Vernalization of Sugar-Beet Seed[1]

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Recent work (4)[3] concerning plant development has indicated that some of the stages through which plants must pass before reaching sexual maturity may be induced while the embryo is still within the seed. This work has shown that reproductive development can be hastened by a process known as vernalization which consists of storing seed at cool temperatures while it is held in a partially sprouted condition. The present report is the result of a study to determine some of the factors in sugar-beet seed vernalization. Applications are considered in connection with hastening the reproduction of breeding stocks grown under greenhouse conditions and of increasing the reproductivity of certain varieties of beets when planted for commercial seed production.

Definition of Terms

The economic varieties of sugar beets are biennials. During the first season they normally produce a non-reproductive type of growth and large quantities of stored food. For induction of reproductive development, sugar beets require a period of cool temperature (thermal induction) with, or followed by, long daily exposures to light (photoperiodic induction). Since the effects of thermal and photoperiodic induction overlap, their combined effect on the reproductive development of beets has been referred to as photothermal induction (7). The production of a seedstalk, which is taken as the first outward indication of reproductive growth, is termed "bolting."

Varieties of sugar beets vary widely in bolting tendency. Beets which require prolonged periods of photothermal induction before they form a seedstalk are termed non-bolting types. Those which require only short periods of photothermal induction before bolting are called fast-bolting or easy-bolting types.

Materials and Methods

Seed of several varieties of beets, varying widely in bolting tendency, were used. Non-bolting varieties, however, were used for the more extensive tests. The uniform, non-bolting variety, R. and G. Old Type (S.L.C 5638), was used extensively in greenhouse studies, but its use in field plantings was limited because of its susceptibility to curly top. Other seedlots used were from the curly-top resistant varieties developed in the breeding program of the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. D. A.

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[3]Figures in parentheses refer to Literature Cited.

All lots of seed were first disinfected by soaking- in 10-pereent commercial formalin solution for 10 to 15 minutes, then washed for at least 15 hours and air-dried. Washing of seedlots prior to cold storage was thought necessary since previously reported studies (12) had shown that water-soluble substances are present in sugar-beet seedballs, which produce a toxic effect during the germination process. This toxic effect results chiefly from ammonia which is hydrolyzed from nitrogenous substances by enzymatic action associated with germination (9).

After the preliminary washing treatment, separate seedlots were weighed and soaked over night in water or in certain chemical solutions. They were then placed on weighed metal screens and slowly and uniformly dried to the moisture percentage noted. Moisture was calculated on the basis of air-dried seed rather than total moisture content. The screens were then placed in galvanized cans where the humidity was maintained at a high level.

After germination had progressed to the desired stage—usually 5 to 10 percent of the seedballs having radicles barely visible—the seed was placed in cold storage. Temperatures were maintained at 33° to 36° F. unless otherwise noted. The seed was stirred occasionally during storage to aerate it and to maintain uniformity of moisture content.

All greenhouse plantings and most of the field plantings were made using moist seed which was kept cold until planted. Greenhouse plantings were made in rows 7 to 8 inches apart, and the beets were thinned to about 20 plants to 2½ feet *of* row. Most of the field plantings were in rows 22 inches apart and the beets were left unthinned.

Greenhouse temperatures were so maintained as to induce some bolting in plants from untreated seed. Daily maximum temperatures were frequently reached as peaks caused by brief periods of sunshine before ventilators were opened. Minimum temperatures were reached much more gradually. For this reason the mean temperature was generally within 5 degrees of the minimum. Average monthly minimum greenhouse temperatures during the tests are presented in table 1.

Table 1.—Monthly average of daily minimum greenhouse temperatures during tests with vernalized sugar-beet seeds.

	October	November	December	January	February	March
	°F	°F	°F	°F	° F	°F
1937-1938 (table 2)	41.5*	40.8	43,6	42.1	43.3	44.3
1938-1939 (tables 3 and 4)	42.3	42.4	51.4	46.0	45.0	**47.1**

•First readings recorded October 11, 1937.

Since effects of thermal induction are dependent upon associated or subsequent photoperiodic induction, plants grown in the greenhouse were grown under long photoperiods. Photoperiods were controlled by using artificial light to supplement the natural day length. Tungsten filament Mazda lamps equipped with reflectors were placed over the plants at distances to give light intensities of at least 25 foot candles. Bolting plants in the greenhouse were pulled and counted at frequent intervals to make room for the remaining plants.

