CIRCULAR No. 377 NOVEMBER 1935
UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

VERNALIZATION EXPERIMENTS WITH FORAGE CROPS

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INTRODUCTION

Studies reported by a number of investigators have called attention to the influence of environmental factors during the germination phase of growth on the later development and fruiting of the plant. This general phenomena has been designated "vernalization." It also is referred to under the term "iarovization" or "yarovization."

The economic objective of vernalization is the shortening of the vegetative period and increasing yields of both seed and forage. The hypothesis of Lysenko,[1] who has been the chief proponent of the application of this principle, may be stated as follows: The conditioning for sexual reproduction and vegetative growth in a plant may occur in the seed when the embryo has started development but has not yet, or scarcely has, broken the seed coat, if proper environmental conditions are provided the seed at this time.

Thus vernalization is practically a seed treatment that influences the plant in its later stages of development. The seed is started into growth by the application of a limited amount of moisture and then subjected to other controlled factors: Temperature, light, darkness, and time. The growth of the seed is arrested or controlled by limiting the amount of moisture, and in the case of seed treated at low temperature the cold is a limiting growth factor.

In the case of winter wheat and other so-called "winter annuals", the vernalization process consists of adding water to the seed in an amount that will scarcely or just bring the seed into visible germination. This will require a 1- to 2-day period with the temperature of the processing chamber kept at 10° to 12° C. The seed is then transferred to a temperature of 3° to 5° and the moisture maintained by addition of water when necessary, and the seed frequently stirred. The time required in the cool room will vary, depending upon the temperature and variety of seed, but from 35 to 45 days is average. It has been pointed out by Lysenko that vernalization should be

[1] LYSENKO, T. D., [IAROVIZATION IN AGRICULTURE.] Odessa Ukrainskii Inst. Selectii Biull. Iarovizatsii nos. 1-3, illus. 1932. [In Russian.]

completed in total darkness. Other investigators [2], however, have shown that this is not necessary or at least not for some seeds.

In the case of plants requiring high temperatures, such as corn, foxtail millet, soybeans, Sudan grass, and sorghum, the vernalizing process, according to Lysenko, is very much the same as with low-temperature plants. In his experiments, where high temperatures were used, sufficient moisture was added to the seed to induce swelling or germination, and the swollen or germinated seed was then kept for from 5 to 10 days at a temperature of from 20° to 30° C., depending upon variety.

The requirements for several different kinds of seed as given by Lysenko are shown in table 1.

TABLE 1.—*Requirements to induce vernalization for different kinds of seeds, according to Lysenko* [1]

Crop	Ratio of water to weight of seed	Temperature during vernalization	Time of exposure	Crop	Ratio of water to weight of seed	Temperature during vernalization	Time of exposure
	Percent	° *C.*	*Days*		*Percent*	° *C.*	*Days*
Corn	30	20–30	10–15	Sorghum	26	25–30	8–10
Millet	26	25–30	5	Soybeans	75	20–25	10–15
Sudan grass	26	25–30	8–10				

[1] See footnote 1.

Since the publication of Lysenko's results, workers in the United States have attempted to duplicate his experiments. Many data have been published regarding the effect of low-temperature treatments [3] but few regarding high-temperature treatments, and in the latter case Lysenko's findings have not been substantiated.[4]

MATERIALS AND METHODS

The factors entering into the vernalization treatment were moisture, temperature, light, and time. Ordinary commercial seed of the various crops was used which in most cases had a high percentage of germinable seed. In the case of crotalaria and hairy vetch, however, hard seeds were present in varying amounts, which increased the percentage of moisture absorbed, as the entire amount of moisture applied was taken up by fewer seeds than would have been the case had no hard seeds been present.

The amount of water added to the various lots and varieties varied with the amount necessary to induce germination and further in accordance with the object of the experiment. Stoppered bottles were at first used to maintain the moisture content of the seed at a definite percentage, but these later were replaced by Petri dishes. With the lapse of time there usually was a gradual loss of moisture from the

[2] SPRAGUE, F. S. EXPERIMENTS ON IAROVIZING CORN. Jour. Agr. Research 48: 1113–1120, illus. 1934.

[3] MARTIN, J. H. IAROVIZATION IN FIELD PRACTICE. U. S. Dept. Agr., Bur. Plant Indus., 13 pp. 1934. [Mimeographed.]

MCKINNEY, H. H., and SANDO, W. J. EARLINESS AND SEASONAL GROWTH HABIT IN WHEAT. Jour. Heredity 24: 169–179, illus. 1933.

