CHEMICAL TREATMENTS TO HASTEN AND INCREASE GERMINATION OF BLACK LOCUST SEED

By

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PREFACE

Various treatments to hasten and increase germination of black locust seed were conducted by the writer, and the conclusions drawn therefrom are presented here in an effort to evaluate them. During the course of the experiments, three important results were realized: first, further support was given to arguments for the use of sulfuric acid; second, practically no correlation could be found between swelling and germination percentages; and third, the use of detergents with water may be found satisfactory in treating certain lots of seeds.

The reported results are entirely valued by sound statistical method. Of necessity, the tests had to be limited in number, as well as in variations in the method of treatment. The conclusions point up hitherto fairly well known facts on pretreatments, as well as opening the door to further research along these lines.

It is with pleasure that I express my obligation to Leigh J. Young, Professor of Silviculture of the School of Forestry and Conservation, for his helpful advice given during the experimental period. Gratitude is also due to B. A. Soule, Associate Professor of Chemistry, for his contributions on techniques in the handling of chemicals, to R. S. Johnson, Instructor in Statistics, for the statistical methods used in this paper and to F. C. Stahl, Chief of the State of Michigan Seed Testing Laboratory, Lansing, Michigan, for his time spent demonstrating the methods used in government seed testing.

CHEMICAL TREATMENTS TO HASTEN AND INCREASE GERMINATION OF BLACK LOCUST SEED

Statement of the Problem

The recently increased use of black locust (*Robinia pseudoacacia* L.) in various parts of the country to control soil erosion requires the production of many seedlings annually. To take care of this need, nurserymen have seeded black locust extensively but have been unable to obtain satisfactory germination. This is due to a very hard, impermeable seed coat which is characteristic of other members of the Leguminosae.

A high percentage of any lot of black locust seed is sound and capable of germination without delay if the embryo can obtain sufficient moisture to germinate. The problem resulting from a delay in germination may be seen most clearly after an examination of nursery plantings of black locust. After sowing, a portion of the seeds will germinate within the first month with successively smaller numbers germinating during the later months and throughout the succeeding three or four growing seasons. This condition results in uneven-aged nursery stock. Any attempt made to reseed the area after transplanting the initial crop of seedlings will be met with this constant, though dragged out, germination of black locust which acts on later seedings much as common weeds do on flowers in a garden. Furthermore, economic loss is suffered when any large quantities of viable seeds are left to rot in the seed beds. "Replies to a questionaire sent out by the Forest Service in 1935, comprising reports from 40 nurseries located throughout the United States, indicate that on an average not more than 8% of sown seed produce nursery seedlings." (7, page 1.)

Conclusions Reached in Earlier Experiments

Several treatments have been developed to remedy this condition. The object of the treatments has been to make the seed coat more permeable to moisture by softening the seed coat, by reducing the volume of the seed coat, or by removing a waxy covering on the surface of the coat. Specifically, the most widely used treatments and the most important are (1) soaking the seed in water of varying temperatures for different lengths of time until a large percentage are swollen, (2) scarification by mechanical means, (3) the use of wax solvents to remove the wax coating, and (4) steeping the seeds in concentrated sulphuric acid (specific gravity 1.84).

Scarification or abrasion of the seed coat is one of the oldest methods and has, in some cases, been very successful. Hurst and others (4)* have invented disk and barrel

*Figures in parentheses refer to References cited, listed at the end of this report.

scarifiers especially designed for the treatment of leguminous seeds. Impermeability is due to the thickness, as well as to the porosity, of the seed coat. Therefore, the major problem in using scarification is to determine the severity of treatment to suit the requirements of individual lots of seed. The mechanical treatment has the advantage, however, in that the seeds are left in a hard condition and thus can be stored or seeded mechanically with no extra care.

The hot water treatment has been recommended by Toumey and Korstian (10), who advocate soaking in water at a temperature of 190° to 195° F., using four to five times as much water as seed and setting the vessel aside until most of the seeds have swollen. Mattoon (5) suggests initial immersion of the seeds at 160° F. and permitting them to soak for 20 hours at cooler temperatures. Toumey and Durland (9) advocate soaking seeds in hot water from 5° to 10° F. below the boiling point for one or more days to get increased germination percentages. However, they have demonstrated that overlong soaking in hot or warm water reduces germination percentages of the seed. The hot water treatment has been moderately successful with some strains of seeds, but the disadvantage lies in the swollen condition of the seed which necessitates immediate sowing, care in handling, and the abandonment of any large scale mechanical sowing methods. The advantage is in safety of treatment in so far as the nursery worker is concerned.

A small number of experiments have been conducted by McKeever (6) using xylene, acetone, and similar wax solvents in the belief that the coating of wax on the seed coat was delaying the penetration of moisture. The results indicated a correlation between the quantity of wax and the germination, since germination was increased with the removal of the wax.

The sulphuric acid treatment has been advocated by Heit (2) and (3), Meginnis (7), and others who claim that the highest germination has been obtained by using this method.

The sulphuric acid treatment has three main advantages: (1) it has given the best results for most lots of seed, with germination figures of sulphuric acid treated seeds as much as three times greater than untreated seeds of the same lot; (2) the treated seeds may be dried and stored for a period of time with little loss of viability, and (3) since the seed has taken on no water it is still in a hard, firm condition and therefore may be planted by mechanical means any time after treatment.*

There are two disadvantages in using sulphuric acid: Because the acid tends to carbonize at temperatures above 75°F, care must be exercised in conducting the treatments to avoid higher temperatures. Sulphuric acid, when combined with heat, will burn away the seed coat and destroy viability through contact with the embryo in a much shorter time than

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*Meginnis conducted tests which indicated that treated seed may be stored up to one year, depending on condition of seed and severity of treatment.

when sulphuric acid is used at room temperature. Due to the difference in volume of seed coats from seeds of different lots, small scale experiments must be run to determine the length of treatment to insure maximum germination of all seeds in the individual lots. In addition, the extreme heat generated by sulphuric acid when in contact with a physical object necessitates caution on the part of the worker making the treatments. This is an important disadvantage which can be reduced to a minimum only by providing adequate instructions in its use or by developing a better and safer treatment.

A New Approach through the Use of a Detergent

The use of a detergent in combination with water to overcome impermeability has never been attempted. This chemical, similar to "soapless soaps," reduces surface tension and enables water to penetrate more quickly. Soaking in ordinary water produces significant results in the majority of cases. However, it takes successive treatments to penetrate the harder seed coats. This procedure consumes many man-hours in sorting the swollen from unswollen seeds. If sorting is not done, as is usually the case, the seeds which swelled on the first treatment and were presumably viable are rotting while other seeds take on moisture. When this happens there will be more seeds rotting from overlong soaking than there are seeds swelling, with the resultant trend away from the maximum germination point.

The introduction of a small amount of "Water-wetter"* into water at room temperature should enable a larger percentage of seeds to take on moisture in a shorter time, thereby overcoming the loss of viable seed due to overlong soaking and subsequent rotting. After treatment, the seeds would be in a swolen condition, necessitating care in handling and abandonment of mechanical sowing. The advantages of the treatment lie in the ease and safety of the treatment in so far as the nursery worker is concerned, as well as in reduction of losses due to carbonization attendant upon using sulphuric acid.

The discussion to follow deals with tests made and conclusions drawn therefrom to serve as a check on the work done by previous writers and to experiment with a detergent-water solution. The experiment and discussion which follows include (1) the establishment of adequate controls, (2) soaking in water for various lengths of time, (3) the correlation between swelling and germination, (4) the use of a 0.1% solution of detergent and distilled water, (5) the use of a 0.1% solution of detergent and city water, and (6) the steeping in sulphuric acid (specific gravity 1.84) for periods of up to two hours.

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*Trade name of the O. E. Linck Co., Clifton, N. J.

The Establishment of Adequate Controls to Serve as a Check for Experiments

Methods.- Seven lots of seed were used in these experiments. Sources and dates of collection are listed with their identifying letter as follows:

Lot A - by F. W. Schumacher in Massachusetts
Lot B - Shawnee National Forest, Ill., in 1940
Lot C - Wayne National Forest, Ohio, in 1941
Lot D - Jackson County, Indiana, in 1941
Lot E - Harrison County, Indiana, in 1944
Lot F - Clark County, Indiana, 1945
Lot G - Clark County, Indiana, in 1946

Samples of 50 seeds each were selected at random from all lots. Only apparently sound seeds were drawn for the tests without regard to size, color, shape, or other characteristics. Purity percent of all lots was calculated to be between 90 and 95%. The tests were run under as nearly similar temperature conditions as possible, the temperature being that of a room 75° F. plus or minus 10°F.

The control lots were set up to germinate in 3½" petri dishes with two layers of moistened filter paper in the bottom. Germination counts were made every 24 hour period to obtain not only the final germination percent, but also to obtain the percent germination from day to day. The dishes were watered at the time of counting by a fine sprayer so that moisture was always present. Molds were present to a small extent in all but a few isolated tests but were considered to be of insufficient quantity to interfere with seed germination. The tests were allowed to run until five successive days had passed with no germination.

