A METHOD FOR DETERMINING SEED VIABILITY BY ELECTRICAL CONDUCTIVITY MEASUREMENTS

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THESIS

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By

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A METHOD FOR DETERMINING SEED VIABILITY BY ELECTRICAL CONDUCTIVITY MEASUREMENTS.

I. INTRODUCTION.

The first use of electrical conductivity in physiological research dates back to 1836 when Eduard Weber (1) at Halle studied the electrical resistance of the body and the effect upon it of temperature and moisture. Following him, came du Bois Reymond (2) with his studies on the resistance of muscles; Ranke (3) who showed that the resistance of plant and animal tissues decreased upon death; Stewart (4), Roth (5), Burgarszky and Tangl (6) who studied the resistance of red blood cells; and many others who need not be mentioned here but whose work will be found discussed in any good standard textbook.

Of the numerous recent workers, only a few will be briefly referred to. Osterhout (7-11), through his thorough studies on the resistance of living and dead tissues of several marine algae and his conclusions based on these studied as an indirect means of measuring permeability, gave a big impetus to the use of conductivity methods in physiological problems. Green and Larson (12) and Johnson and Green (13) in their work on the conductivity of bacterial and yeast cell suspensions respectively showed that, at death of these organisms, a change in resistance took place due to an alteration in the permeability of their membranes. Such men as Washburn (14-15), Taylor and Acree (16), Hibbard and Chapman (17), and Green (18) deserve mention as having made electrical con-

ductivity methods accurate and attractive in the investigation of many physiological phenomena.

Following Osterhout's important findings in his studies on the permeability of Laminaria and especially the difference in conductivity of living and dead tissue of the same, Dr. E. A. Bessey, of the Department of Botany, Michigan Agricultural College, conceived the idea that electrical conductance measurements might constitute a means of determining seed viability that would be a decided step in advance of the present methods of running germination tests, which require at least several days and, in the case of some grass seeds, several weeks. Promptly, work was begun by Doctors Bessey and Hibbard to investigate the problem suggested by this idea. When but fairly begun, the war intervened and the work was dropped. The idea, however, persisted and in the fall of 1923, aided by the liberality of the Ferry Seed Company in contributing a fellowship to carry on the work, the investigations were resumed by the author.

The problem briefly is this. To determine a correlation, if there be any, between seed viability and electrical conductivity. If a relationship between the two could be established, attention would then be directed toward making resistance measurements a practical means of determining the percentage of germination of any sample of seed. It is believed that a correlation has been found which is fundamental and which, with further amendment and improvement, can be practically used. However, much work remains to be done and it is realized that this is but a preliminary investigation.

II. MATERIALS AND METHODS.

A. Description of apparatus.

The apparatus used was practically the same as that recommended by Hibbard and Chapman (17) with several minor changes. As shown by these authors, the apparatus is capable of great precision and yet is easily operated. The average time required to make a reading is about two minutes.

The source of current employed was a 60-cycle rotary converter actuated by the college circuit of 220 volts (D.C.). In connection with the converter, a variable transformer was employed which cut the current down to 6 volts (A. C.). This was then led directly to the bridge and galvanometer. The converter and transformer were placed under the table at which the work was carried on so as to prevent them from exerting an influence on the sensitive galvanometer. A switch was attached to the side of the table to facilitate the starting of the converter. Originally, a radio oscillator was used as a source of current in connection with a telephone tuned to the frequency generated by the oscillator. This, however, was early discarded because of the difficulty of obtaining an accurate "minimum" in measuring high resistances, and because it is impossible to use the telephone successfully except in an absolutely quiet place such as a telephone booth or individual laboratory. An attempt was made to use the galvanometer as a detector with the oscillator but the latter did not generate sufficient current to actuate the galvanometer coils. Although a high frequency and an extremely pure sine wave such as the oscillator gives are desirable, Green (18) and Hibbard and Chapman (17) have shown that very good results may be had by using a frequency of 60 cycles.

The bridge used was a Leeds and Northrup product and was of the

Kohlrausch roller type which has proved very satisfactory wherever used. The bridge wire is 470 cm. long. The scale is divided into a thousand divisions which, in turn, are divided into halves that can be read accurately to a fifth, making the error from bridge readings negligible for all purposes of this work. By removing two plugs, the bridge wire can be extended where extreme accuracy is desired in measuring low resistances. In this work, the "short" bridge was exclusively used. Prior to use, the bridge had been sent in to Leeds and Northrup to be calibrated.

The known resistances were of the "plug decade type." The plug-controlled "five decade box" put out by Leeds and Northrup has a range of from .1 to 20,000 ohms and an accuracy of 1/20 per cent for all but the 1 ohm coils (which have an accuracy of 1/10 per cent). Such a box was used throughout these experiments and proved very satisfactory. Care was taken to keep the plugs and "plug holes" clean at all times.

An alternating current galvanometer of the Rowland electrodynamometer type was employed to determine when a balance had been reached. This instrument, also a product of Leeds and Northrup, has proved very satisfactory. The stationary coil of the galvanometer is placed in the main circuit and the swinging coil across the bridge, just as is the telephone in the Kohlrausch method. The maximum allowable current of the fixed coil is 1/5 of an ampere for 10 second periods--the value just reached by the current from the transformer. The swinging coil has a maximum allowable current of 1/10 ampere for 10 second periods. Alternating current galvanometers of this type must be protected against external electric fields. The main offender in producing such a disturbing factor was the rotary converter until it was placed under the table, as previously stated. A key was inserted in the line running from the bridge to the swinging coil.

This enables the operator to break the circuit as soon as a deflection of the galvanometer scale is noticed. After an adjustment of the bridge is made, the key is again pressed down to make contact--just long enough to get a deflection. This is continued until a balance is reached. By closing the circuit for an instant only at each trial, the galvanometer is protected and, moreover, errors from heating and polarization are avoided.

To avoid too great deflections of the galvanometer, when readings were first begun and the difference between the unknown resistance and the known resistance in the box was likely to be great, a plug resistance box was put in the main line to enable the operator to cut down the current to the stationary coils of the galvanometer. Usually, a resistance of 300 ohms was inserted until, after two or three trials, balance was approached; then this resistance was taken out to secure greater sensitivity for final bridge adjustments.

The electrolytic cells ultimately used were of the immersion type (see figure 3). They were made by Eberbach Brothers of Ann Arbor, Michigan, according to the designs and specifications furnished them. For the first measurements made in this work, a U-shaped cell was improvised with electrodes 1 cm. in diameter and 8 cms. apart, but this was soon discarded as the immersion cells proved better adapted to the work in hand.

Prior to use, the electrolytic cells were thoroughly cleaned and the electrodes platinized according to the method recommended by Findlay (19). When ready for use, the cells were immersed in the solutions to be measured. The solutions were in Pyrex beakers which, following the addition of the cells, were placed in a constant temperature water bath regulated to 25°C. The water bath was essential since temperature changes

produce marked differences in resistance readings.

Since all readings were run in pairs, two electrolytic cells of exactly the same specifications and made by the same man were used. These were checked against one another in conductivity water and agreed to within 5/10 of 1 per cent, which was close enough for this work, especially since this error was compensated for in using first one, then the other, in making readings on successive samples of seed.

The apparatus is illustrated in figure 1, and a diagram of the "set-up" ultimately used is shown in figure 2.

B. Materials.

Only two kinds of seed were used in this work as it was thought better to experiment intensively with a few sorts than superficially with many sorts. Timothy and red clover were chosen as they represent two very common and widely different types of seed--timothy having a very large endosperm and red clover none at all. Furthermore, they are of convenient size to work with.