——— SANDO, W. J., SWANSON, A. F., HUBBARD, V. C., SMITH, G. S., SUNESON, C. A., and SUTHERLAND, J. L. FIELD EXPERIMENTS WITH VERNALIZED WHEAT. U. S. Dept. Agr. Circ. 325, 8 pp. 1934.

[4] SPRAGUE, F. S. See footnote 2.

KIRK, L. E. DIVISION OF FORAGE PLANTS, REPORT OF THE DOMINION AGROSTOLOGIST. Canada Expt. Farms Rept. 1934.

seed and additional water was added to keep the percentage relatively constant. The amount of moisture added to the seed in all cases was determined by weight and expressed as a percentage of the air-dry seed.

FIGURE 1.—White lupine. The vernalized plants (*B*) are in pod and well matured while the check plants (*A*) show no sign of bloom.

For cold treatment of seed, an ordinary refrigeration room maintained at a constant low temperature (0° C.) was used. For high-temperature treatment a small electric oven thermostatically controlled was used in some cases, while in others a steam-radiator heated room served this purpose. In no case, however, did the temperature vary much except when intentionally induced.

Seed treated at low temperature in the refrigeration room or at high temperature in the electric oven were in constant darkness. Seed treated at high temperature in the steam-radiator heated room were subjected to a variation from total darkness to the intermittent light of night and day. The duration of treatment with both high and low temperatures was more or less arbitrarily determined, but consideration was given to experience of others doing similar work so far as data were available, and an attempt made to use optimum time rather than to use time as a variable in the experiment.

In the case of high-temperature treatments molds always gave trouble and, while the use of disinfectants was attempted, no satisfactory way was found for completely overcoming this difficulty. Seedings in the greenhouse were made in large pots using an excess of seed, and after the seedling plants were established the stands were reduced to the same number of plants per pot for the different treatments. Plantings in the open field and coldframes were made in rows with the stands approximate. Plantings in the open field were made at the Arlington Experimental Farm, Rosslyn, Va., and the coldframe and greenhouse plantings were made in Washington, D. C.

EXPERIMENTAL RESULTS

In table 2 is given the results from vernalized seed of white lupine (*Lupinus albus*), crimson clover (*Trifolium incarnatum*), hairy vetch (*Vicia villosa*), Austrian Winter field pea (*Pisum arvense*), double cut red clover (*T. pratense*), and white sweetclover (*Melilotus alba*). The vernalized seed was in the cold chamber for 40 days (Mar. 16 to Apr. 25). One check lot of seed was kept in ordinary storage and sown dry at the time of seeding the vernalized seed, while a second check lot was kept moistened for 6 days (Apr. 17 to Apr. 23) and then dried for 2 days (Apr. 23 to Apr. 25) before sowing on April 25, 1934, at which time the vernalized and dry check seed was sown.

In the case of the white lupine the check lots of seed, while making as good growth as the vernalized seed, excepting the one lot in the greenhouse, did not in any case come into bloom, while the vernalized seed in all lots blossomed and developed seed pods (fig. 1).

Crimson clover check lots in all cases failed to blossom. This also was the case with the vernalized lots planted in the open field and coldframes, but the vernalized lot in the greenhouse bloomed and made a larger growth than the check plants (fig. 2).

The vernalized lots of hairy vetch seed bloomed earlier than the dry seed check, while the swelled seed lot was in bloom at practically the same time as the vernalized lot.

In the case of Austrian Winter field peas the vernalized lots blossomed decidedly earlier than the check lots.

The red clover lot of seed that was vernalized and grown in the greenhouse blossomed earlier than the check lots; the lots in the coldframes all came into bloom on the same date, while all lots in the open field failed to blossom.

None of the vernalized or check lots of white sweetclover blossomed, and the vegetative growth was about the same for all treatments.

With reference to conditions for growth in the greenhouse, open field, and coldframes, it should be recorded that in the open field conditions were less favorable for growth than in the greenhouse and in coldframes where artificial watering was practiced; and in the case of the greenhouse plantings, temperature conditions were more favorable for rapid growth during the early period of development. The effect of the vernalization is most definitely shown in the greenhouse plantings. Only in the case of hairy vetch is the development of the preliminarily moistened and then dried seed advanced and this only in the coldframe and field-planted lots.