Results.- Total percentage of germination of the control lots is shown in Table I, page 10. With the exception of lots C and G, germination percentages were between 20% and 35%. In one case, lot B, the germination amounted to 44%. The curves showing germination percentages by days or percentage acceleration by days in charts 1 through 7, indicate that normal germination of black locust seed closely follows the pattern of a normal distribution curve for the period of the experiment.* The tail on the right of the curve points out the intermittant germination of the seeds which is characteristic.

The purpose of this entire experiment is to analyze the effects of various chemical treatments on seed coat impermeability, making pertinent conclusions on each lot separately. From an examination of the curves in charts 1 through 7, a difference in dispersion and in kurtosis**

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*Statistically, there is a slight skewness to the right, since the right tail could progress to infinity whereas the left tail could not start from a point less than zero.

**Kurtosis expresses the degree of peakedness of a curve. Dispersion is the variability among the seeds in time of germination.

Table I. Final Germination Percentages of Controls by Sample Numbers[1]

Lot	Sample Numbers 1	2	3	4	5	6	Total Germ.	Average[2] Percent Germ.
A	13	6	13	11	11	12	66	22.0
B	24	26	26	23	15	20	134	44.6
C	9	6	5	7	8	4	39	13.0
D	18	12	22	19	12	14	97	32.3
E	9	8	10	8	5	18	58	19.1
F	19	13	15	23	19	20	109	34.9
G	5	1	9	8	3	6	32	10.5

(1) Each sample contained 50 seeds.

(2) Average Percent Germination found by combining the 6 samples of 50 so that $N = 300$, ΣX = No. of seeds germinated or 66; and using formula

$$\bar{X} = \frac{\Sigma X}{N} \times 100$$

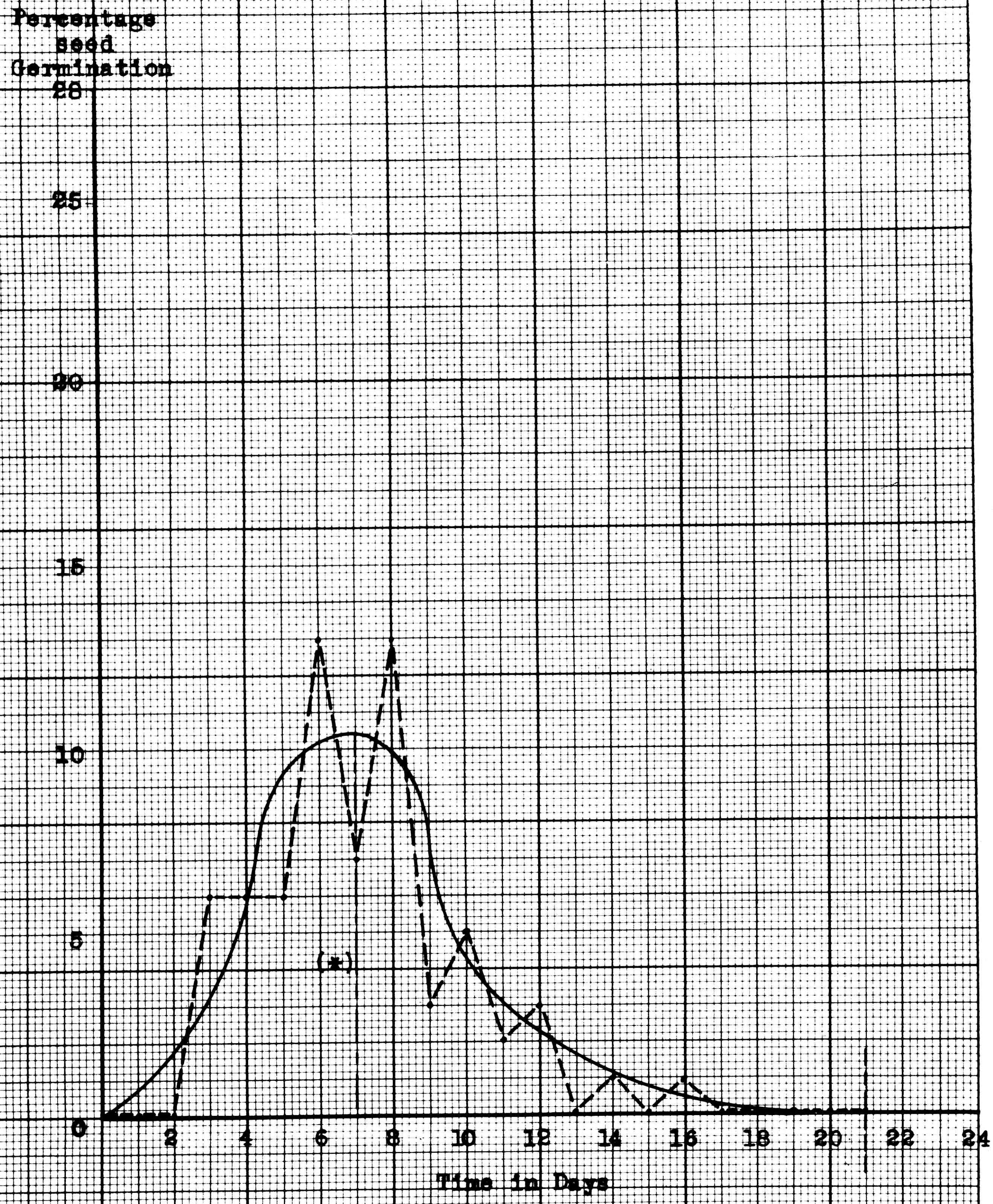


Chart 1. Control Lot A - Percentage Germination by Days

* (Vertical dotted line in charts 1 through 7 represents the modal, or typical, time in the germination period.)