The seeds were obtained from various sources. The D. M. Ferry Seed Company supplied 10 pounds of timothy and 10 pounds of red clover from their 1923 crop. A quart jar of 1915 timothy was obtained for the work from the same source. Attempts were made to locate very old seed of each kind to be used with the highly germinable seed furnished by the Ferry Seed Company in making up mixtures of different percents of germination. The various attempts to procure old seed proved futile, no seed older than 1918 being found in the time allotted. Some old class exhibits in the Botany Building were gone through and, finally, a sample of red clover dated 1893 was uncovered. No old timothy was found. The four lots of seed mentioned formed the nucleus of seed used in the experiments of this work.

The germination of each lot of seed was very carefully determined. After being thoroughly mixed, 10 samples of 100 seeds each were counted out from each lot. These were placed in moist chambers and the percentage germination calculated for each sample; then, the average of the ten taken as the percentage germination of that particular lot. Beginning after three days, the seeds began to germinate and every few days the sprouted seeds were taken out until, after two weeks, the final counts were made. The germination percentages for the four lots calculated, in this way, follow:

Lot #1, 1915 crop timothy (from Ferry Seed Company) --- 73.9%

" #2, 1923 " " " " " " --- 89.8%

" #3, 1923 " red clover " " " " --- 91.8%

" #4, 1893 " " " (from old Botany Building sample) --- 3.6%

Since no timothy of very low germination could be found, different methods of killing these seeds were tried out--first, by etherization; second, by chloroform; and, lastly, by heat. The results of these experiments are tabulated below:

Table 1. Effect of Etherization on Germination.

Seed	Average percent germination (3 series) after exposure to saturated ether vapor (under sealed bell jar) for					
	12 hrs.	24 hrs.	36 hrs.	48 hrs.	60 hrs.	7 days
Lot 1(1915 timothy)	81.7	78.6	77.5	74.0	77.2	70.6
Lot 2(1923 timothy)	- -	- -	- -	- -	- -	78.3

Table 2. Effect of Chloroform on Germination.

Seed	Average percent germination (3 series) after exposure to saturated chloroform vapor (under bell jar) for			
	2 days	4 days	6 days	8 days
Lot 2(1923 timothy)	71.4	71.2	62.0	63.5

Table 3. Effect of Dry Heat Treatments on Germination.

Seed	Percent germination after following heat treatments			
	3 days at 80°C. dry heat	3 days at 90°C. dry heat	7 days at 80°C. dry heat	7 days at 90°C. dry heat
Lot 1(1915 timothy)	13.0	None	None	None
Lot 2(1923 timothy)	- -	Slight	Slight	None

The conductivity water used throughout this work was triple distilled. The condensed steam from the college power plant was redistilled in a Barnstead automatic still which yielded water having a specific resistance of 45,000 ohms. This, in turn, was redistilled in an all-glass still yielding what shall hereafter be referred to as "conductivity water" of a specific resistance of 125,768 ohms (or specific conductivity of 7.95×10^{-6}). Although not conductivity water in the strictest sense of the word yet it was a very pure and high grade of distilled water and satisfactory for this preliminary investigation.

C. Method.

Various methods of handling the seeds were tried out but the method that will be here described is the one finally adopted. The others will be briefly mentioned under the experiments discussed farther on. Both kinds of seed were handled in the same general way except for minor differences, but they will be separately considered.

After working with amounts varying from .5 gm. to 5 gms., the former (used with 100 cc. of conductivity water) was selected as the best. Smaller amounts, while perhaps giving better results, would hardly be fair samples. A .5 gram sample of timothy contains between 1600 and 1800 seeds which, when the seed lots are well mixed, should give a fairly representative sample from the standpoint of germination. A number of .5 gram amounts were carefully weighed out on a standard Becker scale, using weights standardized by the United Stated Bureau of Standards, and stored in small coin envelopes properly labeled.

When ready to run a test, two of the envelopes--the tests were run in pairs--were opened and the seeds emptied into separate Pyrex beakers of 125 cc. capacity. The time was carefully noted and, on the minute, 100 cc. of conductivity water was added to one of the beakers and, after a three-minute interval, 100 cc. to the other. This interval was maintained throughout until the reading was made. Fifteen minutes after adding the conductivity water, the solutions were well stirred for three minutes, an attempt being made to stir both samples uniformly. After another interval (of thirty minutes) the solutions were again stirred--this time for two minutes. Following this stirring, the electrolytic cells were lowered into their respective solutions and the whole allowed to come to equilibrium before the resistance reading was made. The reading was made exactly one hour after the addition of the conductivity water.

One gram of clover was chosen as the best amount to work with. Such an amount contains between 650 and 800 seeds. A number of samples were weighed out and stored in properly labeled coin envelopes just as in the case of timothy. When a test was run, two of the envelopes were opened and their contents emptied into separate Pyrex beakers of 125 cc.

capacity. On the minute, 100 cc. of conductivity water was added to one of the beakers and, after a four-minute interval, the same amount to the other. Immediately after the addition of conductivity water, the solution was stirred for two minutes. Fifteen minutes after this addition, the solution was again stirred for two minutes. The solution was stirred for two minutes each time at the end of the two following successive half-hour periods. The cell was introduced into the beaker after the last stirring and, shortly afterwards--exactly one and an half hours after the addition of conductivity water--the reading was made.

A clearer idea of the methods followed for timothy and red clover may be gained from the schedules on pages 16 and 24 respectively, which are the forms in which the data for all tests were recorded.

III. EXPERIMENTS AND RESULTS.

When this work was first begun, it was thought that the most logical way to attack the problem was to measure the resistance of the seeds themselves, much in the same manner as Osterhout measured the resistance of the Laminaria discs. But, when carried into practice, this method is beset with difficulties. As mentioned by Osterhout, the surface of the discs in contact with the electrodes is a great source of error and cannot be kept constant. He obviated this error by holding the discs off from the electrodes a short distance so that the current traveled from the electrodes through a small chamber of sea water before reaching the discs. With small seed, such as timothy and clover, this is not easily done. It was thought that, if the seeds were placed in the bottom of a small U-shaped cell and the electrodes lowered down the sides of the "U" until just above the seeds, this difficulty would be surmounted. But, now, the

difficulty of obtaining uniform packing of the seeds and of keeping some of the seeds from rising upward until they rested against the electrode surface presented itself. One complexity led to another and attention was turned to discover a better method. Several schemes were tried before the method finally used was discovered and adopted. In the following experiments, the results obtained from these preliminary methods are reported together with the final method.

Experiment 1.

The electrolytic cells used in this work were made according to the same design and specifications yet it is impossible to make cells so alike that they will give exactly the same resistance reading for a given solution. So an experiment was carried on to check the readings of the cells on hand--four of them--to determine the two cells that would give closest agreement. For this purpose, several readings were taken, with each cell, in conductivity water kept at a constant temperature of 25°C. The cells had all been previously cleaned and the electrodes platinized according to the method recommended by Findlay (19). They were immersed in 100 cc. conductivity water contained in Pyrex beakers that had been carefully cleaned and rinsed with conductivity water. The beakers containing the conductivity water and cells were placed in the water bath, regulated to 25°C., fifteen minutes before the readings were made. Several readings were made with each cell following the same procedure.

The readings obtained were carefully recorded. Comparison of them showed that cells "1" and "4" gave the closest agreement and most constant readings. The electrodes of cells "2" and "3" showed bright areas where the platinum black had not been deposited due either to impurities in the platinum or to dirt that had not been removed from the electrode

in the cleaning. The electrodes of cells "1" and "4" showed a uniform velvety layer of platinum black and this must have accounted for their greater constancy. They were used in all further experiments. The average resistance of the conductivity water, according to the three separate readings of cell "1", was 125,685 ohms; according to the readings of cell "2", 125,851. The difference between the average reading of the two cells is .13 of 1 per cent, which is the largest possible error that can result from their use. This was deemed allowable in this preliminary work, especially so since relative rather than absolute resistances were sought.

