THE STIMULATION OF GERMINATION
OF
GREEN MANURE SEEDS

REPORT
OF AN EXPERIMENT
ON
"THE STIMULATION OF GERMINATION
OF
GREEN MANURE SEEDS"

by

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1. Introduction.

The primary function of a green manure crop is to supply organic matter to the soil, and for this reason leguminous plants are almost exclusively used for the purpose. In order that their cultivation should be economic, however, they must satisfy the need for a crop which will not only yield a high percentage of green matter per acre, but also provide a good thick cover over the soil in the shortest possible time. In so doing they will suppress weed development, and counteract loss of soil moisture by evaporation, and at the same time prevent scouring and erosion in hilly land. Hence an even and a rapid "take" are essentials in a green manure crop.

The species of plants most widely employed are seldom grown on a very expensive scale, so that a regular seed supply is rarely available, resource being had to occasional collection of the seed when opportunities offer. The seed supply is therefore very uneven, in varying stages of maturity, and unreliable. Such immature seed possesses frequently a very hard testa, and only germinates with great difficulty or not at all. This has lead to considerable trouble in planting up green manures, so that in the absence of an improved seed supply, measures of treating the seed so as to increase the germination capacity have had to be adopted. The trial of such measures, either previously employed or suggested by present circumstances, forms the subject of the following report.
2. Scope of the Problem.

Various experiments in past have been made with a view to stimulating the germination of seeds under laboratory conditions, but few attempts have been recorded of methods applicable on an agricultural scale.

The methods of approach fall broadly under four heads:

(1) Soaking in water or dilute salt solutions.
(2) Acid treatment.
(3) Refrigeration.
(4) Mechanical treatments.

The response to soaking the seed in pure water, and dilute solutions of such salts as potassium nitrate, 5% phosphate solutions, and other potash salts (3, 11) is apparently slight, though in a few specific cases the method might yield results comparable with acid treatment. Muelen (7) for example found that germination was enhanced in the case of Centrosema pubescens by soaking in water at about 60°Celsius (?) - a temperature easily obtained by using equal quantities of boiling and well water - for an unspecified time, and sowing either directly the water has cooled (in monsoon weather) or left overnight (under moderate rainfall). Duvel (3) stresses the fact that excess water and prolonged soaking may have an injurious effect, by reducing the oxygen supply and causing an accumulation of carbon dioxide to the limit of toxicity. In this connection Takiguti (13) after making the same observation, finds that in the case of Brassica napella addition of hydrogen peroxide, and to a lesser extent sulphuric acid, absolute alcohol, and hot water, accelerated germination by increasing its oxygen supply. Water-soaking followed immediately by rapid drying apparently retards germination.

Kidd and West (5) point out that soaking seed in water for periods longer than 24 hours can have no effect upon germination, and that the period of dormancy cannot by such means be reduced beyond the limits imposed by the nature and environment of the parent plant. The size of the seed (and hence its maturity), the time of harvest-
ing, and conditions of storage, all have a considerable influence upon the dormancy period, which can to a greater or less extent depending upon the species be modified by artificial treatment. Finally they stress the importance of studying the effect of various treatments upon subsequent growth of the plant, and no confining attention solely to the stimulation of germination. This is also recognised by Parnell (9).

(2) The use of aids has been more widely exploited than any other method, and it can safely be said that in general it has met with the greatest degree of success. Sulphuric acid (conc.) is usually employed - Whitcomb (14) affirming that this is the only fat solvent which will increase the permeability of the seed coat, though he was unable to obtain any positive results by its use in the germination of a limited number of leguminous plants except with sweet clover in the hull. Sampson (10), on the other hand, declares that most seeds will respond to sulphuric acid treatment, while the few exceptions he considers may feasibly be propagated by vegetative methods. In the latter category he includes Dolichos hosei, and Indigofera endecaphylla which have been so treated at Paradeniya. Blackman (1) proved the efficacy of sulphuric acid (conc.) in delinting cotton seed, and found that such treatment could safely be applied without impairment (but not apparently with enhancement) to seed in bulk. He further showed that longer immersion in weaker acid not only was inferior to conc. acid treatment, but was liable to be injurious if the acid was allowed to dry on the seed, thus concentrating it and burning the testa and embryo.

Maas (6), working exclusively with green manure seeds, carried out extensive tests on a number of species, and noted the effect of different dilutions of, and varying periods of immersion in sulphuric acid upon the rate of germination. The differential response of the species over untreated seeds was very marked in all cases, and in general he found that there was a specific period of immersion in a given concentration in each instance. This is in contrast to Nye's (8) assertion that the longer the seeds are left in acid the higher is the germination - apparently irrespective of species. As will be seen subsequently the findings of the present tend to support
Maas' point of view. In the course of the latter's experiments, the seeds were separated out according to colour differences, thus discriminating between mature and immature, and hard and "soft" seeds. This method of experiment, while serving to emphasise the variability of response occasioned by immaturity and other factors, does not give a clear ideal as to the effect of the treatment on bulk samples under conditions more nearly approximating to field trials.