It seems evident that lupines require a shorter period of cold to induce normal development than some other winter annuals. The

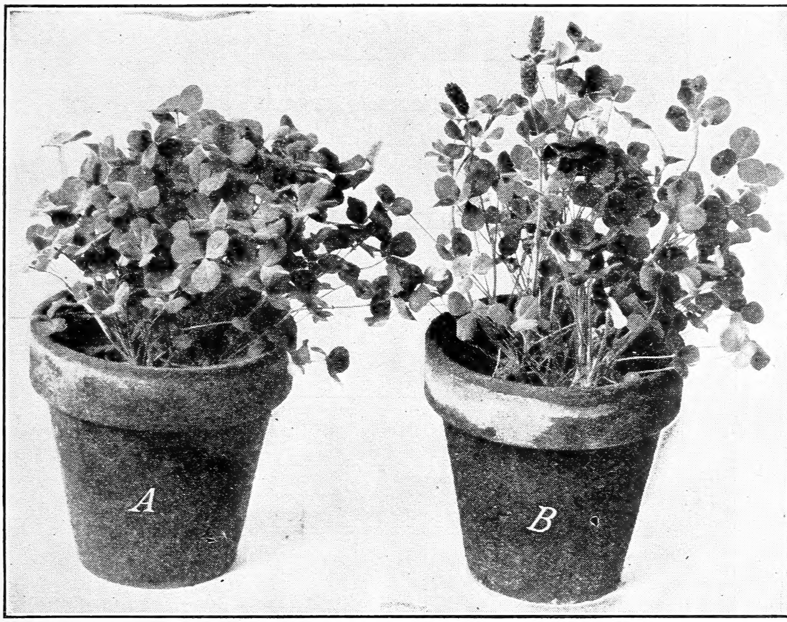

FIGURE 2.—Crimson clover. The vernalized plants (*B*) are in bloom and later matured while the check plants (*A*) never bloomed.

intensity of the cold and the time of exposure required probably are contingent on other environmental factors. The white sweetclover in this experiment showed no effect of the cold treatment and red clover showed but little if any, while the hairy vetch and field pea were only slightly influenced. This may have been due to insufficient time or insufficient growth activity during the cold period. Johnson [5] reports that sweetclover seedlings 3 inches high gave greater response, for the same period of cold treatment, than did three-leaf seedlings, while swollen seed under the same conditions gave no response. That all winter annuals may require some cold for their best development and that all may differ somewhat in this requirement is at least reasonably possible.

[5] JOHNSON, I. J. THE PHYSIOLOGICAL EFFECT OF LOW TEMPERATURE ON THE BIENNIAL GROWTH OF SWEETCLOVER (MELILOTUS ALBA). Sci. Agr. 12: 746–748, illus. 1933.

TABLE 2.—*The effect of cold and drying treatments of moistened seed on subsequent growth and development*

Species	Treatment		First bloom			First pods			Final height of plants		
	Water added	Subsequent treatment	Greenhouse	Cold-frames	Open field	Greenhouse	Cold-frames	Open field	Greenhouse	Cold-frames	Open field
	Percent								*Inches*	*Inches*	*Inches*
Lupinus albus	0	0	None	None	None	None	None	None	12	18	24
	125	Dried	June 23	do	do	do	do	do	12	18	24
	150	Cold	June 5	June 13	June 1 [1]	July 12	July 12	June 25	20	18	24
Trifolium incarnatum	0	0	None	None	None	None	None	None	7	5	5
	110	Dried	do	do	do	do	do	do	7	7	5
	130	Cold	July 2	do	do	None [2]	do	do	10	5	5
Vicia villosa	0	0	June 23	do	do	None	do	do	24	36	24
	75	Dried	None	June 23	June 29 [1]	do	do	July 10	24	36	24
	100	Cold	June 12	do	June 25	do	do	do	24	30	36
Pisum arvense	0	0	None	do	do	do	do	do	18	30	12
	80	Dried	do	do	do	do	do	do	18	30	12
	100	Cold	July 12	June 13	June 15 [1]	do	June 23	June 25	18	30	16
Trifolium pratense	0	0	do	July 11	None	None [2]	None [2]	None	[3] 10	12	6
	100	Dried	do	do	do	None	None	do	[3] 10	12	6
	125	Cold	July 6	do	do	do	do	do	[3] 10	10	6
Melilotus alba	0	0	None	None	do	do	do	do	30	30	12
	100	Dried	do	do	do	do	do	do	30	30	12
	120	Cold	do	do	do	do	do	do	30	30	12

[1] Plantings in the field were not watched sufficiently close to get the exact day of blooming and first pod, but dates are approximate.
[2] Pods may have formed, but seed did not develop.
[3] At time of early bloom; was larger later.