Experiment 2.

This experiment was performed to determine the resistance of the conductivity water used. The conductivity water as previously mentioned under "Materials", was triple distilled, the last time in an all glass still. The distillate came through the condenser hot enough to rid itself of ammonia vapor. The water was kept in very old five-gallon glass bottles. After standing in these bottles (tightly corked) for two weeks, the water showed only .6 of 1 per cent decrease in resistance. The error resulting from such a decrease, in measurements of solutions of 8,000 to 14,500 ohms resistance--the working limits in this investigation--would be very small and compatible with the aims of this paper, namely, to point out a fundamental relationship. The error mentioned above was further decreased by making up fresh conductivity water every six or seven days.

The following table gives the results of three readings with the two cells adopted.

Table 4. Resistance (in ohms) of Conductivity Water.

	Reading			
	First	Second	Third	Average
Cell "1"	125,709	125,649	125,696	125,685
Cell "4"	125,974	125,782	125,798	125,861

Average reading (both cells) = 125,768.

Conductivity = $\frac{1}{125768}$ or 7.95×10^{-6}

Experiment 3.

After abandoning the method of measuring the resistance of the seeds themselves, the matter of relative absorption and excretion of salts, as indicated by conductivity measurements, was considered as a possible means of determining seed viability. Five-tenth gram amounts of timothy were soaked in NaCL and KCL solutions of varying concentration for different lengths of time. The dry seeds were placed in an improvised sieve made out of a 10 cm. length of large glass tubing (4 cm. in diameter) with a partition of paraffin across one end of it that was perforated with holes small enough to keep the seeds from passing through. The sieve, containing the seeds, was introduced into a beaker containing a definite amount of the salt solution. After standing in this a certain length of time, the seeds were washed by pouring a given amount of conductivity water through the sieve to remove the salt from the surface of the seeds. The outside of the sieve was also thoroughly cleansed of the salt. The sieve plus the washed seeds was then placed in a beaker containing 70 cc. of conductivity water and the electrolytic cell (immersion type) within the sieve. After a short interval, a resistance reading was made and, thereafter, at regular intervals. The readings measured the resistance of the conductivity water plus the salts excreted by the seeds plus the seeds themselves.

This method was unsatisfactory because (1) it gave very erratic results, (2) required too much time, and (3) because it was difficult to perfect a technique whereby each lot of seed would be given exactly the same treatment. Moreover, in some cases, almost all of the seeds rose to the surface of the salt solution and it was impossible to submerge them. In other cases, very few would rise to the surface. This inconstancy, no doubt, contributed toward the erratic results obtained which were further

augmented by the great amount of manipulation. Although very little work was done with this method because of its complicated nature and because it required too much time, it is not improbable that a technique could be developed that would make it easier as well as shorter. It was deemed advisable, however, to investigate some other methods.

Experiment 4.

Another method of attacking the problem from an entirely different angle was next tried and will be described in this experiment. It is the method which, with the modifications successively described herein, was finally adopted. The relative outward diffusion of electrolytes from dead and living seeds is made the basis of determining seed viability. The steps in the development of this method are taken up under the following subdivisions:

A. The first work with this method was done as follows: .5 gram samples of seed were put in clean Pyrex beakers of 125 cc. capacity and 100 cc. of conductivity water added. After an half hour, readings were begun and, thereafter, at intervals. These readings represent the resistance (in ohms) of the conductivity water plus the soluble salts that pass from the seeds out into the water plus the seeds themselves. For the sake of brevity, this resistance is hereafter referred to as "Solution Resistance." It is interesting to note the gradual drop in resistance of the two lots of seeds recorded in the table. Lot 1 is 73.9 per cent viable timothy; lot 2, 89.8 per cent viable timothy. The experiment was started at 2:05 P.M.

Table 5. Solution Resistances (in ohms) of 2 lots of timothy at various time intervals. 25°C.

	2:35 P.M.	3:05 P.M.	3:35 P.M.	4:05 P.M.	6:05 P.M.	7:30 P.M.	9:15 P.M.	10:15 P.M.	9:30(next) A.M.(day)
Lot 1	19076	16831	15919	15375	12816	11877	10856	10529	8484
Lot 2	19291	17870	17351	16320	14160	13353	12844	12339	8650

From the preceding table, it can be seen that the drop in resistance is steady throughout the course of the experiment but that, at no point, was there any marked difference between the resistance of the two lots, and that it took considerable time for the resistance to drop from 19076 to 8484 ohms, viz. 19 hours.

B. Diffusion of salts in water is a comparatively slow process. It was thought that if stirring were employed it would not only bring about the drop in resistance in a shorter period but it might also accentuate the differences in resistances between the two lots of seed at any particular time. The effect of stirring is brought out in table 6. The method used was exactly the same as that described under "A" except that, immediately after the addition of 100 cc. of conductivity water, the whole was vigorously stirred with a glass rod for five minutes. At the end of this time only a few seeds remained on the surface, thus obviating another undesirable feature of the previous method, for it is evident that all seeds should be submerged if accuracy is to be obtained. The experiment was started at 10:13 A.M.

Table 6. Influence of Stirring on Solution Resistances (in ohms) of Timothy at Various Time Intervals. 25°C.

	A. M.			P. M.			
	10:43	11:13	11:43	12:13	1:13	2:13	3:13
Lot 1	9389	8628	7761	7176	6844	6630	6410
Lot 2	16447	14883	11776	11462	9980	9105	8570

A comparison of tables 5 and 6 shows that, where the solution of seeds and conductivity water is stirred, the resistance drops at a much faster rate and that, at any one time, there is a greater difference between the two lots of seeds. The greatest difference occurs at 10:43 or after the seeds have been in the water one-half hour. Almost as great a

difference, however, is observed after the one-hour interval (at 11:48). It would appear that an half hour was too short a time to expect an accurate difference to show itself in the relative rate of outward diffusion or leaching of electrolytes from the two samples of seeds especially since the stirring is done only at the beginning of the test--after that natural diffusion is the only force operative in the distribution of the electrolyte through the solution. Consequently, it was decided to leave the seeds in the conductivity water for one hour and to stir the solution just prior to reading, allowing sufficient time, however, for equilibrium to be established.

C. The following two schedules, taken from the record sheets kept in this work, will show in the clearest manner just how and when the stirring was done in the method finally adopted. This is but a slight modification of the method of "B". The tests, it will be seen, were run in pairs.

Schedule 1.

2:38 - .5 gm. Lot 1 put in beaker with 100 cc. conductivity water	2:41 - .5 gm. Lot 2 put in beaker with 100 cc. conductivity water
2:53 - Solution stirred 3 minutes	2:56 - Solution stirred 3 minutes
3:23 - Solution stirred 2 minutes	3:26 - Solution stirred 2 minutes
3:28 - Electrolytic cell immersed in solution	3:31 - Electrolytic cell in beaker
3:38 - Reading: Solution Resistance=9849 ohms	3:41 - Reading: Solution Resistance=12658 ohms

Schedule 2 will be found on next page.

Schedule 2.