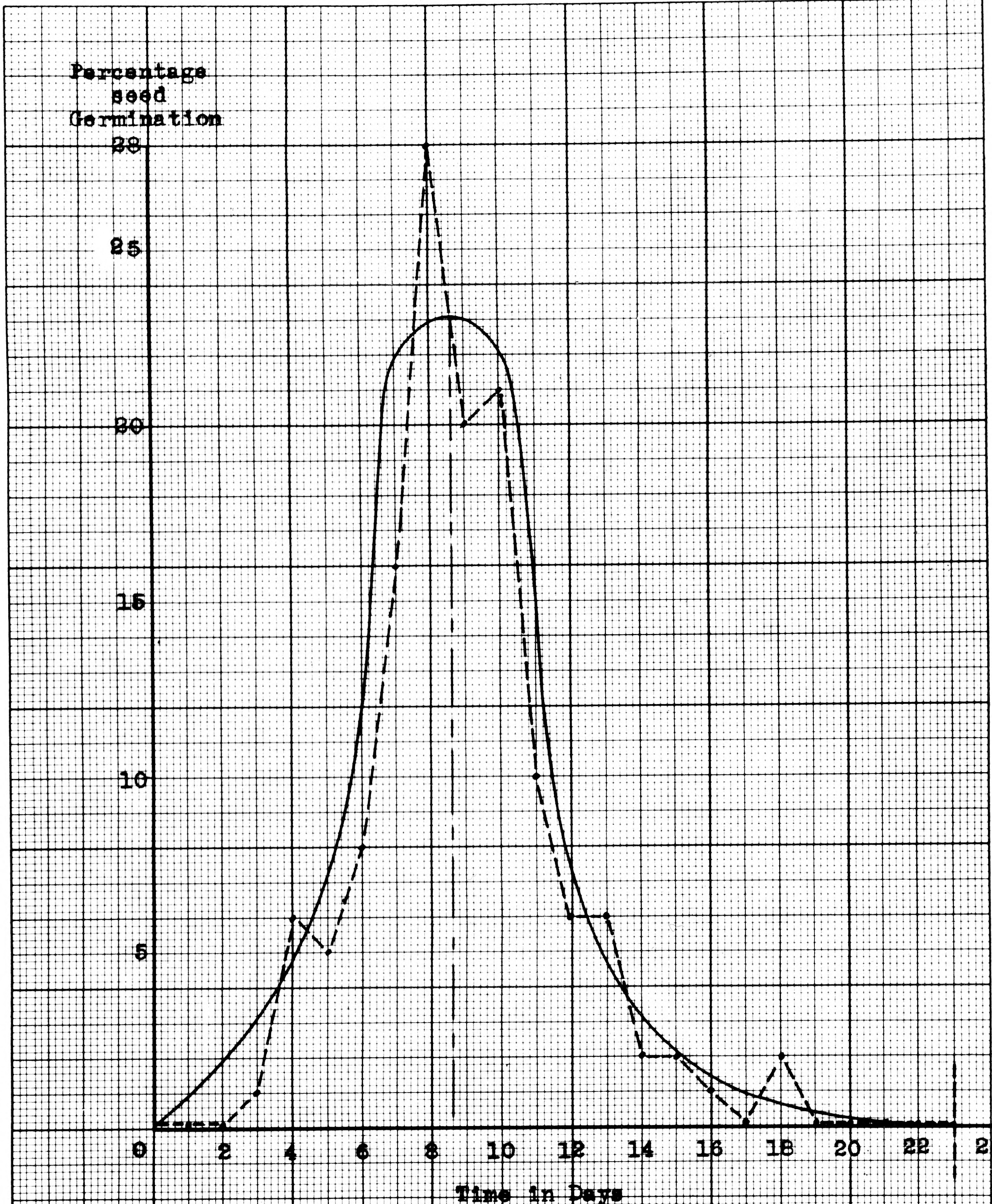


Chart 2. Control Lot B - Percentage Germination by Days

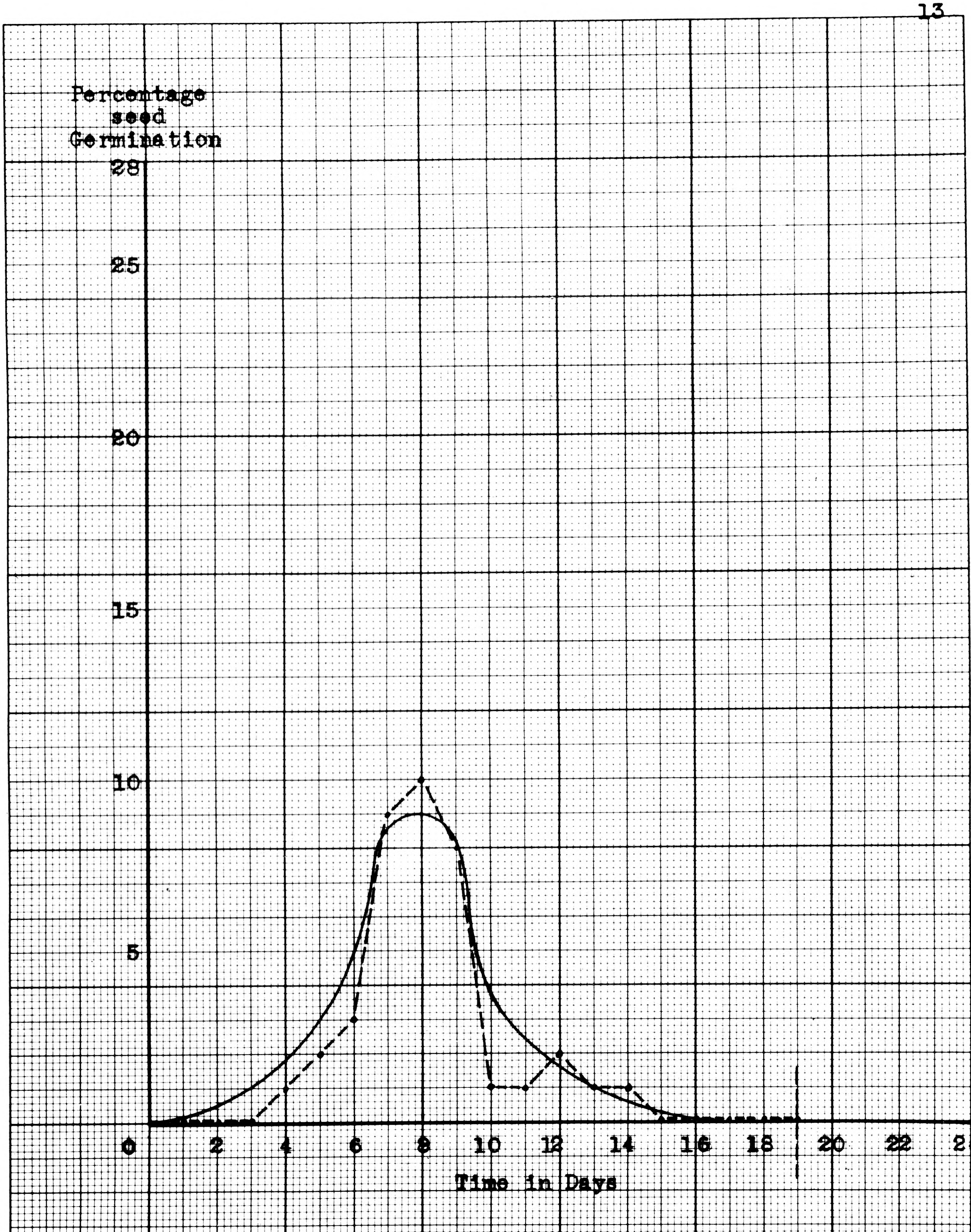


Chart 3. Control Lot C - Percentage Germination by Days

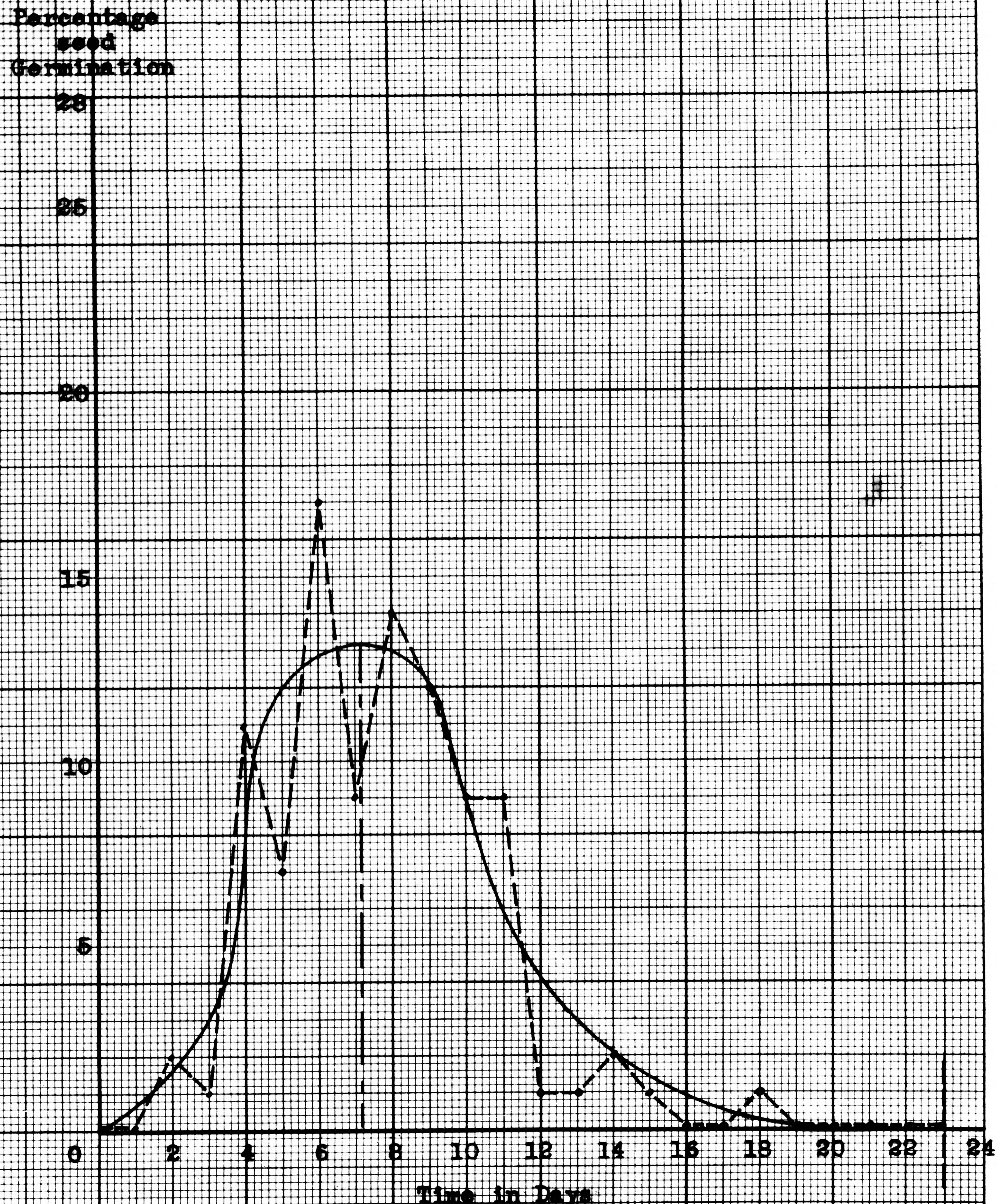


Chart 4. Control Lot D - Percentage Germination by Days

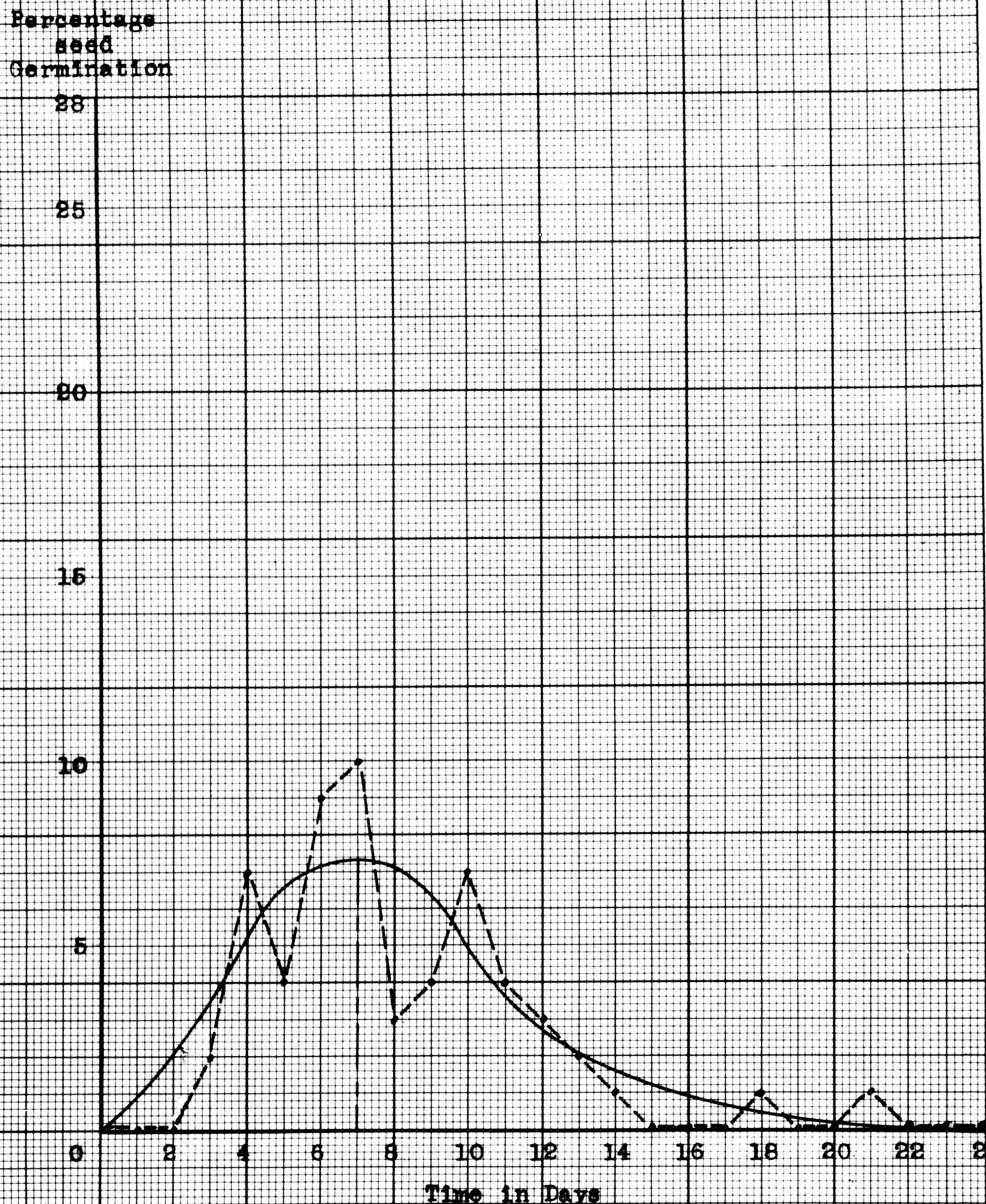


Chart 5. Control Lot E - Percentage Germination by Days

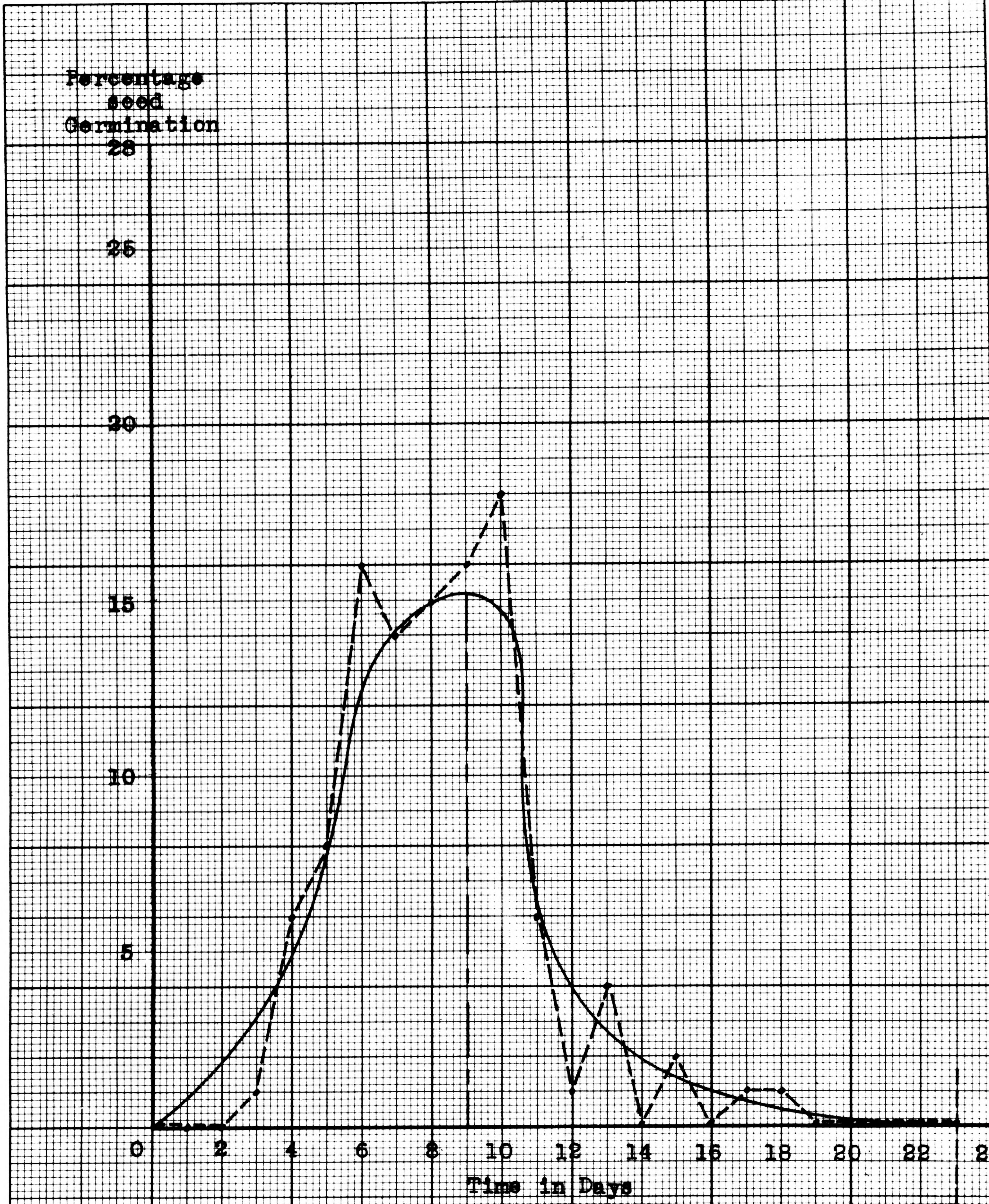


Chart 6. Control Lot F - Percentage Germination by Days

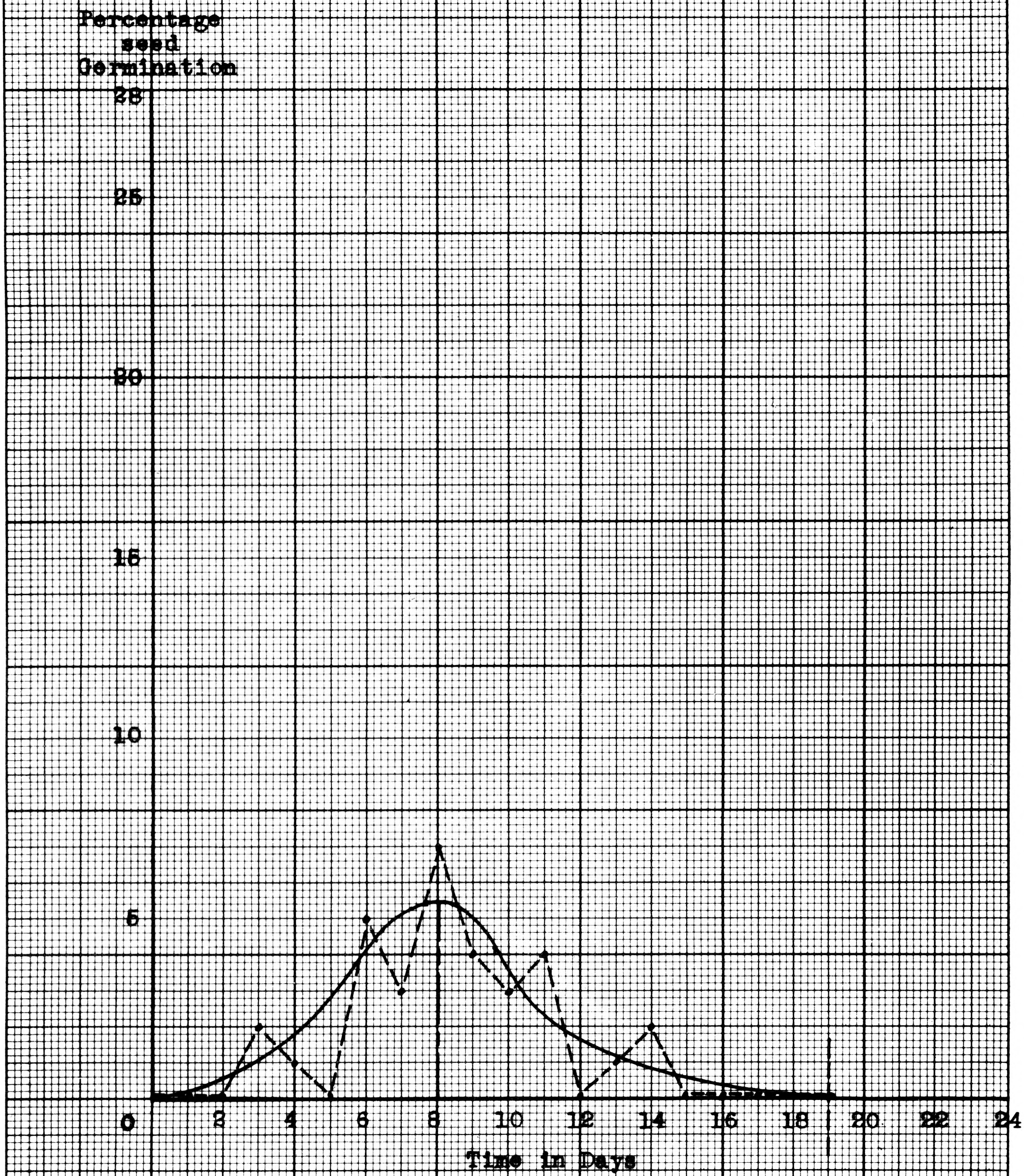


Chart 7. Control Lot G - Percentage Germination by Days

will be seen by comparing one lot with another. However no worthwhile result can be achieved by statistically analyzing these differences due to the fact that it is impossible to isolate the influences of each of a number of variables which may account for any or all of the differences. These variables are age, permeability of seed coat and geographical location, which includes such factors as soil, weather conditions, exposure, and climate.