In tables 3 to 5 are given the results of tests with varieties of foxtail millet grown in a greenhouse, and in table 6, tests with this crop grown in the open.

As shown in table 3, the vernalized seed sown March 15 started into growth ahead of the check, and the plants were slightly larger throughout the period of development. The two lots, however, headed at the same time. That sown April 5 was a little slower than the check in starting and gave a very thin stand. At time of thinning, when the plants were about 4 inches high, the vernalized plants were stockier than in the check, owing to the thicker stand of the latter. Later, the vernalized plants were larger than those of the check but were delayed in coming into head. In the May 10 planting, the check was as early as the vernalized lots, and the variation in total growth is not significant.

TABLE 3.—*German foxtail millet sown in greenhouse*

Treatment	Vernalization			Culms headed on date indicated				Length of culms[1]			Remarks
	Days	Ratio of water to seed	Temperature	May 13	May 15	May 17	June 13	Longest	Total	Average	
	Number	*Percent*	° *C.*	*Number*	*Number*	*Number*	*Number*	*Inches*	*Inches*	*Inches*	
Check	------	------	------	1	3	5	13	34	375	28.8	Maturing June 13.
Vernalized[2]	5	22	20–30	1	3	7	13	38	419	32.2	Maturing June 13; quickest in starting.
				June 13	June 23	July 1					
Check	------	------	------	6	7	7		39	229	32.8	
Vernalized[3]	5	22	32	4	7	7		40	246	35.1	
Do.[4]	5	22	32	0	5	7		51	297	42.4	
				July 15	July 17	July 20	Aug. 2				
Check	------	------	------	1	1	3	[5] 8	60	387	48.4	Latest starting.
Vernalized[6]	5	22	20–23	0	1	2	4	66	361	51.6	
Do.[6]	5	22	20–23	2	3	6	7	60	413	59.0	Started first.
Do.[6]	7	26	20–23	1	1	2	6	53	348	49.7	Intermediate starting.

[1] All plants were mature when harvested.
[2] Vernalized in alternating light and darkness and then dried 6 weeks and sown Mar. 15, 1933. Total=13 plants.
[3] Seed sterilized and vernalized in total darkness and then dried and sown Apr. 5, 1933.
[4] Seed not sterilized but vernalized in total darkness and then dried; slowest starting; latest maturing, sown Apr. 5, 1933. Total=7 plants.
[5] The check by error had 8 instead of 7 plants.
[6] Vernalized in alternating light and darkness and then dried and sown May 10, 1933. Total=7 plants.

TABLE 4.—*Vernalized seed of foxtail millet varieties sown in greenhouse in mid-winter*

Variety and treatment	Vernalization: Ratio of water to seed	Vernalization: Temperature[1]	Vernalization: Days	Culms	Length of culms: Longest	Length of culms: Total	Length of culms: Average	Date first head	Heads on date indicated: Mar. 12	Mar. 17	Mar. 19	Mar. 26	Apr. 2
	Percent	°*C.*	*Number*	*Number*	*Inches*	*Inches*	*Inches*		*Number*	*Number*	*Number*	*Number*	*Number*
Common [2]	30	32	6	7	10.0	49	7.0	Mar. 9	3	7	7	7	7
Check				7	11.0	50	7.1	Mar. 17	0	1	5	7	7
Common [2]	30	32	6	8	20.0	133	16.6	Mar. 15	0	6	8	8	8
Check				8	20.0	144	18.0	Mar. 17	0	1	3	8	8
Hungarian [2]	26	32	6	12	11.0	97	8.0	Mar. 9	8	12	12	12	12
Check				13	8.0	71	5.5	Mar. 15	0	4	10	13	13
Hungarian [2]	26	32	6	5	17.5	69	13.8	Mar. 12	3	4	5	5	5
Check				5	24.0	71	14.2	do	2	4	5	5	5
Common [3]	30	32	6	8	17.0	116	14.0	Mar. 26			0	4	8
Check				8	23.5	170	21.3	Mar. 19			1	6	8
Hungarian [3]	26	32	6	12	18.0	136	11.3	Mar. 26			0	7	12
Check				12	22.5	217	18.0	Mar. 19			1	11	12

[1] Temperature at night was decreased to about 24° C.
[2] Sown Jan. 11, 1934, the checks being planted on the same date.
[3] Dried 8 days subsequent to treatment before seeding Jan. 19, 1934, the check being planted on the same date.