3:57 - .5 gm. Lot 1 put in beaker with 100 cc. conductivity water	4:00 - .5 gm. Lot 2 put in beaker with 100 cc. conductivity water
4:12 - Solution stirred 3 minutes	4:15 - Solution stirred 3 minutes
4:42 - Solution stirred 2 minutes	4:45 - Solution stirred 2 minutes
4:47 - Electrolytic cell immersed in solution	4:50 - Electrolytic cell immersed in solution
4:57 - Reading: Solution Resistance=9696 ohms	5:00 - Reading: Solution Resistance=12700 ohms

Other readings were made with the two lots of timothy to determine the constancy of the readings. The readings for "Lot 1" varied from 9400 to 10531 ohms; for "Lot 2", from 12537 to 12852 ohms. At first glance, these variations appear too large to be satisfactory but when one considers only the two most important sources of the variations--stirring and the taking of seed samples--they do not seem so. The stirring was all done by hand and all solutions were stirred as nearly alike as it was humanly possible to do, yet it is not unlikely that one solution was stirred a little more vigorously or, perhaps, for a little longer period than another and that, in this way, variations in readings that should agree were produced. The difficulty of securing two samples of seed, from the same lot, having exactly the same germination percentage, can be readily appreciated; also, the variations in resistance readings that would be produced by samples not having the same germination percentage. In the case of lot 1, the difference between the largest and smallest resistance readings obtained amounts to 9 per cent of the smallest reading; in the case of lot 2, to 4 per cent. These variations can be largely attributed to the "personal error" of the experiment, which with the improvement of the method

and the introduction of mechanical stirring devices should be materially decreased.

D. This part of the experiment was performed to determine whether the drop in resistance was caused by the leaching of electrolytes from the interior structure of the seeds, or merely by some mineral salts adhering to the surface of the seeds. The latter was thought possible, because of the extremely quick drop in resistance but improbable because of the constancy, within certain limits, of the results obtained. Yet it seemed a point requiring proof.

The method followed was the same as in "C" except that the seeds were washed, prior to use, in order to remove any electrolyte on the surface of the seeds. The seeds were placed in a beaker with 50 cc. of conductivity water and vigorously stirred for one minute to give them a thorough washing. They were then filtered off on ash-free filter paper and again washed by pouring 50 cc. of conductivity water over them. The bottom of the filter paper was punctured and the seeds washed into a beaker with a few cubic centimeters of conductivity water. The solution in the beaker was made up to its full amount by adding what remained of the 100 cc. of conductivity water, and the experiment continued as outlined under "C." The following results were obtained.

Table 7. Solution Resistance (in ohms) of Timothy Seeds at end of 1 hour and 6 hour intervals (after a preliminary washing). 25°C.

Time immersed	Lot 1 (73.9%)	Lot 2 (89.8%)
1 hour	14,616	23,442
6 hours	10,732	14,122

Referring to the schedules under "C", it is seen that the same general relationship of "solution resistances" between lots is maintained in the above table; e.g., in schedule 1 under "C" the resistance for

lot 1 is 9849 ohms and for lot 2, 12,638 ohms; in the preceding table, the resistance for lot 1 is 14,612 ohms and for lot 2, 25442 ohms. It is also apparent, from the resistance reading at the end of the 6-hour interval, that the fall in resistance is not so rapid as it is when no preliminary washing takes place (compare tables 6 and 7). This is probably due to the loss of salts during the washing period.

Experiment 5.

Three ways of artificially killing seeds were tested--etherization, chloroforming, and dry heat. The best results were obtained by heating in an oven for seven days at 90°C. in the case of timothy. Two lots of timothy were killed in this manner--lot 1 (73.9% viable) and lot 2 (89.8% viable). Both lots were put in and taken out of the oven at the same time, receiving the same handling in every way. It was intended to use these artificially killed seeds of both lots in making up mixtures of various germination percentages from 0 to 89.8%, but before using them this present experiment was undertaken to compare them from the standpoint of relative resistance. The method outlined under "C" of Experiment 4 was used. All readings were made at the end of one hour and at a temperature of 25°C.

Table 8. Solution Resistances (in ohms) of Artificially Killed Seeds.

Lot Number	Test Number			
	1	2	3	4
1	8,963	8,643	8,870	8,425
2	11,983	11,782	11,883	11,852

The table shows that the solutions from the two lots of "dead" seeds offer vastly different resistances to the passage of an electric current. It shows, further, that seeds artificially killed by dry heat

cannot be used in making up samples of different percentage germination.

Experiment 6.

The method of procedure described under "C" in Experiment 4 gave such constant readings after many trials that it was adopted as the best method found in the course of these investigations. In this experiment, it was used in measuring the solution resistances of .5 gm. samples of timothy of viability varying from 73.9 per cent to 89.8 per cent by increments of 5 per cent--i.e., 73.9 per cent, 75 per cent, 80 per cent, 85 per cent and 89.8 per cent. Since the results of Experiment 5 showed that it was not possible to use seeds artificially killed by heat and, since no very old timothy of low germination could be found, it was impossible to extend the limits of this range in germination.

The samples mentioned above were made up by mixing the seeds of lot 1 (73.9% viable) and those of lot 2 (89.8% viable) in varying proportions by weight. The weight of each lot of seed to be used in getting a sample of a certain percent germination was calculated according to the formula

$x(73.9)+.5-x(89.8)=.5(y)$, where x equals the grams of 73.9 per cent seed to be used and y equals the percentage germination desired in the mixture. For example, to find out how many grams of 73.9 per cent and 89.8 per cent seed must be mixed to secure .5 gm. sample of 85 per cent germination, one would write the equation

$$x(73.9)+.5-x(89.8)=.5(85)$$

Solving, we find $x=.151$ or the number of grams of seed of lot one (73.9%) that must be used. Then, $.5-.151=.349$, the number of grams of seed of lot 2 (89.8%) to be used. By mixing these amounts of lot 1 and

lot 2, a sample of 85 per cent germination is secured. Three samples of each percentage germination were carefully made up according to this formula.

The solution resistances of the samples of various per cent germination were measured according to the method outlined under "C" of Experiment 4. All samples were stirred as uniformly as possible and, to insure this, the strokes made with stirring rod were timed. Forty-five strokes were made per quarter minute and, at the end of each such period, the direction of stirring was reversed. The results obtained are tabulated in table 9.

Table 9. Solution Resistances of Timothy. 25°C.

Per cent germination	Test 1	Test 2	Test 3	Average
73.9	10,331	9,400	9,485	9,772
75.0	10,089	9,930	10,070	10,027
80.0	10,617	10,998	11,122	10,912
85.0	11,211	11,627	11,363	11,400
89.8	12,600	12,852	12,337	12,590

The results of the above table are graphically represented in figure 4.

Experiment 7.

The resistance of a solution is greatly affected by any change in temperature. Although all of the readings of this work were made at a temperature of 25°C., a constant temperature bath being employed that kept the temperature to within 1/10 of a degree of that point, it was considered worthwhile to compute the temperature coefficient of the solution consisting of timothy seed in conductivity water.

Four readings were taken at each of three temperatures--20°, 25°, and 30°Centigrade--in the two lots of timothy. The average of each

four readings was taken as the basis for further calculation. The following table gives the readings taken, using the method described in "C" of Experiment 4.

Table 10. Solution Resistances (in ohms) of Timothy Seeds at 20°, 25°, and 30° Centigrade.

	20°C.	25°C.	30°C.
Lot 1 (73.9%)	11,284	9,849	8,568
	11,086	9,696	8,474
	11,109	9,485	8,482
	11,097	9,749	8,630
	Average=11,144	Average=9,682	Average=8,526
Lot 2 (89.8%)	13,783	12,638	11,234
	13,902	12,700	11,016
	13,891	12,600	11,170
	13,844	12,827	11,224
	Average=13,855	Average=12,691	Average=11,161

In the case of lot 1, the resistance at 25° is 13.6 per cent higher than at 30°; at 20°, it is 15.1 per cent higher than at 25°. In the case of lot 2, the resistance at 25° is 13.7 per cent higher than at 30°; at 20°, it is 9.2 per cent higher than at 25°. The average percentage increase for a five-degree drop in temperature is 12.9 per cent, or the average percentage increase per degree drop is 2.6.