This brings one to a consideration of how best acid treatment can be applied in the field - an aspect of the subject which has received very little attention, but of obvious importance. Methods must necessarily be simple and suited to the materials likely to be available on small plantations; the extension of seed treatment to the extensive commercial scale adopted in the case of sugar beet is clearly not justified at present. The only description of such a simple apparatus which has been found in the literature is that of Cameron (2). His technique, evolved for the treatment of cotton seed in N. Rhodesia, is as follows:— From a 5-gallon drum 4 sections were cut out leaving a skeleton casing which was lined with steel mesh ½" thick, and on top placed heavy lead plates. This contained the seeds, and the whole was placed in a larger drum containing the acid. It was left for 5 mins. in the acid, and subsequently washed quickly in water 5 times. The sulphuric acid could be used 3 times, and 30 lbs. of seed treated at a time. The cost of treatment was estimated at 5d. per lb., but it could be reduced to 2d. per lb. if larger quantities of acid were used. The method further was claimed to screen the seed and eliminate the bad from the good. It is thought that this type of apparatus should receive further trial, with modifications such as in the size of the steel mesh to suit particular conditions.

(3) Spencer (12) has shown that in the case of two leguminous oil-seeds - soya-bean, and ground-nut - cool storage at about 55°F had a markedly beneficial effect upon their germination when stored for periods longer than 4 months. Such a result is very suggestive to the present problem, and although facilities for such treatment are not universally available this aspect should be definitely borne in mind.
The utilisation of mechanical treatments, such as milling and other forms of scarifying the seed coat has not as yet any definite place in seed treatment. Numerous experiments have shown that prick ing and scraping has a beneficial effect in certain cases, but seldom are the methods employed applicable on a field scale. Maas (6) for example made small cuts in the seeds with a scalpel, and others have used pricking with a fine pin as a means of increasing the water absorption of the coat. Heat treatment, employed by Whitcomb (14) and others was shown to give results comparable with scarifying. In all such cases, however, the response was never found to approach that resulting from acid treatment. The chief difficulty would appear to lie in gauging the severity of treatment which has to be accorded any one sample in order to obtain a significant response, and at the same time not injure the embryo. It is said that a method adopted in seed stations in Europe is to shake up the seed in a tin lined internally with emery paper. To what extent this method would effect such hard seeds as are found among the green manure species is a matter of some doubt, but it would certainly merit a trial.

A mechanical treatment was also attempted. The seed was mixed with about an equal quantity of sand, and placed between the folds of a cloth. This was then rolled with a cylindrical stone and allowed to remain for some hours. The seed was then washed in running water to prevent undue heating from taking place. Washing was continued for about 2 minutes, after which the seed was roughly dried on filter paper, and subsequently transferred in rows.

3. Method of the Experiment.

Five species of leguminous green manures were originally chosen for examination:

A petri-dish trial, using moist filter paper placed in the bottom of the dish as the medium of germination, was carried out to determine the approximate range of each species in relation to the strength of acid employed and the period of immersion. 25 seeds of each species were taken at random from the seed tin, and sufficient sulphuric acid (conc.) added to cover the seeds completely. At the end of the fixed period, the acid was poured off, and the seed rapidly washed in running water to prevent undue heating from taking place.
of 5 to the petri-dish, the lid being replaced. The moistness of the filter paper was kept as nearly constant as possible in each dish, and throughout the experiment. Counts of the number of seeds germinated (First appearance of the radicle) were made daily and recorded.

B. A seed-box trial was made subsequently, using well-composted soil as the germination medium. At first, difficulty was experienced owing to the presence of mildew which seriously upset the validity of the results. Sterilisation of the soil by heating it on a thin metal plate had little or no counteracting effect. It was then recommended that the boxes should be placed in a better-lit place, and further that a thin layer of well-sieved soil should be sprinkled over the seed. As a further precaution the seed was dried for 24 hours subsequent to acid treatment. These measures proved sufficient in reducing the incidence of mildew to a negligible extent. Each box was divided into two halves - on one side were placed 100 untreated (control) seeds, and on the other 100 treated seeds - in rows of 10 to facilitate counting. Germinations were recorded daily as soon as the cotyledons had emerged from the seed.

A mechanical treatment was also attempted. The seed was mixed with about an equal quantity of coarse sand and placed between the folds of a cloth. This was then rolled with a cylindrical stone and considerable pressure applied. In all cases it was practically impossible to crush the seeds injuriously, however great the pressure applied by hand. Very little scarifying effect could be detected by eye-inspection. A preliminary experiment was frustrated by rats which got at the boxes, and the subsequent experiment could only be made on three of the species.