TABLE 5.—*Vernalized seed of foxtail millet varieties sown in greenhouse in late summer*

Variety and treatment	Vernalization[1]: Ratio of water to seed	Vernalization[1]: Temperature	Vernalization[1]: Days	Culms	Length of culms: Longest: Aug. 29	Longest: Sept. 13	Longest: Final	Length of culms: Total	Length of culms: Average	Date first head	Heads on date indicated: Sept. 28	Oct. 4	Oct. 6	Oct. 11
	Pct.	°*C.*	*No.*	*No.*	*In.*	*In.*	*In.*	*In.*	*In.*		*No.*	*No.*	*No.*	*No.*
Siberian [2]	39	22	5	7	¼–½	7	19	99.5	14.2	Sept. 28	5	6	7	7
Check [2]				7	1	9	15	87.0	12.4	do	5	7	7	7
Common [2]	32	22	5	7	½	6	30	169.0	24.1		0	4	6	7
Check [2]				7	1	7	27	153.0	21.8		0	7	7	7
Hungarian [2]	27	22	5	7	½	7	24	118.0	16.9		0	6	6	7
Check [2]				7	1	9	23	140.5	20.0	Sept. 28	4	7	7	7
					Sept. 13	Oct. 11	Final			Oct. 1	Oct. 14	Oct. 16	Oct. 18	Oct. 23
Siberian [3]	39	22	5	3	¾–1	7	15½	38	12.7	0	0	2	2	3
Check [3]				3	1½	9	21	58	19.3	1	3	3	3	3
Common [3]	32	22	5	2	1–2	12	26	50	25.0	0	1	1	2	2
Check [3]				2	1–2	8	23	39	19.5	0	0	0	1	2
Hungarian [3]	27	22	5	5	1–2	10	15	62	12.4	1	4	4	4	5
Check [3]				5	1–1¾	10	21	85	17.0	0	2	2	5	5

[1] Seed dried subsequent to vernalization.
[2] Sown Aug. 24, 1933 and harvested Nov. 7, 1933.
[3] Sown Sept. 5, 1933 and harvested Nov. 7, 1933.

TABLE 6.—*German foxtail millet vernalized and sown in the open in rows 20 inches long and 6 inches apart, and harvested Aug. 18, 1933*

[Maturity in checks and vernalized seed in all cases was practically the same]

Treatment	Vernalization			Germination	Culms	Height		Date first head
	Days	Ratio of water to seed	Temperature			July 5	Final	
	Number	*Percent*	° *C.*		*Number*	*Inches*	*Inches*	
Check [1]				Good	54	26	64	July 23
Vernalized [2]	5	22	22	Fair	30	30	72	Do.
Do.[2]	5	22	22	Poor	17	30	68	Do.
Do.[2]	7	26	22	Fair	29	30	68	Later.
Check [1]				Poor	16	19	64	Aug. 1
Vernalized [3]	6	26	22	Good	49	21	64	July 29
Check [1]				Fair	33	20	64	July 23
Vernalized [4]	6	26	22	Good	55	19	68	July 29
Check [1]				do	61	16	64	Aug. 1
Vernalized [5]	7	26	22	do	69	16	64	Do.
Do.[6]	7	26	22	do	72	16	68	Do.
Do.[5]	9	26	22	do	32	16	68	Do.
Do.[6]	9	26	22	do	45	16	64	Do.

[1] Checks were seeded on the same date as the vernalized seed.
[2] Vernalized in alternating light and darkness and then dried before seeding, May 22, 1933.
[3] Vernalized in darkness and seeded without drying, May 29, 1933.
[4] Vernalized in alternating light and darkness and seeded without drying, May 29, 1933.
[5] Vernalized in darkness and seeded without drying, June 1, 1933.
[6] Vernalized in alternating light and darkness and seeded without drying, June 1, 1933.

In table 4 the temperature was varied day and night with 32° C. for the day and 24° for night. The seed sown January 19 differed from that sown January 11 in that it was dried subsequent to the vernalizing treatment. The results with the three varieties used in this experiment show no injury from drying and no effect from the vernalization.

The plantings of August 24 and September 5 (table 5) differ only in treatment of the seed subsequent to vernalization. Seed of the August 24 planting was dried slowly for 4 days, while in the September 5 planting the drying was continued for 15 days. The results of the two plantings are similar and show no effect from vernalization.