Suppose a reading is made when the temperature is 27°C. and it is desired to know what the reading for the same sample would be at 25°C. Since the average increase per degree drop is 2.6%, the percentage increase from 27° to 25°C. would be 2x2.6 or 5.2, the factor by which the reading at 27° must be increased to give the reading of the same lot at 25°. Or stated algebraically,

$$R_{25^\circ} = R_{27^\circ} + .026(27-25)R_{27^\circ}$$

A more general expression of the interpolation of a reading at any temperature--within the limits of 20° - 30°C.--to a temperature of 25°C. would be

$$R_{25^{\circ}C} = R_x o + .026(x^{\circ} - 25^{\circ})R_x o$$

$$= R_x o + (.026x - .65)R_x o$$

$$= R_x o(1 + .026x - .65)$$

$$= R_x o(.35 + .026x)^{*}$$

Attention was now turned to applying the method, adopted as the best in the case of timothy, to another type of seed, namely, red clover. The following experiments deal with the efforts made in this direction.

Experiment 8.

The first thing to determine in applying the method to red clover was the best amount of seed to use and, also, the length of time the seed should be allowed to remain in the conductivity water before making a reading. This experiment deals with these two more or less interlocking phases. It was decided, from the first, to use "stirring" since it hastens the even distribution of electrolytes throughout the solution.

A. Seeds used were samples of lot 3 (91.8% viable) and lot 4 (5.6% viable). Schedule 5 (on next page) outlines the procedure and gives the results obtained at certain time intervals. All readings were made at 25°C.

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*This formula does not give absolute accuracy nor is this claimed for it. It is included here to show that the method of seed viability determination herein given could be used even though no means for keeping the temperature constant were at hand. (See Discussion of Results)

Schedule 3.

9:30 - .5 gm. Lot 3 put in beaker with 100 cc. conductivity water	9:33 - .5 gm. Lot 4 put in beaker with 100 cc. conductivity water
9:45 - Solution stirred 2 minutes	9:48 - Solution stirred 2 minutes
10:15 - Solution stirred 2 minutes and electrolytic cell immersed	10:18 - Solution stirred 2 minutes and electrolytic cell immersed
10:30 - Reading: Solution Resistance=56,035 ohms	10:33 - Reading: Solution Resistance=38,387 ohms
10:45 - Solution stirred 2 minutes	10:48 - Solution stirred 2 minutes
11:00 - Reading: Solution Resistance=40,185 ohms	11:03 - Reading: Solution Resistance=22,706 ohms
11:15 - Solution stirred 2 minutes	11:18 - Solution stirred 2 minutes
11:30 - Reading: Solution Resistance=26,999 ohms	11:33 - Reading: Solution Resistance=15,755 ohms
11:45 - Solution stirred 2 minutes	11:48 - Solution stirred 2 minutes
12:00 - Reading: Solution Resistance=19,187 ohms	12:03 - Reading: Solution Resistance=9,749 ohms
1:45 - Solution stirred 2 minutes	1:48 - Solution stirred 2 minutes
2:00 - Reading: Solution Resistance=10,770 ohms	2:03 - Reading: Solution Resistance=4,076 ohms.

The same procedure was again followed using .5 gm. samples of the same seeds but this time the first two stirrings were of three minutes duration. At the end of the first hour, the solution resistance of Lot 3 was 46,512 ohms; of Lot 4, 28,443 ohms. At the end of two hours, the solution resistance of Lot 3 was 21,726 ohms; of Lot 4, 10,850 ohms. The results were similar to those in the preceding table except that the readings were somewhat lower but the drop was proportionally the same

during the first two hours.

B. Some tests were run, using 1 gram samples and taking readings at the end of the one and one and an half hour periods. The solutions were stirred every fifteen minutes (for two minutes), and immediately after the last stirring--fifteen minutes before the reading was taken--the electrolytic cell was immersed in the solution. All readings were made at 25°C. The following table shows the results obtained.

Table 11. Solution Resistances of Red Clover for 1 hr. and 1-1/2 hrs. periods. 25°C.

Resistance (in ohms) at end of	Lot 3 (91.8%)		Lot 4 (3.6%)			
	Lot 3	Lot 4	Lot 3	Lot 4	Lot 3	Lot 4
1 hour	27633	18162	- -	- -	- -	- -
1-1/2 hours	17473	9653	16021	9739	18679	9218

The readings at the end of the 1-1/2 hour period were at a better "working level" than at the end of the 1 hour period. In other words, the resistance values were at a level low enough that slight mistakes in weighing the samples or in measuring the conductivity water did not produce too large errors, yet they were high enough that an appreciable difference existed between the readings for the two lots. The agreement between the readings for samples of the same lot is not so close as desired, however.

C. It was decided to use 1 gram samples of red clover seeds and to allow them to remain in the conductivity water for 1-1/2 hours before reading the resistance, then to modify the time and interval of stirring to find out which would give the most constant results. The details of this work need not be given, but the method finally adopted is made clear, and some results obtained by its use are recorded in the following schedule. (Schedule 4 on next page)

Schedule 4.

8:30 - 1 gram Lot 3 put in beaker with 100 cc. conductivity water	8:34 - 1 gram Lot 4 put in beaker with 100 cc. conductivity water
8:45 - Solution stirred 2 minutes	8:49 - Solution stirred 2 minutes
9:15 - Solution stirred 2 minutes	9:19 - Solution stirred 2 minutes
9:45 - Solution stirred 2 minutes and electrolytic cell immersed	9:49 - Solution stirred 2 minutes and electrolytic cell immersed
10:00 - Reading: Solution Resistance=17,695 ohms	10:04 - Reading: Solution Resistance=9,273 ohms

Other readings obtained, using precisely the same method, were: for lot 3, 17,551 ohms, 17,990 ohms; for lot 4, 9,182 ohms, 8,669 ohms.

This method gave, by far, the most constant readings obtained with red clover. Some variation in the readings, of course, cannot be avoided since there are bound to be some samples that are not representative of the germination of the lot of seed from which taken.

Experiment 9.

This experiment deals with the application of the method of "C" under Experiment 8 to samples of red clover ranging in germination from 3.6 per cent to 91.8 per cent. The amount of each lot to use in a sample of, say, 40 per cent germination was calculated from the formula

$x(3.6)+1.-x(91.8)=1.(40)$, which is identically the same as that used in making up the timothy samples.

The method followed is exactly the same as that given under "C" of Experiment 8. The following table records the results that were obtained.

(See table 12 on next page)

Table 12. Solution Resistances (in ohms) of Red Clover Seed. 25°C.

Percentage Germination	Test 1	2	3	4	Average Reading
3.6	9173	9132	8056	8669	8770
10.0	8417	8200	9007	7955	8395
20.0	8939	8618	9441	8208	8814
30.0	9979	10504	9423	8000	9478
40.0	9557	9015	11413	8770	9689
50.0	10976	10832	10504	9126	10360
60.0	9500	9619	12120	9820	10265
70.0	12776	11436	12625	10231	11767
80.0	11814	11933	13585	10621	11863
90.0	14056	15506	14906	12549	14254
91.8	13427	14300	14529	15291	14387

The above table shows that there is an increase in resistance as the germination becomes greater and, but for two exceptions, the increase is fairly regular.

The data included in Table 12 are shown graphically in figure 5.

