STIMULATION OF GERMINATION OF DORMANT LETTUCE SEED BY SULPHUR COMPOUNDS

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(WITH ONE FIGURE)

Numerous agents have been reported as effective in promoting germination of dormant lettuce seed. BORTHWICK and ROBBINS (**1**) found that the percentage of lettuce seed germinating at temperatures above 25° C. is increased by high oxygen pressure. THORNTON (**17**) reported that good germination was obtained at temperatures as high as 35° C. if the surrounding atmosphere contained from 40 to 80 per cent. of carbon dioxide. SHUCK (**14, 15**), FLINT (**7**), and FLINT and MCALISTER (**8**) have reported on the influence of light on the germination of dormant lettuce seed.

DENNY (**2, 3**) and MILLER (**13**) have studied the influence of certain sulphur compounds on the breaking of dormancy in the potato tuber. That certain sulphur compounds are also effective in breaking dormancy in lettuce seed was reported by the writers (**16**). The present paper is a more complete report of studies made on the influence of chemicals in stimulating germination of lettuce seed under conditions that result in poor germination if the seed is not given some special treatment.

Material and methods

In making these studies, aqueous solutions of the chemicals were used as media in which the seeds were germinated. The tests were all made in 95-mm. Petri dishes. A piece of filter paper was first placed on the bottom of each dish. Fifty seeds were scattered on the paper and two ml. of the solution to be tested were added by means of a measuring pipette. The dishes were covered and placed immediately in a germinator in darkness at 24° to 26° C. The dishes were removed and the germinated seeds counted after 72 hours. Each test was run in quadruplicate.

The chemicals that have been tested for their influence on the germination of lettuce seed include acetamide, thioacetamide, allyl urea, allyl thiourea, ammonium sulphate, ammonium thiocyanate, asparagin, calcium thiocyanate, potassium thiocyanate, potassium cyanide, potassium ferricyanide, potassium ferrocyanide, semi-carbazide hydrochloride, thio-semicarbazide, sodium nitrate, sodium thiocyanate, sulphuric acid, urea, thiourea, ammonium nitrite, hydrazine, and sulphanilic acid.

Only chemically-pure materials were used. Each chemical was first tested in various strengths of aqueous solution to determine the concentration giving the highest percentage of germination. A few of the materials were injurious at concentrations as low as 0.1 per cent. Nearly all were injurious in 1.0 per cent. solutions.

Six of the chemicals tested gave very marked increases in germination over the untreated checks. The chemicals having a marked stimulating influence and their optimum concentration are as follows: thioacetamide, 0.4 per cent., and thiourea, allyl thiourea, thio-semicarbazide, ammonium thiocyanate, and potassium thiocyanate, 0.5 per cent. Sodium thiocyanate and calcium thiocyanate gave some significant increase in germination on some lots of seed but neither of these had the stimulating influence exhibited by the six above-mentioned chemicals. All of the other chemicals retarded germination in most cases except in very dilute concentrations.

Experimentation

Relative effectiveness of chemicals in stimulating germination

After the optimum concentration of the chemicals had been determined, a series of tests, hereafter known as series I, was made, using the six most effective chemicals—allyl thiourea, thioacetamide, thiourea, thio-semicarbazide, ammonium thiocyanate, and potassium thiocyanate. The results from this series of tests are given in tables I and II.

TABLE I

Germination of chemically-treated seed of four lettuce seed stocks
Series I

Treatment	Germination in percentage of 200 seeds in 4 replications				Mean
	Seed stock number				
	1	2	3	4	
	%	%	%	%	%
Check*	79.0	4.0	0.0	100.0	45.75
Thiourea	99.5	78.0	18.0	100.0	73.86
Thio-semicarbazide	93.0	55.0	30.0	98.0	69.00
Thioacetamide	87.0	70.5	0.0	96.0	63.38
Allyl thiourea	67.5	63.0	0.5	76.0	51.88
Ammonium thiocyanate	51.0	47.5	0.5	95.5	48.63
Potassium thiocyanate	45.5	15.0	0.5	94.0	38.63
Mean	74.64	47.71	7.0	94.14	

* Differences required to show significance are as follows: treatments, 5.58 per cent.; stocks, 4.24 per cent.

Four lots of seed were used. Three of these showed some degree of dormancy when untreated and the fourth was a non-dormant lot giving a check test of 100 per cent. germination on filter paper moistened with water. The three dormant lots were numbered 1, 2, and 3, and the non-dormant stock no. 4. The dormant lots gave check germination tests of 79.0, 4.0, and 0.0 per cent., respectively. The non-dormant lot was included to determine the influence of the chemicals on seed not normally dormant.