The Relationship between Swelling and Germination Percentages after Soaking in Water at 70° F.

Method.- Samples of seed from each of the seven lots were selected at random and soaked in tap water at 70° F. for different lengths of time. Time periods selected were twenty-four hours, four days, and eight days. Germination media were the same as in the controls. The tests were allowed to run until five successive days had elapsed with no germination. Records were kept on swelling percentages as compared with germination percentages for the same test.

Results.- Table II, page 19, shows the comparisons between swelling and germination percentages of all lots. It will be seen that four of the seven lots, including the hardest coated lot (G) and the softest coated lot (B)

Table II. Swelling Percentages Compared with Germination Percentages in Water Soaking Tests

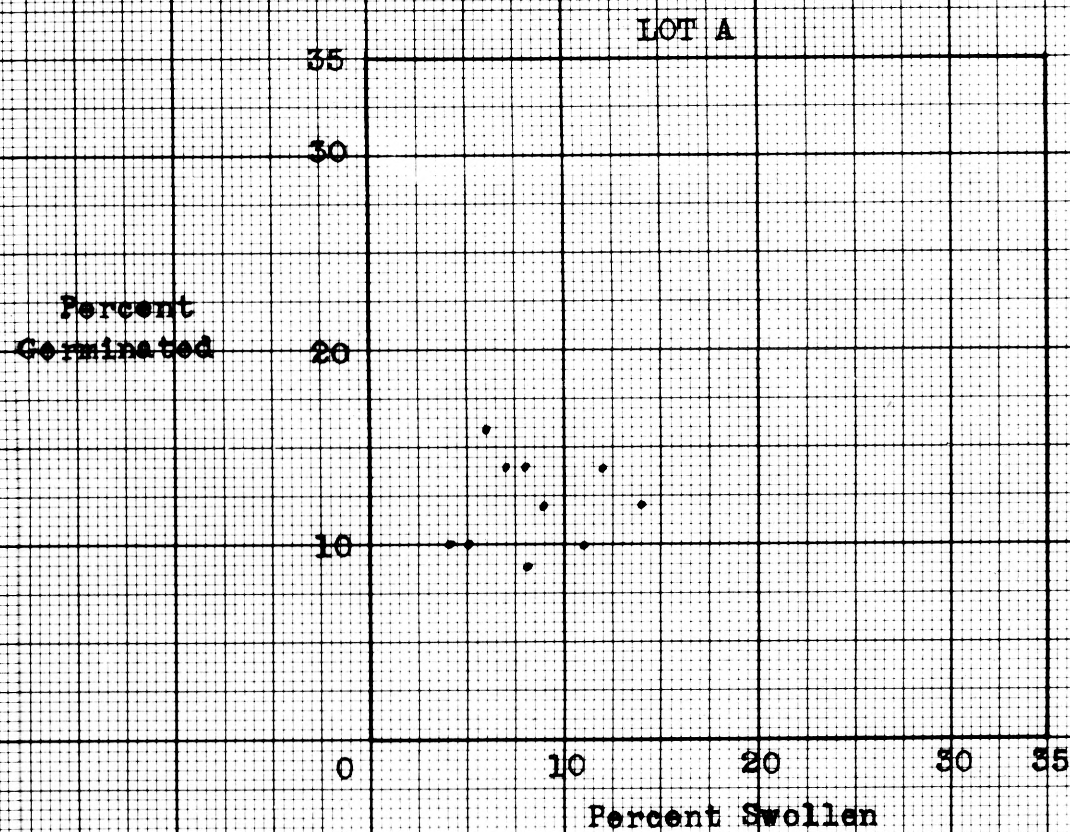
	Seed Lot Number						
	A	B	C	D	E	F	G
Control							
Germ. %	22	45	13	32	19	35	11
1 Day							
Germ. %	22	48	13	39	14	44	21
Swell.%	22	73	28	37	56	39	34
4 Days							
Germ. %	31	41	6	36	17	45	16
Swell.%	32	84	25	40	45	65	46
8 Days							
Germ. %	25	45	6	40	15	37	12
Swell.%	30	92	24	49	35	54	31

showed about twice as much swelling as germination.

According to Meginnis (7) a very apparent positive correlation* between swelling and germination was observed where seeds were soaked in water at temperatures around 170° F. If this correlation were present in the results of the experiments shown in Table II, the percentages would form a scatter diagram in such a fashion that the dots would arrange themselves in a semblance of a straight line, which would slope upwards and to the right. However, in Charts 8, 9, and 10, it will be seen that the position of the dots which are computed from plotting germination percent over swelling percent, do not form a straight line in any direction; rather, they are arranged in a curcular cluster.** This indicates very obviously that there is no correlation between swelling percent and germination percent when seeds are soaked in water at 70° F. The only possible exception to this occurs in the case of Lot C, where there appears to be a slight correlation. To check this mathematically, the coefficient of correlation was computed. This was found

*Positive correlation:- the greater number of seeds that swell will result in a proportionate increase in germination.

**No data obtained for Lot F.



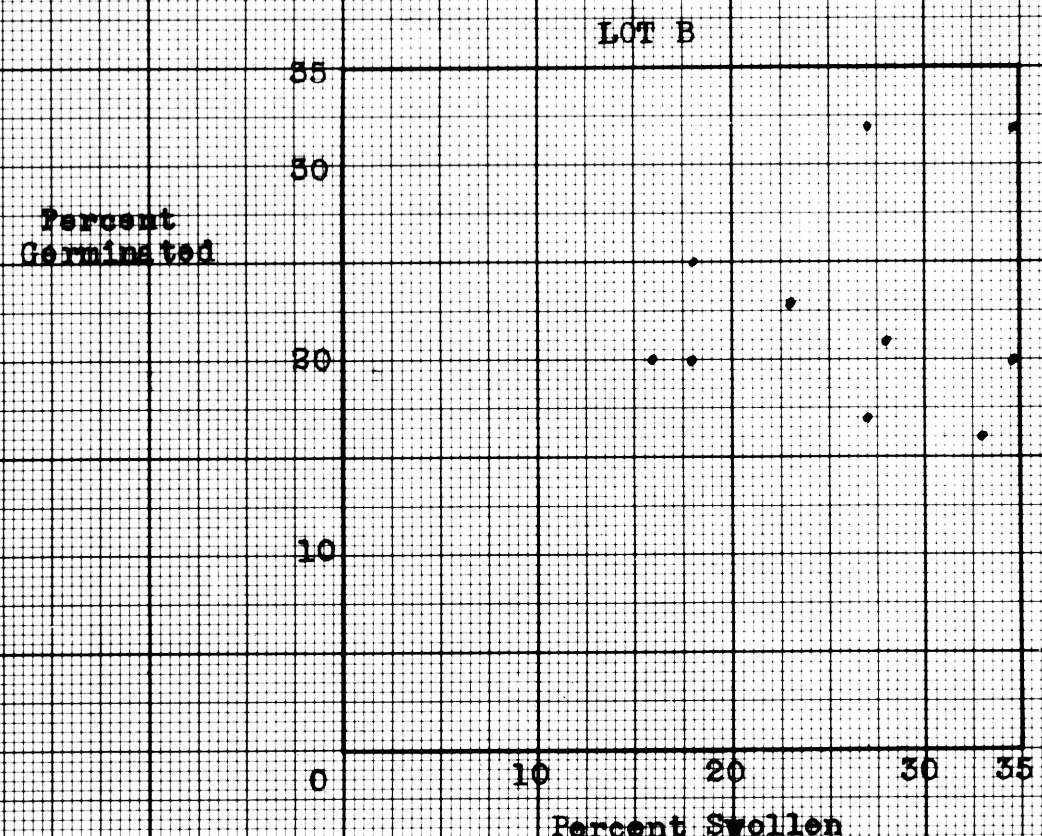
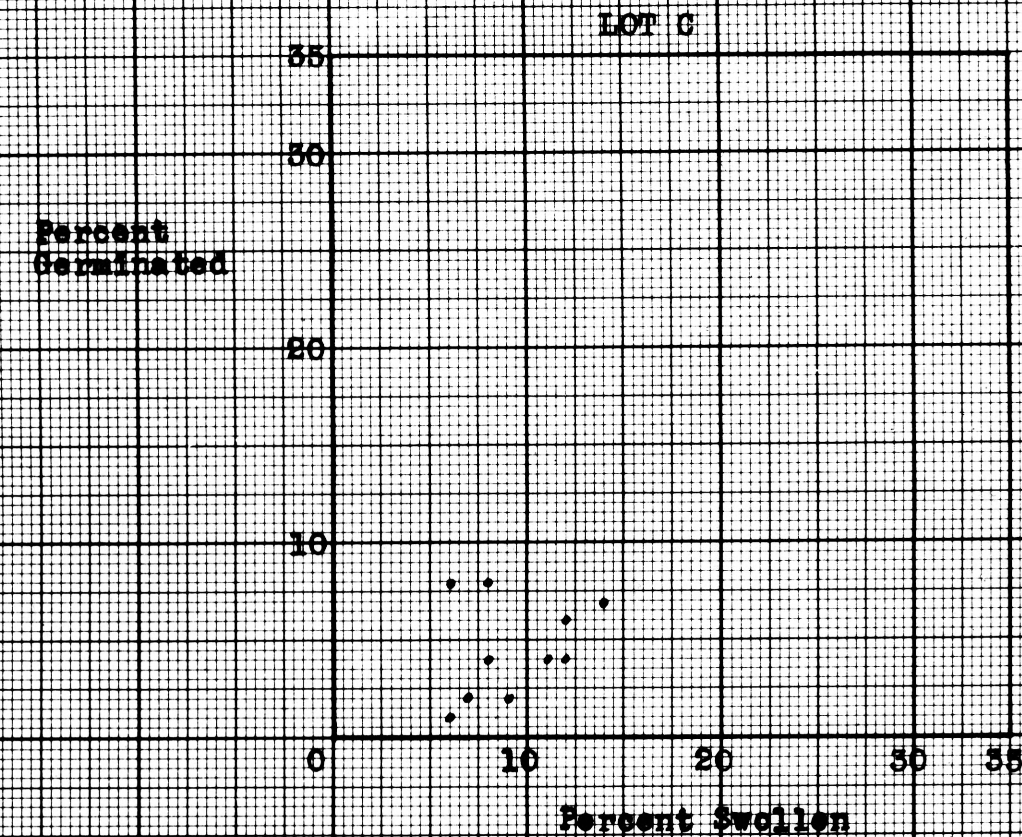