IV. DISCUSSION OF RESULTS.

In the past ten years, much work has been done dealing with the determination of life or death by means of electrical conductivity measurements. Many different plant and animal tissues were subjected to such electrical determinations and, in some cases, with noteworthy results. A thorough review of the literature available failed to reveal a single instance, however, in which seeds were the subject of investigation. As

far as is known by the author, this is the first work in which it was attempted to determine seed viability by electrical conductivity measurements.

The two methods prelimi-/narily used have been fully described and discussed under "Experiments and Results." Since they have been discarded, they need not be again discussed. The discussion is limited, then, to the finally adopted method and to the results obtained by its use.

In table 9, are shown the results obtained with timothy of various percentage germination at a temperature of 25°C. It is readily seen that there is a progressive increase in solution resistance with viability increase. However, there are some discrepancies. Several readings for a certain percentage viability are too high. This, however, is to be expected as will be explained in the consideration of a specific case. In one test of 73.9 per cent seed, a solution resistance of 10,331 ohms was found, which is not only higher than either of the other readings obtained for seeds of that viability but also higher than any reading for 75 per cent seed, and almost as high as the readings for 80 per cent seed. This can only be explained by attributing it to a personal error made in technique, or by supposing that the particular sample of seed, giving the higher reading, was of higher germination than the average sample of that class. The latter is a very valid supposition, for, when the germination of the lots of seed were originally determined by sprouting, it was found that the different samples used would vary over a range of 7 per cent for lot 3 to 22 per cent for lot 1. The germination of lot 1, according to the average of ten tests of 100 seeds each, was 73.9 per cent; the highest test being 83 per cent, the lowest, 61 per cent. This difference was obtained despite the very thorough

mixing of seed lots prior to sampling. So it does not seem unreasonable to suppose that the sample giving the reading 10,331 ohms was of germination considerably higher than 73.9 per cent.

More conclusive proof would have been offered in support of the correlation between electrical resistance and seed viability had it been possible to extend the range of germination percentages of the samples of timothy in table 9. It was originally intended to use artificially killed seeds and, by mixing these with seeds of lot 2 (89.8%), to make up .5 gram samples ranging in viability from 0 to 89.8 per cent. But, as the data in table 8 show, artificially killed timothy seeds from two different lots did not give the same resistance. Yet, the readings for either lot are so surprisingly constant that errors in method or technique could not be blamed. Neither lot of killed seeds showed the least sign of germinating when tested and were believed to be dead. Being dead, accordingly, they should show very nearly the same resistance. Since they did not, the conclusions reached were that either the seeds were not killed (but brought into dormancy) or that the changes that take place in the natural death of a seed were not affected by the artificial death produced. Or it may be that, because of the suddenness of artificial death, the seeds retain part of the chemical constitution of live seeds which, however, is lost in the longer process of naturally dying. Since the readings for the killed seeds did not agree, it was thought best to dismiss both artificially killed lots and to use only seeds that had lost their vitality in a natural manner. Very old timothy seeds were sought in the hopes of getting some of very low germination. When none was to be had, the tests were confined to the limits represented by the germination of lots 1 and 2.

An interesting sideline of the resistance measurements, taken

with timothy, was the determination of what produced the conductivity of the solution. After a test had been run, using a lot 1 sample, the solution was filtered to remove the seeds, and a portion of the filtrate sent to the Experiment Station Chemist for analysis. His report showed that 14 per cent of the mineral content of the solution was P_2O_5, that an appreciable amount of Ca was present (probably as $CaHPO_4$), and that there were traces of SO_4, Mg, and K present. Due to the minute quantity of mineral matter present in the solution, he succeeded in making a quantitative test for P_2O_5 only; for the others, only qualitative tests were made.

The most important point brought out by the data is that a difference does exist between the solution resistances of seeds of high and low germination (see figures 4 and 5). That is the fundamental relationship that must form the basis of any future work that looks toward the practical application of this method in the determination of the germination percentage of any lot of seed.

The same method was used in measuring the solution resistance of red clover as was used for timothy except that 1 gram of the former was used and that the time and interval of stirring were slightly changed (see schedule 4). The readings obtained for samples of red clover, ranging in germination from 3.6 per cent to 91.8 per cent are shown in table 12.

A glance at the data of table 12 suffices to tell one that the resistance increases with the germination but, upon analysis, one finds several discrepancies. Some readings seem too high, others too low. No more can be said regarding these than has already been said concerning those in the table of timothy readings, viz., that they resulted (1) from a sample having a much higher or lower germination than it was

supposed to have, or (2) from a "personal error" in conducting the experiment.

The average readings for red clover when incorporated into a graph (figure 5) show that, with two exceptions, the resistance increase is concomitant with viability increase. That is the important thing for it forms a foundation upon which future work can be based that will make the method practical for determining the viability of red clover. The immediate object of this investigation was not to develop a method whereby seed viability could be measured by electrical conductivity, but to attempt to discover a fundamental correlation between seed viability and electrical conductivity. Once established, such a basic relationship might be put to practical use in the laboratories of seed firms, as a quicker and cheaper means of determining the percentage of germination of any lot of seed, than the present method of running germination tests.

The temperature coefficient of resistance was worked out for timothy merely to show that it is not absolutely essential to have such an expensive piece of apparatus as a constant temperature water bath to conduct the tests; also, to show one way in which a temperature coefficient might be calculated. When a practical method, based on the correlation discussed, is perfected, the work of developing it will have been done in a laboratory where a constant temperature bath is available. All the readings, for a certain kind of seed, will have been taken, then, at the same temperature (e.g., 25°C.) and embodied in a table or graph. If a temperature coefficient be carefully worked out at the same time, a worker in another laboratory could make his test at room temperature and, by means of the temperature coefficient, interpolate the resistance reading obtained to its 25°C. value. Referring to the table or graph containing

the solution resistance values for seeds of various percentage germination at 25°C., he could find immediately the viability of that particular sample. The coefficient will vary in value for seed of different viability but this could, no doubt, be mathematically calculated and included in the formula.

Many flaws can be found in the method described in this paper and the writer is fully cognizant of them. Following are some of the recommendations for future work that will tend toward the elimination of these faults.

1. In this investigation, all the stirring was done by hand. As previously explained, attention was given to stirring each sample just as nearly like the others as was humanly possible. To stir all exactly alike, however, is impossible. Likewise, it is impossible for two men to stir exactly alike. To obviate the unavoidable errors resulting, it would seem a great improvement to stir the solutions mechanically. This would not only give more constant and dependable results but, it is believed, it would also make the method shorter and easier to conduct. It has been shown that the drop in resistance of a solution to the "working level" occurs much more quickly when the solution is stirred than when not stirred. If the solution were stirred continually, by mechanical means, there is no doubt that the drop to the "working level" would require yet less time.

2. It would be advisable to use larger samples of seed and proportionally more conductivity water. The difficulty of getting fair samples, from the standpoint of germination percentage, has been emphasized and it is logical to suppose that the larger the sample the more nearly it will approximate the germination of the well-mixed lot from which it

is taken.

3. The solution resistance of timothy in conductivity water was taken at the end of one hour, i.e., the readings were begun at that time. The average time required to take a reading was two minutes. However, in one case it may have required less than two minutes, in another case, longer. If something occurred that delayed the reading (and it did several times), the solution resistance would be low due to the outward diffusion from the seed over a longer period. It would seem an advantage to draw off a definite amount of the solution just as the one-hour period (or whatever it may be) elapsed. Then, it would matter little whether the readings were made immediately or shortly afterwards.

