</th>
<th>Stronger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada Field</td>
<td>n/32</td>
<td>n</td>
<td>8n</td>
</tr>
<tr>
<td>Alaska</td>
<td>n/32</td>
<td>n/2</td>
<td>16n</td>
</tr>
<tr>
<td>Black Eyed Marrowfat</td>
<td>n/32</td>
<td>n/2</td>
<td>8n</td>
</tr>
<tr>
<td>Extra Early Gradus</td>
<td>n/32</td>
<td>n/2</td>
<td>8n</td>
</tr>
</tbody>
</table>
| Variety | Dry Check | H₂O | N/32 | N/16 | N/8 | N/4 | N/2 | N | 2N | 4N | 8N | 16N | 32N | Conc.
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<tbody>
<tr>
<td>Canada Field</td>
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</tr>
<tr>
<td>Av. Per Cent Gain in Weight</td>
<td>23.06 40.36 36.66 30.30 25.30 28.23 31.20 26.70 6.20 7.35</td>
<td>.04</td>
<td>1.09</td>
<td>.53</td>
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</tr>
<tr>
<td>Av. Per Cent Germination</td>
<td>90.00 86.00 70.60 34.60 35.30 36.60 26.60 20.60 26.60 84.00 90.56 76.00 14.00 5.33</td>
<td></td>
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<tr>
<td>Alaska</td>
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</tr>
<tr>
<td>Av. Per Cent Gain in Weight</td>
<td>56.53 62.70 36.66 38.40 46.96 47.56 40.53</td>
<td>33.43 11.21 10.13</td>
<td>.22</td>
<td>.70</td>
<td>1.91</td>
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<tr>
<td>Av. Per Cent Germination</td>
<td>100.00 94.00 91.30 80.60 60.00 11.30 12.60 13.30 25.30 62.66 62.00 89.33 46.00 32.66</td>
<td></td>
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<tr>
<td>Black Eyed Marrowfat</td>
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<tr>
<td>Av. Per Cent Gain in Weight</td>
<td>44.00 54.60 45.46 37.30 34.93 41.83 30.46</td>
<td>29.10 7.29 3.29</td>
<td>.50</td>
<td>.53</td>
<td>.45</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Av. Per Cent Germination</td>
<td>76.00 67.33 80.00 65.30 46.00 12.30 7.30 6.60</td>
<td>12.60 44.66 54.00 49.33</td>
<td>4.56</td>
<td>1.33</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra Early Gradus</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Av. Per Cent Gain in Weight</td>
<td>60.23 71.53 68.96 59.36 58.56 60.20 48.56</td>
<td>51.96 25.50 6.29</td>
<td>.60</td>
<td>.64</td>
<td>.35</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av. Per Cent Germination</td>
<td>89.33 88.66 89.30 48.00 32.60 6.60 6.00 5.30</td>
<td>8.30 44.60 61.33 46.56</td>
<td>2.00</td>
<td>4.00</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Fig 1  Average Per Cent Increase in Weight

Canada Field

Alaska
Fig II Average Per Cent Increase in Weight

Black Eyed Marrowfat

Extra Early Gradus.

Conc
Fig. III  Average Per Cent Germintion

Canada Field

Alaska
Fig. IV. Average Per Cent Germination.

Black Eyed Marrowfat.

Extra Early Gradus.
The increase in weight in water in no instance equals that found after treatment with n/32. The imbibition for all varieties treated with increasing concentration, exhibits first a decrease in the rate of imbibition, then an increase and finally again a decrease. Black Eyed Marrowfat and Extra Early Gradus when soaked in water resulted in a lower per cent of germination than when treated with n/32 for an equal time. In the case of Canada Field and Alaska the water treatment produced a greater per cent of germination than when treated with n/32. The per cent of germination in these varieties was greater in the control than in the treated, though the rate of germination was not so rapid.