Chart 7. Scatter Diagrams to Illustrate Correlation Between Percent of Seeds which have Germinated and Percent of Seeds which have Swollen.



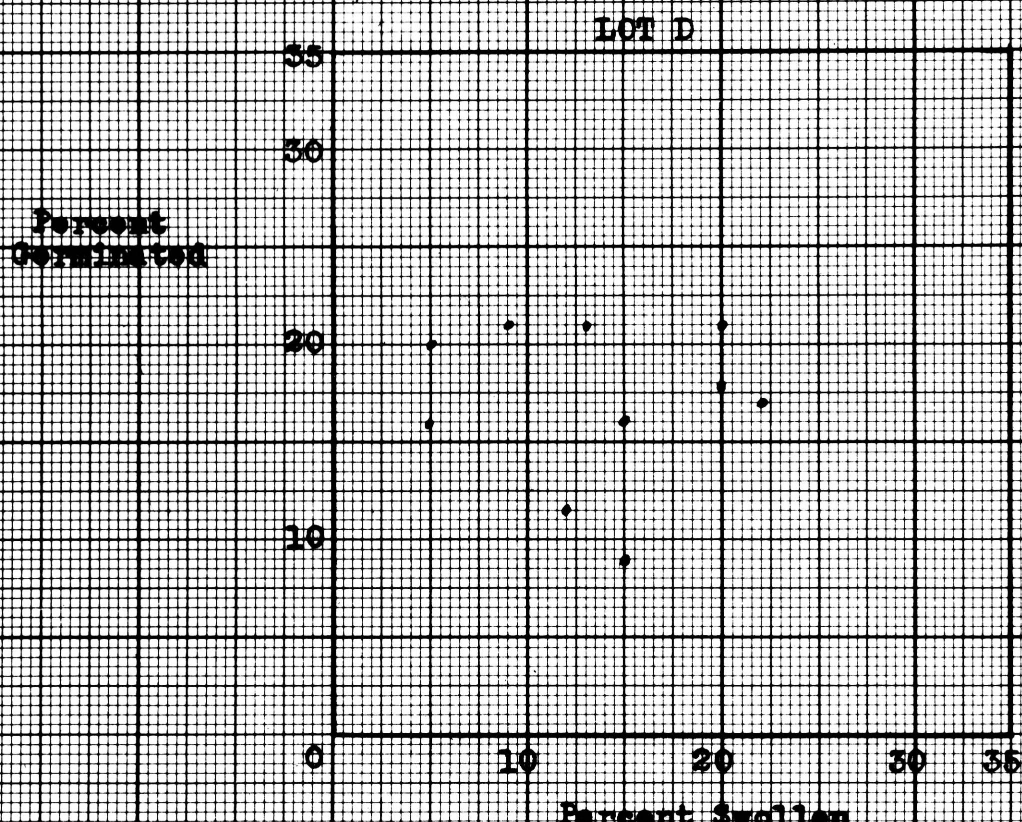
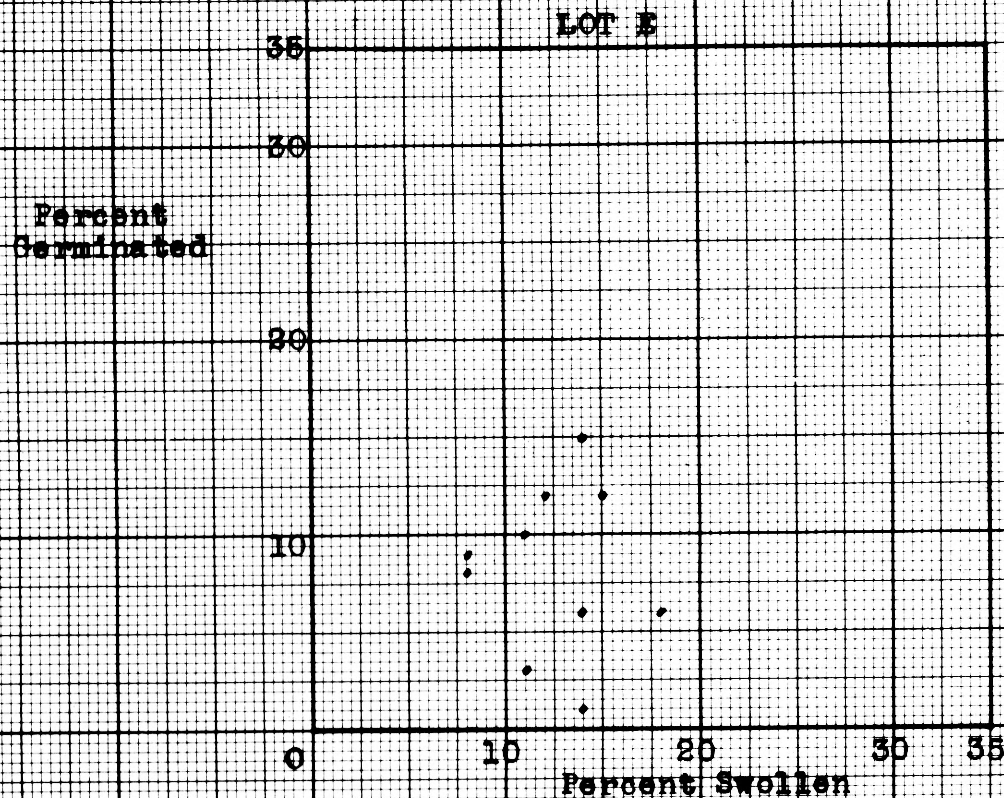


Chart 8. Scatter Diagrams to Illustrate Correlation between Percent of Seeds which have Germinated and Percent of Seeds which have Swollen.