4. The conductivity water used in this work was made up fresh every six or seven days. It was always prepared in precisely the same manner and by using the same distilling apparatus. Successive lots agreed so closely in resistance that the difference between them was ignored in calculating the solution resistances, since relative rather than absolute results were sought. But, if the method were used in different laboratories, it would be extremely difficult for all to make or secure conductivity water of the same resistance. A means of calculating the solution resistance is needed that takes into consideration the resistance of the conductivity water used, which will enable workers in various laboratories to use conductivity water of their own making and, for the same lot of seed, to obtain identical results.

5. Very satisfactory results were obtained with the electrolytic cells used but there are several reasons why an open type of cup, such as is used with the soils bridge, would prove more satisfactory. In the

first place, the cells are very fragile. Because the electrodes are enclosed in glass (see figure 3), they are very difficult to clean prior to replatinizing or to keep clean. Furthermore, they are difficult to make so similar that any two cells will give the same resistance reading for a given solution. An open cup made of hard rubber would seem much more desirable. It would be cheaper, less fragile and easier to keep clean. Because of its open nature, it would not be difficult to make the electrodes of exactly the same dimensions and the same distance apart.

6. For future work, it is essential that old samples of timothy and red clover seeds be procured that will be of all varying degrees of viability. The solution resistance of a sample of red clover that is, we shall assume, 50 per cent viable could then be compared with that obtained for the 50 per cent samples used in this work, which were made up by mixing red clover seeds of lots 3 and 4. In other words, the solution resistances obtained for artificially-mixed samples could be compared with natural samples having the same germination percentages. This seems a very important consideration.

7. Seeds from various sections of the country, having the same viability should also be secured to see what influence soil has on the electrolyte content or composition of seed as indicated by comparative solution resistance measurements.

8. It is possible to conceive a sample of, say, 50 per cent germination giving the same solution resistance as a 70 per cent sample. The seeds of both samples might contain a like number of dead cells (having permeable membranes) but due to the more fortuitous location of such dead cells (farther removed from the germ) in the one sample, a higher viability might obtain, although of a weaker nature. This possible relationship needs to be investigated.

V. SUMMARY.

These investigations dealt with an attempt to determine seed viability by an electrical conductivity method.

The seeds used were timothy and red clover.

Three different methods were used in studying the possible correlation between seed viability and electrical resistance. These were:

1. Measuring the resistance of the seeds themselves.
2. Comparing the relative absorption and excretion of salts.
3. Measuring the relative outward diffusion of electrolytes as indicated by conductivity readings.

Methods one and two were discarded.

Method three was adopted.

The readings obtained by its use seem to indicate that a correlation between seed viability and electrical conductivity exists which, with future improvement, may be capable of practical application.

VI. ACKNOWLEDGMENT.

The writer wishes to express his deep appreciation to Doctors R. P. Hibbard and E. A. Bessey for the many kind suggestions and the very helpful criticism that they gave him concerning his work; also to Doctor Robinson for much help in connection with the apparatus used, and to Prof. R. N. Haskell for his valuable assistance in dealing with the mathematical aspects of the problem.

ADDENDUM.

At the last moment, two old samples of timothy were obtained from the Albert Dickinson Seed Company. The samples gave solution resistance readings of 8,048 and 8,551 ohms, which are both considerably below the lower limit of the range covered by the artificially-mixed samples. This indicated that their germination should be expected to be considerably less than 73.9 per cent. Germination tests with some of the soaked seeds (200) used for the resistance measurements gave 40 per cent and 33 per cent germination respectively. It has been found, by comparison with germination tests using ordinary dry seeds, that such previously soaked seeds give only approximate results, so that the percentages reported are merely indicative of a germination considerably lower than 70 per cent.

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VIII. DESCRIPTION OF ILLUSTRATIONS.

Figure 1. Complete setup.

Figure 2. Scheme of connections of apparatus used in these investigations.

Figure 3. Electrolytic cell of immersion type used to measure solution resistances.

Figure 4. Graph showing the solution resistances for timothy of various per cent germination. No average curve was computed for these data because of the narrow range over which the germination percentages extend.

Figure 5. Graph showing the solution resistances for red clover of various per cent germination. The curve of best fit was computed by the method of "least squares."

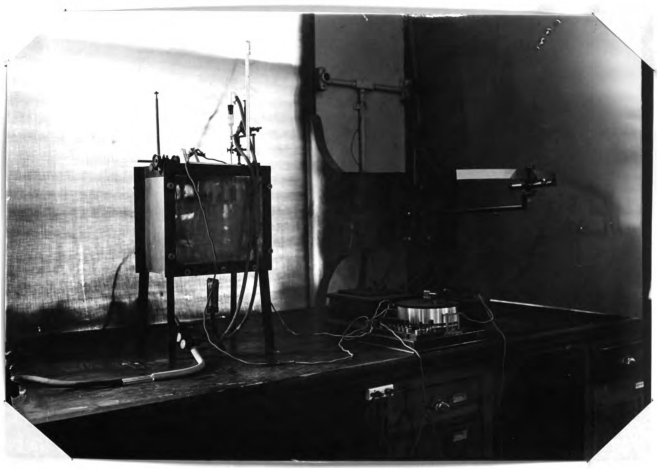

Figure 1. Complete setup.

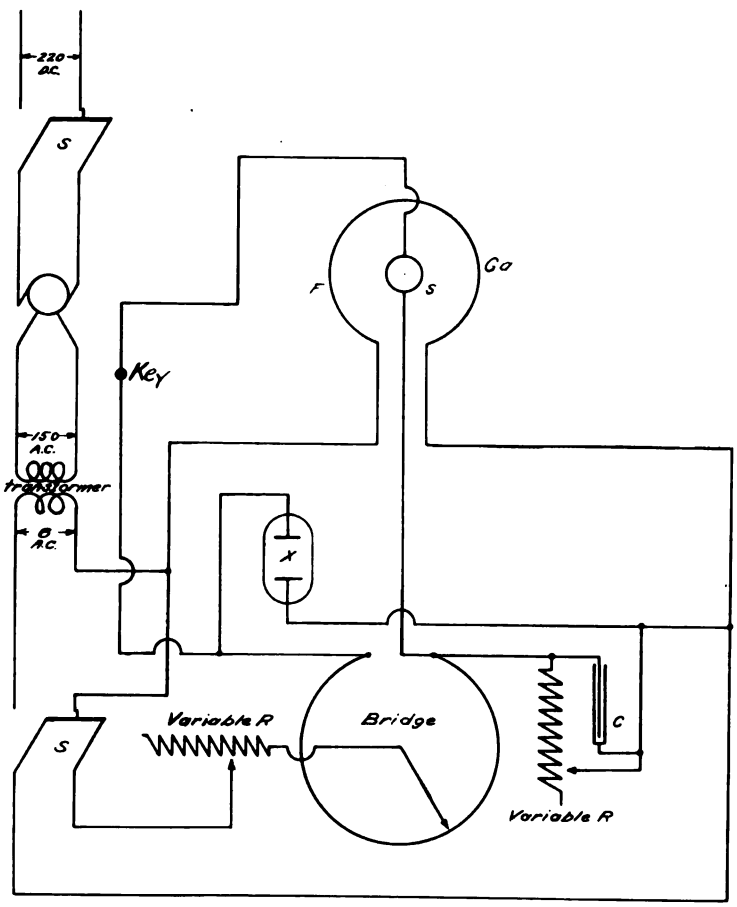

Figure 2. Diagram of scheme of connections.

Figure 3. Electrolytic cell.