The plantings made in the open (table 6) show some differences, but these are not sufficiently consistent to be considered significant. The tallest plants were from vernalized seed, but the time of maturing was about the same in all lots. In the May 29 seeding, one lot of vernalized seed was taller than the check while the other was equal. In this planting, the date of appearance of the first heads showed a week's difference in the two check lots, but no difference could be noted in the time of maturing of these and vernalized lots. The two tallest lots in the June 1 seeding were from vernalized seed, but no difference could be noted in time of maturing.

The results with Sudan grass given in table 7 are much the same as with the foxtail millet and gave little or no indications of vernalizing effects. While in the September 5 seeding one vernalized plant was the earliest in bloom, the behavior of the other plants was such that this is not significant.

TABLE 7.—*Vernalized seed of Sudan grass sown in greenhouse and harvested Jan. 31, 1934*

Name and treatment	Vernalization			Plants	Date first head	Heads on date indicated			Length of culms			
	Days	Ratio of water to seed	Temperature			Dec. 4	Dec. 21	Final	Longest: Sept. 13	Longest: Final	Total	Average
	Number	*Percent*	° *C.*	*Number*		*Number*	*Number*	*Number*	*Inches*	*Inches*	*Inches*	*Inches*
Sudan grass	[1] 3	45	22	5	Nov. 20	3	5	6	12	57.50	293	48.83
Check				5	Nov. 27	2	5	7	14	59.00	339	48.43
Sudan grass	[2] 3	45	22	4	Dec. 4	1	2	4	2–3	57.50	196	49.00
Check				4	Later	0	2	6	4–5	37.66	226	37.66

[1] Then dried slowly through 6 days and planted Aug. 24, 1933, the check being planted on the same date.
[2] Then dried slowly through 6 days and kept dry 12 days before planting Sept. 5, 1933, the check being planted on the same date.

It has been brought out in these trials, as in some earlier unpublished work, that in the case of grasses and in certain legumes, seed that have been slightly sprouted and again dried will start into growth quicker than unsprouted seed. Legumes which have epigeous cotyledons cannot be sprouted and dried without injury, but in the writer's experience legumes in which the cotyledons are hypogeous can be sprouted and dried several times without serious injury and when sprouted, dried, and again moistened, start growth more quickly than unsprouted seed.

It has also been shown that the capacity of seed to absorb moisture varies greatly in different varieties as well as in different species, and that the amount necessary to induce germination is about three-fourths of the seeds' total absorption capacity. The amount of moisture necessary for germination, therefore, can be ascertained approximately by determining the total absorption capacity. The absorption capacity of seed of a number of plants as determined in the course of these experiments, expressed in percentage of the ordinary air-dry weight of the seed, is given below:

	Percent
Agrostis alba (redtop)	96
Cajanus indicus (pigeonpea)	129
Chaetochloa italica:	
(Common foxtail millet)	32
(Hungarian foxtail millet)	28
(Siberian foxtail millet)	39
Crotalaria spectabilis	159
Crotalaria striata	176
Dactylis glomerata (orchard grass)	113
Festuca elatior (meadow fescue)	100
Hedysarum coronarium (sulla)	132
Lolium multiflorum (Italian ryegrass)	67
Lespedeza sericea	125
Lupinus albus (white lupine)	152
Medicago sativa (common alfalfa)	130
Melilotus alba (white sweetclover)	120
Pisum arvense (Austrian Winter field pea)	110

	Percent
Soja max:	
(Biloxi soybean)	136
(Manchu soybean)	130
(Peking soybean)	100
Sorghum vulgare sudanense (Sudan grass)	45
Sorghum vulgare saccharatum (sorgo)	40
Trifolium incarnatum (crimson clover)	130
Trifolium pratense (red clover)	126
Vicia monantha (monantha vetch)	96
Vicia pannonica (Hungarian vetch)	101
Vicia sativa (Oregon common vetch)	100
Vigna sinensis (Groit cowpea)	137

SUMMARY

Seed of white lupine, crimson clover, hairy vetch, and Austrian Winter field pea vernalized or started into growth by the addition of moisture and then kept for a period of 40 days at 0° C., when subsequently planted came into flower and fruit, while seed not so treated remained in the vegetative stage or came into bloom at a later date. Seed of white sweetclover and red clover showed no response, but this may have been due to insufficient treatment.

Seed of foxtail millet, Sudan grass, soybean, and crotalaria moistened and kept for a period of 5 to 9 days at high temperatures showed in most cases decreased vigor and in no case did such treatment advance the time of maturing.

Seed moistened and started into growth activity and subsequently dried started into growth sooner than seed not so treated.

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U. S. GOVERNMENT PRINTING OFFICE: 1935

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