TABLE II

ANALYSIS OF VARIANCE OF DATA FROM SERIES I, TESTING THE RELATIVE INFLUENCE OF SIX CHEMICALS IN STIMULATING GERMINATION OF LETTUCE SEED

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE
Stocks	3	9,984.72
Treatments	6	603.68
Replications	3	50.48
Stocks × treatments	18	275.06
Error	81	15.61
Total	111	

The different seed stocks varied greatly in their response to treatments, as indicated by the large mean square value for stocks in table II.

The highly significant variance for interaction between stocks and treatments shows definite differences among stocks in response to a given treatment. Variance due to treatment alone is not significantly greater than interaction so it is not possible to generalize with reference to the relative effectiveness of treatment for any specific lot of seed. Variance due to stocks, however, is significantly greater than interaction, showing that these stocks, in general, maintain their relative responsiveness to individual treatments.

IMPORTANCE OF SULPHUR IN THE COMPOUND

Of the six chemicals having a marked stimulating influence on germination, two are inorganic—ammonium thiocyanate and potassium thiocyanate —and four, allyl thiourea, thioacetamide, thio-semicarbazide, and thiourea, are organic. The compounds in the former group have the CNS linkage in common and those in the latter group all have the amino NH_2 linked to the CS. All six have sulphur in common. The importance of sulphur in the compound is indicated by the results obtained from series II of tests in which the thio-compounds were tested with similar compounds in which sulphur is absent. In this series, thioacetamide was compared with acetamide, thiourea with urea, allyl thiourea with allyl urea, and potassium thiocyanate with potassium cyanide. In each case the most effective concentration of the chemical was used. No ammonium cyanide or semi-carbazide was available for testing with ammonium thiocyanate and thio-semicarbazide. The results of this series of tests are presented in tables III and IV. In each of the four comparisons made, the absence of sulphur from the compound not only resulted in no stimulation of germination but in every case retarded germination in concentrations of 0.1 per cent. or higher.

These results (tables III and IV) show clearly the need of sulphur in the compound for its stimulating influence on germination. The presence of sulphur in the compound is not the only condition necessary, as is shown by the failure of ammonium sulphate, calcium sulphate, potassium sulphate,

TABLE III

GERMINATION OF LETTUCE SEED TREATED WITH CHEMICALS WITH AND WITHOUT SULPHUR
SERIES II

TREATMENTS*	GERMINATION IN PERCENTAGE (200 SEEDS IN 4 REPLICATIONS)			MEAN
	SEED STOCK NUMBER			
	1	2	3	
	%	%	%	%
Thiourea	96.0	56.5	8.5	53.67
Urea	20.5	0.0	0.0	6.83
Allyl thiourea	89.5	39.0	0.5	43.00
Allyl urea	8.0	0.0	0.0	2.67
Thio-acetamide	96.0	48.0	0.0	48.17
Acetamide	18.5	0.0	1.5	8.33
Potassium thiocyanate	84.0	5.0	0.0	29.67
Potassium cyanide	19.0	1.0	0.5	6.83
Check	22.5	1.0	2.0	8.50
Mean	51.00	16.78	1.44	

* Differences required for significance are as follows: treatments, 3.54 per cent.; stocks, 2.32 per cent.

and sulphuric acid in any concentration between 0.1 and 1.0 per cent. to promote germination. The presence of C, N, and S in some specific linkage is apparently necessary.

TABLE IV

ANALYSIS OF VARIANCE OF DATA FROM SERIES II, TESTING THE INFLUENCE OF SULPHUR IN THE COMPOUND

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE
Treatments	8	1,267.40
Stocks	2	5,793.03
Replications	3	4.06
Treatments × stocks	16	404.63
Error	78	4.71
Total	107	

Although the interaction between stocks and treatments is significant with reference to error, the variances due to stocks and treatments are both significantly greater than interaction. Thus, the influence of treatments and the behavior of stocks were generally quite consistent.

Discussion

Many tests in which chemicals were used show that thiourea is the most generally effective material in promoting germination of dormant lettuce seed. Thiourea in the 0.5 per cent. concentration resulted in some stimulation of germination in every lot of dormant seed on which it has been used.

In some cases it has given nearly 100 per cent. germination on seed that was completely dormant in water under the same conditions of temperature and light.

The other five compounds—allyl thiourea, ammonium thiocyanate, potassium thiocyanate, thio-semicarbazide, and thioacetamide—were more variable in their effect on different lots of dormant seed. On some lots their stimulating effect was very great, while other lots were not stimulated by the chemical.