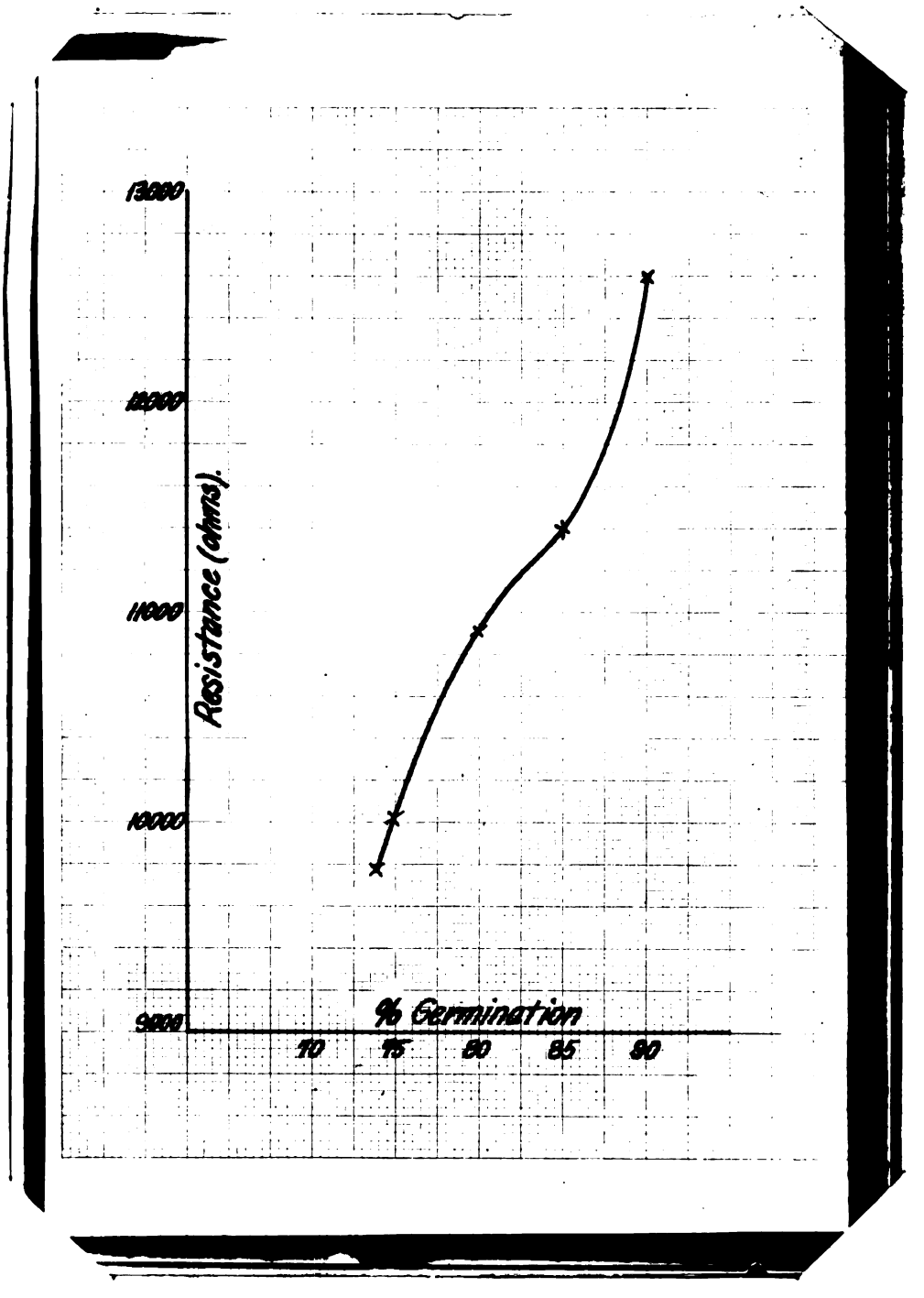

Figure 4. Graph showing solution resistances for timothy.

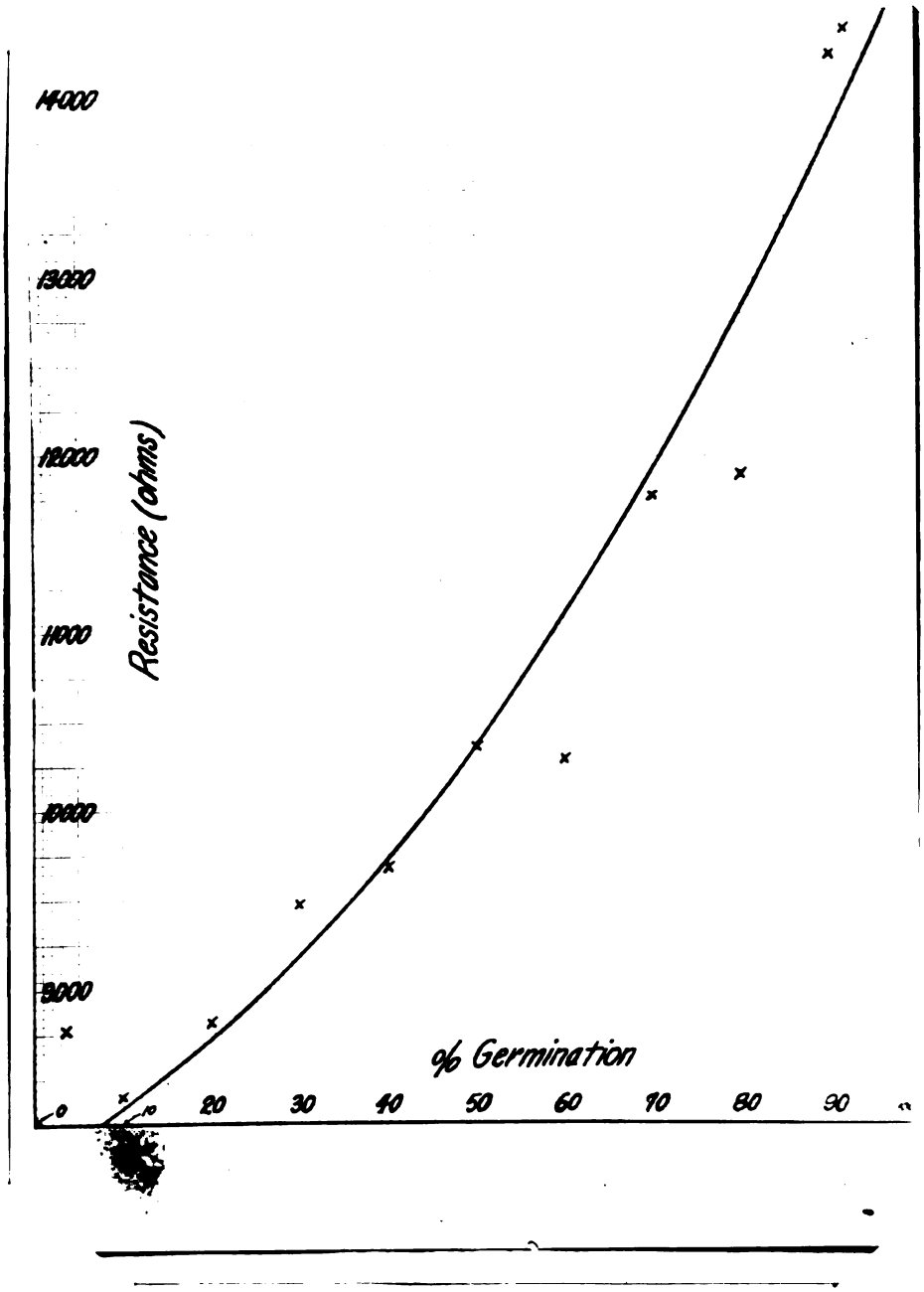

Figure 5. Graph showing solution resistances for red clover.