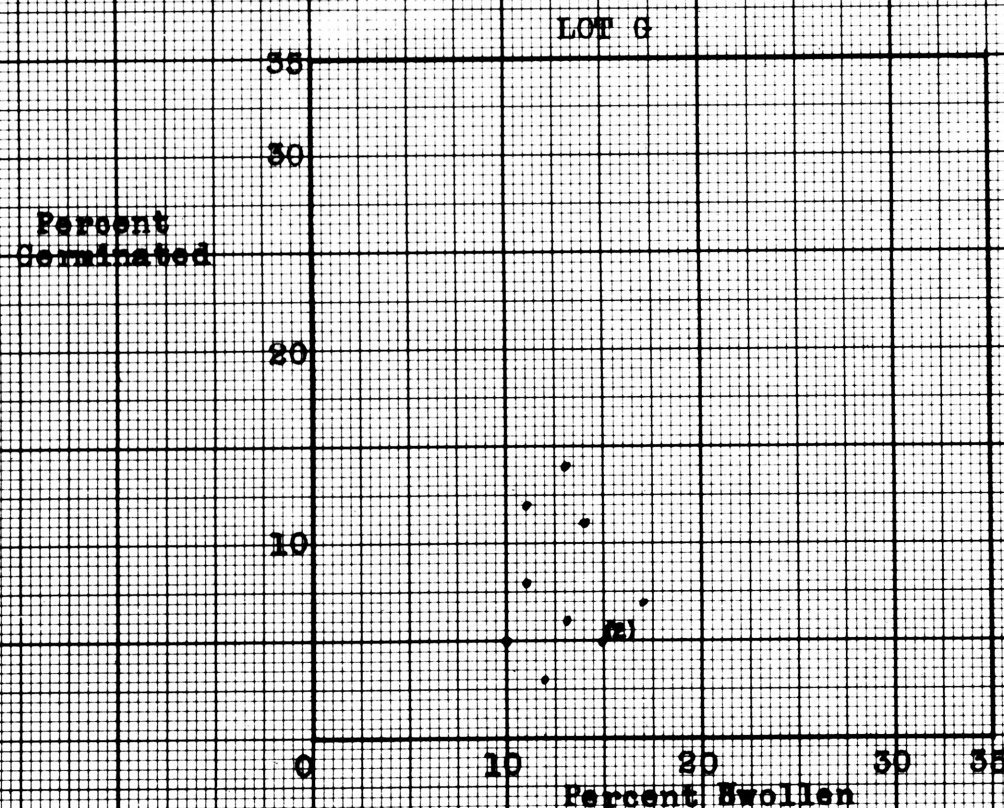


Chart 9. Scatter Diagrams to Illustrate Correlation between Percent of Seeds which have Germinated and Percent of Seeds which have Swollen.

to be .27 and since plus or minus one is considered perfect correlation, it may be seen that there is a very low coefficient of correlation between swelling and germination percentages in Lot C.* It should be noted that the lots cannot be compared with each other due to the inherent differences between lots.

Marked deterioration of some seeds and subsequent rot due to overlong soaking would seem to account for the decrease in germination after soaking for longer periods, even though swelling percentages remained constant or continued to increase. During the period of germination, those seeds that were swelled up when the soaking time expired did not necessarily germinate; rather, other seeds not swollen at the time of transfer to the germinating media took on moisture and germinated.

Final germination figures when compared with the germination of the controls showed no significant difference. In the belief that this lack of significance actually was due to overlong swelling, further tests were made using a solution of water and a detergent which, when combined with water, would hasten the absorption of moisture, thereby decreasing the time required to soak the seeds. This would eliminate the loss of otherwise viable seeds due to over-exposure to water.

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*Sample calculation to obtain the coefficient of correlation is shown in Appendix at the end of this paper.

Comparative Tests Using Seed soaked in a 0.1% Solution of water-wetter and city water, and water-wetter and distilled water

Methods.- Using the same germinating media previously described and under similar conditions, samples of seeds of all lots were placed in test tubes and soaked in a 0.1% solution of water-wetter and city water,* and water-wetter and distilled water. Seeds were immersed in this solution for six, twelve, twenty-four, forty-eight, and ninety-six hours. Tests were carried on until there was no germination for five successive days.

Results.- Analysis of the data reveals that differences between germination percentages of treated seeds and those of controls varies with the individual lot. Table III, page 26, shows average germination percents by treatments. To test significance of results, the standard error of the difference was computed for all lots. Rather than attempt to establish an arbitrary level of significance such as twice the standard error of the difference, the probabilities that the results obtained are due to chance and not due to the treatments are shown.

Suspecting that there may be a chemical reaction between water-wetter and city water due to the high pH shown in the tests made on the city water, further tests were made using a 0.1% solution of water-wetter and distilled water. These comparisons are also shown in Table III.

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* Ann Arbor city water has a pH of between 8.0 and 9.0

Table III. Percentage Germination and Probabilities Computed from Detergent Treated Black Locust Seed.

Seed Lot	Control	% Germination City Water & 0.1% Waterwetter	Optimum time in hrs (1)	Probability	% Germination Dist. Water & 0.1% Waterwetter	Optimum time in hrs	Probability
A	22.0	30	12	1-16 (2)	31	48	1-24
B	44.6	52	96	1-10	54	24	1-20
C	13.0	-- (3)	--	--	16	96	1-4
D	32.3	42	48	1-23	43	24	1-34
E	19.1	23	6	1-5	--	--	--
F	34.9	38	12	1-3	48	12	1-88
G	10.5	23	12	1-32,000	14	24	1-5

(1) Only the best results were selected from five treatments.

(2) i.e. Percent Germination using city water and Water-wetter would exceed the percent germination of the control by this much or more in only one case out of 16, etc.

(3) Dash indicates no increase in germination over that of the controls.

It may be seen from the tests made that there is no method of predicting the results when this solution is used. Each lot of seed gives maximum germination after being soaked in the solution using either city or distilled water for varying lengths of time. Lots A and D show most clearly that either treatment is significantly better than the controls. Lots B, C, and F show no clear cut improvement, while Lot G shows a great significance using the city water treatment but practically none when using distilled water.

Although these data indicate that this method of treatment may be of some value when treating individual lots of black locust seed, no general conclusions can be drawn as to the relative merits of a detergent and water solution. To do this would require comprehensive tests using many different solutions and even mixing detergents in water which varies from high to low alkalinity or acidity.

It is evident that this treatment of black locust seed has some advantages. It is easy to use, is fairly inexpensive, and there is none of the attendant danger to the nursery worker found in using sulphuric acid. The most serious disadvantage lies in the fact that the seeds are in a swollen condition and for that reason must be handled with care after they are removed from the solution.

Although the seeds used in this test were considered viable at the beginning of the experiments, there was an element of doubt present as to whether the seeds actually were viable in the light of the repeatedly poor results obtained. To serve as a check on the viability of these particular seeds and also to check the results of previous writers, an experiment was carried on in which seeds were steeped in concentrated sulphuric acid (specific gravity 1.84).

Tests showing the effect of Sulphuric acid on Germination

Methods.-- Seven lots of seeds were used for these tests. Samples of 100 seeds were selected at random and placed in 6 inch test tubes. The treatment consisted of steeping the seeds at room temperature in concentrated sulphuric acid (specific gravity 1.84), 95 percent pure, for periods of 30, 60, 90, and 120 minutes,* using five parts of acid to one part of seed. The seeds were stirred slightly after the acid was poured in to be sure that all seeds were immersed. After treatment, the seeds were placed in a tea strainer made of fine screen and washed thoroughly in a spray of cool tap water to remove all acid. The treated seeds were spread out and allowed

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*These periods were selected so as to more closely check on Meginnis who used the same time periods, temperatures, and acid concentration.

to dry twenty-four hours. They were then placed in $3\frac{1}{2}$" petri dishes containing moistened filter paper to germinate. Daily germination counts were made. The tests were concluded when germination had ceased for five successive days. The dishes were kept in a laboratory in which temperatures fluctuated from 65° F. to 85° F. Water was sprayed on the seeds daily so as to keep the moisture percent constant.

Results.- As shown in Table IV, page 30, germination percentages were increased in all lots except Lot B, which has proven to contain the highest percentage of soft coated seeds. Lot A showed final germination percentages up to 81% or nearly four times that of the control of Lot A. Lot C showed best germination of 62% after 60 minutes of soaking as against 13% germination of the control. Other lots were similarly affected with Lot G showing over twice as much germination after being steeped in acid for 90 minutes. With the exception of Lot B, germination was two to three times greater regardless of the period of immersion.

The results obtained from lot B would seem to indicate over-long steeping in acid. Only after steeping in acid for 60 minutes did any result which may be considered significant appear. Almost immediately after the seeds were placed in the petri dishes, the two samples

Table IV: Final Germination Averages of Black Locust Seed After Steeping in Concentrated Sulphuric Acid for Varying Time Periods

Seed Lot	Control	Length of Time Steeping (in minutes)*			
		30	60	90	120
A	22.0	68	66	72	81
B	44.6	37	50	35	4
C	13.0	53	62	47	61
D	32.3	68	65	68	74
E	19.1	47	50	47	62
F	34.9	67	60	52	34
G	10.5	21	33	34	14

* Length of time includes the time spent removing the seeds from test tubes, draining the acid away, and washing the seeds in water.

of seed steeped in acid for 90 minutes and 120 minutes showed signs of mold. This was apparently due to too long immersion of the seed with the subsequent destruction of the embryo, as no such large amount of mold had appeared in over 1,000 samples from the same lot of seed under any other treatment. The tests conducted using Lot B at 30 minutes and 60 minutes showed no large amount of mold.

The sulphuric acid treatment, aside from increasing germination of seed, also hastens the germination of the seed.* The mode in all of the control lots was reached on the seventh day after the seeds were placed in the germinating media. Using sulphuric acid, the mode appears by the second day, with a majority of the seeds germinated by the fifteenth day as compared with twenty-two to twenty-five days in the controls. This would indicate that a large percentage of the viable seeds germinate in the first two weeks after planting, thereby eliminating to a great extent the straggling germination of black locust later in the growing season.