The results of the experiments are tabulated below:
4. Experimental Results.

TABLE 1.
PETRI-DISH TRIAL
Germination % after 5 days.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>WATER PERIOD OF IMMERSION</th>
<th>CONC. H₂SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL 35 m.</td>
<td>5m.</td>
</tr>
<tr>
<td>Centrosema sp.</td>
<td>24</td>
<td>44</td>
</tr>
<tr>
<td>Pueraria sp.</td>
<td>8x</td>
<td>12</td>
</tr>
<tr>
<td>Indigofera sp.</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Calapogonium sp.</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Phaseolus sp.</td>
<td>100</td>
<td>76x</td>
</tr>
</tbody>
</table>

(*X = remaining seed killed by fungus)

TABLE 2.
SEED-BOX TRIAL
ACID TREATMENT
Mean Germination % after 10 days.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CONTROL</th>
<th>ACID 10m.</th>
<th>ACID 35m.</th>
<th>DIFFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrosema pubescens</td>
<td>25</td>
<td>82</td>
<td>-</td>
<td>57 ± 3.95</td>
</tr>
<tr>
<td>Pueraria javanica</td>
<td>3</td>
<td>-</td>
<td>26</td>
<td>23 ± 5.40</td>
</tr>
<tr>
<td>Indigofera endecaphylla</td>
<td>4</td>
<td>64</td>
<td>73</td>
<td>60 ± 4.47</td>
</tr>
<tr>
<td>Calapogonium mucunoides</td>
<td>8</td>
<td>44</td>
<td>-</td>
<td>36 ± 6.17</td>
</tr>
</tbody>
</table>

TABLE 3.
SEED-BOX TRIAL
MECHANICAL TREATMENT
Mean Germination % after 10 days.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CONTROL</th>
<th>TREATED</th>
<th>DIFFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrosema pubescens</td>
<td>23</td>
<td>52</td>
<td>29 ± 6.5</td>
</tr>
<tr>
<td>Indigofera endecaphylla</td>
<td>8</td>
<td>31</td>
<td>23 ± 8.3</td>
</tr>
<tr>
<td>Calapogonium mucunoides</td>
<td>10</td>
<td>27</td>
<td>17 ± 6.2</td>
</tr>
</tbody>
</table>

The high germination of Phaseolus Mungo without treatment in the Petri-dish trial did not warrant continuation with this species. It will be noted that Indigofera gave no clear optimum acid treatment in the petri-dish experiment. The seed-box trial
also gave closely similar results with 10m. and 35m. immersions. A graph is therefore appended showing the course of germination of Indigofera under the two treatments. With 10m. immersion the curve rises rapidly between the 3rd. and 5th. days, while with 35m. it shows a gradual increase over the 10 day period. In view of this, it is thought probable that the 35m. period would be more suitable and reliable, as some abnormal stimulus may have caused the sudden rise in the other case. The data for acid treatment in the remaining species are self-evident, each exhibiting quite well-defined optima, and in all instances markedly significant increases over controls.

The results obtained with the improvised mechanical treatment gave unexpectedly promising indications. While the increases are not of the same order as those resulting from acid treatment, they are in each case significantly greater than the controls, even though no clearly visible effect was produced on the seed coat.


The figures given below only apply to experimental conditions, and can only be taken as indicating approximately the order of treatment costs to be expected.

Found that 1 oz. H₂SO₄ would cover 1 oz. seed.

Cost of acid = 9d. per kg. (sp. gr. 2.4 - comm. H₂SO₄) 4-05d.

Hence cost of acid per lb. of seed = 12-4d.

The acid was used about 3 - 5 times for experimental purposes. At the above cost of acid, it is difficult to see how Cameron arrived at his figure of 5d. per lb. including labour and other costs.

6. Conclusions.

Sulphuric acid treatment of seed is clearly beneficial to the germination of the green manure seeds under investigation. They fall under two groups, viz. (1) Centrosema pubescens and Calapogonium mucunoides - optimum period 10 mins.; (2) Pueraria javanica and
Indigofera endecaphylla - optimum period 35 mins. The results are sufficiently promising to warrant a more extensive experiment using an apparatus on the lines suggested by Cameron, and planting the seed out in the field under agricultural conditions.

Mechanical treatments also gave satisfactory results, suggesting that more rigorous methods such as milling would safely yield greater increases in germinating capacity at a smaller cost than the acid treatment.

7. Literature Cited.


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GERMINATION OF
*INDIGOERA ENDECAPHYLLO*
TREATED WITH CONC. H₂SO₄
FOR TWO DIFFERENT PERIODS

% SEEDS GERMINATED

No. days after sowing.