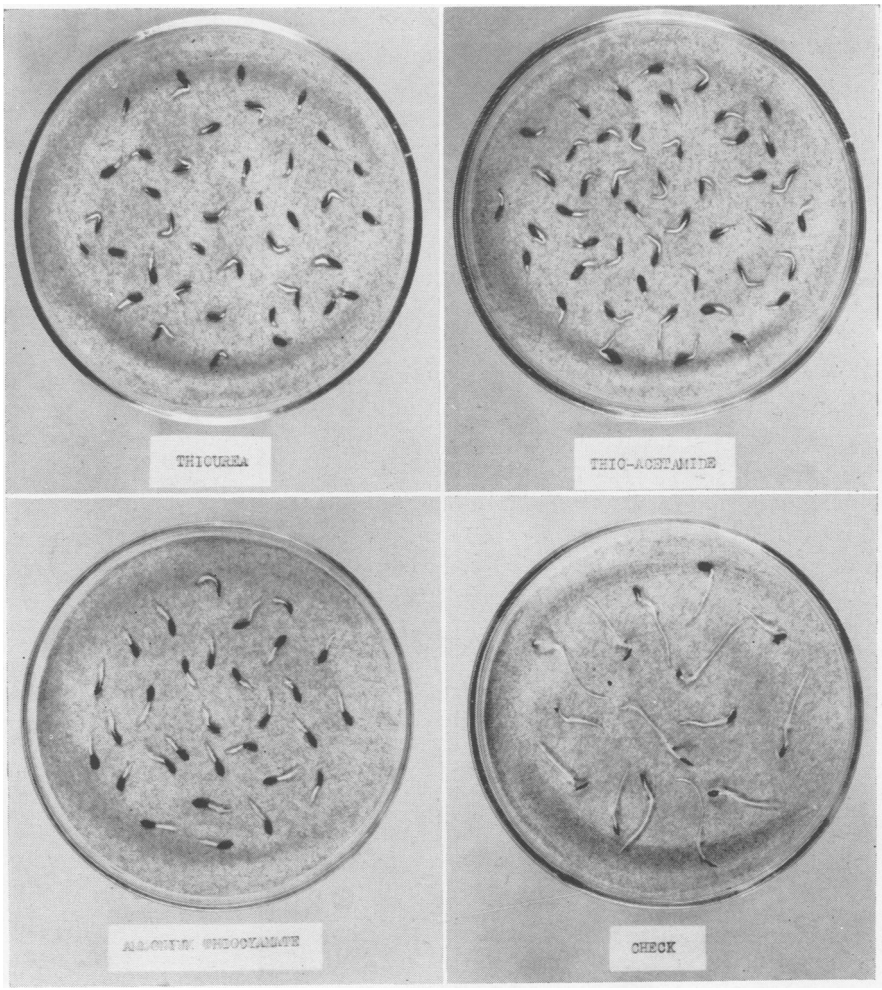

FIG. 1. Sprouted lettuce seed after 72 hours in darkness at 25° C. A, in 0.5 per cent. thiourea; B, in 0.4 per cent. thioacetamide; C, in 0.5 per cent. ammonium thiocyanate; D, in tap water.

In order of general effectiveness in stimulating germination on all of the various seed samples studied, the compounds rank in the following descending order: thiourea, thio-semicarbazide, thioacetamide, allyl thiourea, ammonium thiocyanate, and potassium thiocyanate. The last two named materials have a very marked influence on some lots of seed but on many samples they have little effect. Potassium thiocyanate is the most toxic and causes considerable injury to the radicle in some cases.

All of these compounds except ammonium thiocyanate have a very marked retarding effect on hypocotyl and radicle elongation and on root hair development. With ammonium thiocyanate, radicle and hypocotyl elongation and root hair development are about normal except for a slightly slower rate of growth. The type of growth resulting from the use of thiourea, thioacetamide, thio-semicarbazide, allyl thiourea, and potassium thiocyanate is very similar to that reported by BORTHWICK and ROBBINS (**1**) as resulting when lettuce seed is germinated under high oxygen pressure. The growth-inhibiting effect of some of the compounds used is shown in figure 1.

With the exception of this semi-carbazide, the inhibitory effect of these chemicals is not permanent. When the seeds were removed from the chemical solutions as soon as they had germinated, and planted in soil, the treated lots emerged from the soil and developed as rapidly as the seed germinated in water, with the exception of those germinated in thio-semicarbazide. Seed treated with this material failed to emerge. The rôle of these sulphur compounds in the germination of lettuce seed is not known, and no attempt has been made in the present studies to determine their function in the process of germination. DRYERRE (**6**), HANES and BARKER (**9, 10**), JOHNSON and WORMALL (**11**), DENNY (**4, 5**), MILLER (**13**), and others have studied the effect of certain of the cyanides and cyanates on enzyme activity. Enzyme activity has been found to increase in the presence of KCN and KSCN. The results reported on the effect of these materials on enzyme activity suggest that the sulphur compounds found to have a stimulating influence on the germination of dormant lettuce seed may have some similar function in the process of germination of lettuce seed.

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