The optimum time for steeping each lot of seed in sulphuric acid may be determined so as to get the maximum advantage from the treatment. If the time period is too short, all of the viable seeds will not germinate rapidly

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*Statistically speaking, a hastening of 36 hours will be significant.

because of the hard seed coat. If the time is too long, the seed will be destroyed due to destruction of the seed coat with consequent killing of the embryo.

Comparison of mean germination figures with the standard error of the difference will serve as a general guide to find the optimum length of time to steep the seeds in acid. It will be noted from Table IV that in every lot except B and E, there is more than one time which appears to be the optimum or very close to it. In these cases the significant differences or probabilities that the same results will occur due to chance vary from one out of seven to one out of twenty. These may be called significant differences, although it would be better to regard them as indications of the optimum time required to get maximum germination.

When using sulphuric acid as a treatment to hasten and increase germination, and in other treatments on black locust seed, an advance knowledge of the condition of the seed coat is necessary. Sulphuric acid will increase germination up to three times over untreated seeds. The time required to gain the maximum germination for each lot of seed may be found only by trial and error experiments.

The main advantages of the acid treatment is the increased germination it produces. Aside from this, treatment with sulphuric acid leaves the seed in a sound, firm condition, thereby enabling the nursery man to store the seed for several weeks prior to sowing. The materials needed are relatively cheap, easily obtainable, and make small scale treatments of seed economically feasible, as well as being readily adaptable to treating large lots of seed. The disadvantages are as previously mentioned, the physical danger involved in handling of the seed in the acid by the nursery worker. This may be overcome to a large degree through proper instructions and observation of common precautions when using sulphuric acid. Also, temperatures must be held to about 75° F. If seeds are treated at higher temperatures, sulphuric acid will carbonize and destroy the seeds. If necessary to treat at higher temperatures, the length of period of immersion must be proportionately reduced.

Summary

Laboratory tests conducted at the University of Michigan from October, 1947, to May, 1948, showed that black locust seed is extremely variable in the degree of permeability of seed coats. Variability is shown not only between lots of different origin and with different dates of collection, but also between the individual seeds of the same lot.

Soaking in city water at 70° F. produced significantly better results in most cases. However, these results fell far below the desired germination. The lack of correlation between swelling and germination was also noticed in these experiments. An inverse ratio develops between swelling and germination with the longer time periods of immersion. Although swelling percentages will remain constant or continue to increase, germination percentages will begin to decline as soaking time is increased. This decrease in germination may be attributed to overlong soaking.

Soaking in city water with the addition of a detergent corresponds closely to distilled water and a detergent. Through the use of this solution it was hoped that by the reducing of surface tension, a greater number of seeds would be able to absorb moisture in a shorter time, thereby averting the decline in germination percent due to overlong soaking in water with consequent rotting of the seeds. The results of these tests may be considered significant differences over the germination percentages of the controls. This method has the advantage over sulphuric acid in that there is no danger to the nursery worker when using the detergent to treat seeds. It has the disadvantage of leaving the seeds in a soft and swolen condition.

The best germination was achieved after steeping the hard coated black locust seed in sulphuric acid. This was a very effective means of accelerating and increasing the germination of the seed. With the exception of one lot of seed, germination percentages were from two to three times higher than the germination percentages for the controls and two times better than the seeds treated with a solution of detergent and water. Sulphuric acid is most beneficial in treating seeds with the less permeable seed coats. One lot of seeds with a softer seed coat was nearly destroyed as a result of the strong action of the acid. Acid treatment, in addition to being more effective with hard coated seed, is more practical than treatments which involve the soaking of the seed to produce swelling. Seed treated with acid remains in a sound, firm condition and, therefore, may be more easily sown and can be kept for some time after treatment without loss in viability.

Because of the inherent variability of black locust seed in structure and permeability of outer coats, some lots of seed may require no treatment at all, whereas other lots fail to give satisfactory germination unless the seeds are treated with one of the chemicals discussed here, or are scarified by some method. Preliminary tests conducted on a small scale are necessary to determine which of the treatments will give maximum germination and also to determine the optimum period of treatment.

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APPENDIX

I. Sample calculation to determine whether or not there is a significance between means.

Terminology

N=number of items in the sample

P_1 = true (unknown) percent germination of untreated seeds

P_2 = true (unknown) percent germination of treated seeds

P_{s2} = percentage germination of the control

P_{s1} = percentage germination of treated seeds

P_s = standard deviation of the sample population

T = the number of standard deviations between the difference obtained and the assumed average

$P_{s1} - P_{s2}$ = Standard error of a difference between sample percentages

$$T = \frac{P_{s1} - P_{s2}}{\sigma_{P_{s1} - P_{s2}}}$$

$$\sigma_{P_{s1} - P_{s2}} = \sqrt{\sigma_{P_{s1}}^2 + \sigma_{P_{s2}}^2}$$

$$\sigma_{P_s} = \sqrt{\frac{p \times q}{N}}$$

p = percent germination

q = 100 - percent germination

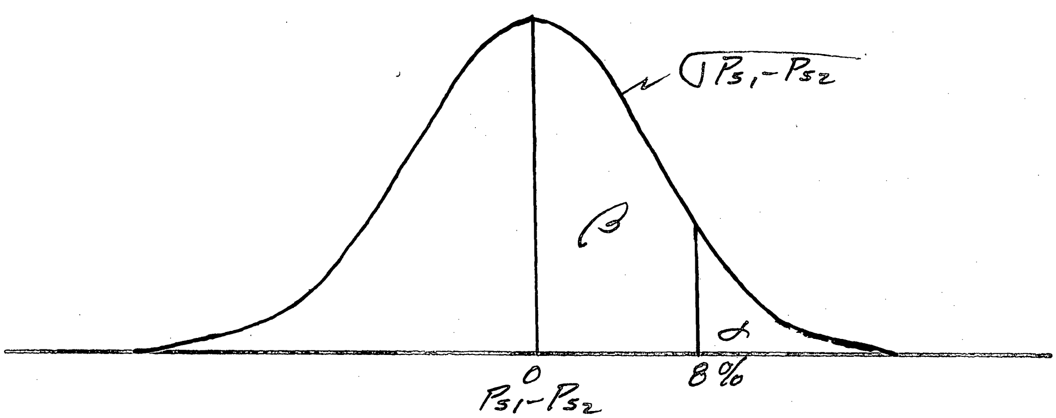
Procedure

The problem is to test whether or not there is a difference between a population of treated seeds and a population of untreated seeds. This is done by determining whether or not there is a "significant" difference between two samples, one sample from each population.

The logic of the procedure for this determination is as follows:

Set up the hypothesis that there is no difference between the population of treated and untreated seeds. (This means that $P_1 - P_2 = 0$.) The difference between sample percentages drawn from these two populations will not necessarily be equal to zero. However, the distribution of $P_{s_1} - P_{s_2}$ will be normal and average zero if an infinite number of samples are taken from each population.

This hypothesis then permits a normal curve of the differences between sample percentages to be drawn:



Control Lot A

$N = 30$

$P_{s_2} = 22\%$

$$\sigma P_{s_2} = \sqrt{\frac{22 \times 78}{300}}$$

City Water + W. W. for 12 Hours

$N = 100$

$P_{s_1} = 30\%$

$$\sigma P_{s_1} = \sqrt{\frac{30 \times 70}{100}}$$

$$\sigma P_{s_1} - P_{s_2} = \sqrt{\frac{22 \times 78}{300} + \frac{30 \times 70}{100}}$$

$$= 5.17; \quad T = \frac{8.0}{5.17} = 1.55$$

From probability tables $\beta = .4394$

Therefore $\alpha = .5 - \beta = .5000 - .4394 = 6.06\%$; $\frac{100}{6.06} = 16$ approx.

Therefore, we can say that in only about 1 case out of 16 could a sample percent germination of treated seeds exceed a sample percent germination of the controls by as much or more than 8% as a matter of chance. If this probability of 1 in 16 is considered sufficiently small, the difference of 8% may be considered "significant" and the original hypothesis (above) is shown to be false.

II. Sample calculation of Lot C to determine the coefficient of correlation between germination percent and swelling percent.

Terminology

Y = percent germinated

X = percent swollen

N = number of items in the sample

Procedure

First the regression line is calculated by using the normal equations (I, II). The unexplained variance and the total variance are then determined and combined to give the correlation coefficient, r = .27.

Y = 8, 4, 2, 8, 4, 4, 6, 1, 7, 2 = 46

X = 6, 8, 7, 8, 12, 11, 12, 6, 15, 9 = 94

X = 94; Y = 46; $\Sigma XY = 451$; $\Sigma X^2 = 964$; $\Sigma Y^2 = 270$

I. $\Sigma Y = Na + b\Sigma X$ II. $\Sigma XY = a\Sigma X + b\Sigma X^2$

I. $46 = 10a + 94b$ II. $451 = 94a + 964b$

$b = .2313432; \quad a = 2.42537$

$Y = a + bX \quad Y = 2.43 + .231X$

$$\sigma_{Ys}^2 = \frac{\Sigma Y^2 - a\Sigma Y - b\Sigma XY}{N} = 5.4097$$

$$\sigma_Y^2 = \frac{\Sigma Y^2}{N} - \frac{(\Sigma Y)^2}{N} = 5.84$$

$$r = 1 - \sqrt{\frac{\sigma_{Ys}^2}{\sigma_Y^2}} = .27$$ or very poor correlation.