The action of the acid in the weaker solutions was not sufficient to cause a marked injury. As the concentration increased, its action upon the seeds became more noticeable. At n/16 in Alaska and n/4 in remaining varieties an increase in imbibition occurred, as shown by an increase in weight. This is shown in figure I and II, by an upward trend in the curve and by a continued downward trend in the germination curve (figures III & IV). In the stronger concentrations (n-8n) the imbibition activity was reduced as shown by a less rapid increase in weight. This fact is revealed in a higher per cent of germination. The injury was less because the acid was unable to overcome the resistance offered by the seed coats. However, as the strength of the acid increased from this point the activity of the acid on the composition of the seed coats was increased. The affect of such activity is shown by a decided decrease in the per cent of germination. Thus in the concentrated acid the total germination was 5.33% - 32.66% - 1.33% and 4% for the varieties Canada Field, Alaska, Black Eyed Marrowfat and Extra Early Gradus respectively.

Pertaining to the activity of the strongest concentrations Arctickovsky (1) states, "That we must not neglect the fact that the chemical activity of various reagents in their strongest concentrations, is likewise very much reduced as pertains to electrolites. Such solutions are not only
relatively but also absolutely more dissociated than the weaker solutions - which gives us the reason for lower chemical activity of the concentration."

His work was done with sulphuric acid and formalin. Peas were used though he does not state the variety. In his results he gives the following table:

Table V.

<table>
<thead>
<tr>
<th>Conc.</th>
<th>n/32</th>
<th>n/8</th>
<th>n/4</th>
<th>n/2</th>
<th>2n</th>
<th>4n</th>
<th>8n</th>
<th>16n</th>
<th>32n</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Germ.</td>
<td>32</td>
<td>76</td>
<td>48</td>
<td>24</td>
<td>49</td>
<td>24</td>
<td>100</td>
<td>100</td>
<td>96</td>
<td>100</td>
</tr>
</tbody>
</table>

The per cent of germination for the last give concentrations (4n - conc.) are far greater than the results I secured for the varieties used. Arcichovsky treated the seeds at 28°C, while the experiments in the present work were done at 35°C.

The per cent of germination of seeds treated in concentrations above those of maximum germination in the stronger concentrations decreased rapidly as shown by table VI:

Table VI.

<table>
<thead>
<tr>
<th></th>
<th>8n</th>
<th>16n</th>
<th>32n</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada Field</td>
<td>90.66</td>
<td>76.00</td>
<td>14.00</td>
<td>5.33</td>
</tr>
<tr>
<td>Alaska</td>
<td>62.00</td>
<td>89.33</td>
<td>46.00</td>
<td>32.66</td>
</tr>
<tr>
<td>Black Eyed Marrowfat</td>
<td>54.00</td>
<td>49.33</td>
<td>4.66</td>
<td>1.33</td>
</tr>
<tr>
<td>Extra Early Gradus</td>
<td>61.33</td>
<td>46.66</td>
<td>2.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

The results are radically different from those secured by Arcichovsky.

The action of the highest concentrations was so great that the viability of a large number of seeds was destroyed. This in turn accounts for the low per cent germination.

In regard to soaking seeds in excess of water, Kidd and West (4) make this statement. The injurious effect is more marked with low temperatures (5° - 10°C) than with the medium temperatures (15° - 20°C). At higher temperatures the amount of injury again increases.

When water was used with these varieties in no instance was the per cent germination as high as resulted from the treatment with n/32, nor was the per cent germination equal to the per cent germinating in the control experiment. When equal periods of immersion are used soaking in water is not so beneficial
in any case as the treatment with the weakest acid solution.

IV. Conclusions

From the results secured the following conclusions are drawn.

1. The effect of sulphuric acid on permeability varies not only with the concentration but also with the variety of peas.

2. Permeability as measured by imbibition decreases, increases, and finally decreases as the strength of the concentration of the acid increases.

3. The per cent of germination is affected by the varying permeability.

4. The rate of germination is hastened somewhat by the acid treatment, with maximum germination occurring at n/32.

5. Seeds fail to germinate when treated with the strongest concentrations because of the marked injury due to high chemical action.

6. Treatment with the weakest concentration (n/32) of sulphuric acid for one hour is most beneficial.

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