Significant differences between treatments were determined by analysis of variance **(3).**

Experimental Results

Greenhouse Experiments.—The first series of vernalization treatments included variations in moisture content of the seed, different periods of storage, and the use of two chemical solutions in which the seeds were soaked before cold treatment. Most lots of vernalized seed produced more bolting plants than the corresponding lots of untreated seed. The moisture content of the seed, the length of the storage period, and the temperature of the storage were interdependent factors and all positively correlated with the extent oP sprouting. Difficulty was experienced with excess sprouting and growth of fungi in prolonged storage, especially at high-moisture content and at the higher temperatures. It appeared that about 35 to 40 percent added moisture was optimum. "With only 25 percent added moisture, sprouting was not initiated and there was no increase in subsequent bolting. Storage at 40° F. increased bolting more than at 36° F. or 32° F. At 40° F. there was no increase in bolting from storage longer than 60 days. The seed that was soaked in a 0.02-percent solution of potassium permanganate produced a greater percentage of bolting plants than those soaked in water alone or in a 0.1-percent solution of manganese sulfate.

The benefit derived from the use of potassium permanganate suggested trials with further chemicals and hormone preparations Listed in table 2, and also suggested increasing the oxygen content of the storage atmosphere. While data in table 2 show that nearly all of the vernalization treatments increased bolting, further benefit was derived from soaking the seed in thiourea, hydrogen peroxide or taka diastase.

Results of further tests with some of the more promising soaking solutions are presented in table 3. Thiourea again increased bolting of plants from vernalized seed more than any of the other solutions used. More uniform and complete sprouting, as well as a healthier appearance of the sprouts, was evident in the **lot** treated with thiourea.

Previously reported results (7) have shown that the extent of sprouting is highly important in connection with results obtained from vernalized sugar-beet seed. More critical data on this factor are

Table 2.—Greenhouse planting of vernalized sugar-beet seed (S. L.. C, 5638). The effect of different chemicals and hormone preparations on the subsequent bolting of plants. All lots stored 86 days at 35 to 40 percent moisture.[1] Planted September 24, 1937, under 17 to 18-hour photoperiods.

Soaking solution	Total plants	Percentage bolters at respective days after planting 52	94	122	150
	Number				
Untreated	251	0.0	0.4	0.4	14.3
Water	128	0.8	3.9	4.7	21.1
0.01 percent potassium permanganate	120	0.8	1.7	4.2	24.1
1.0 percent potassium phosphate (primary;	127	0.0	3.9	6.3	20.0
3.0 percent manganese sulfate	126	0.8	1.6	4.8	33.3
1.0 percenl phosphoric acid	129	0.8	3.1	7.S	26.3
o.5 pcrciMit lakii diastase	317	4.3	7.7	11.1	45.3
0.1 percent thiourea	125	5.6	8.0	12.8	49.6
0.5 percent potassium dichromate	128	2.3	3.9	10.2	27.3
0.1 percent potassium thiocyanafe	126	0.8	3.2	7.1	33.3
1.000 I. units theelol[2]	129	2.3	5.4	6.2	26.3
5000 I. units theelol[2]	126	1.6	5.6	7.1	27.8
2,500 I. units progynon[2]	124	1.6	3.2	S.l	33.9
0.5 percent hydrogen peroxide	129	2.3	10.1	15.5	38.8
1.0 percent hydrochloric acid	125	0.0	0.0	0.8	8.8
l.O percent ammonium nitrate	320	0.8	4.2	5.0	30.0
1.0 percent urea	117	0.0	3.4	5.1	25.6
Difference for significance	Odds 19 :1				13.46
at 150 days after planting	Odds 99:1				17.50

[1]All seedlots in this test were vernalized in an oxygen-enriched atmosphere, but similar lots vernalized in air responded as well.
-International units hormone per 100 ml. water.

presented in table 4. The data in table 4 were obtained from the same vernalization treatments reported in table 3. Each of the five lots of vernalized seed were separated inlo two classes. One class was made up of only those sordballs showing- no visible radicles at the time of planting'. The other consisted of seedballs in which every individual seed within the seedball had sprouted before planting. This segregation was made by removing all sprouted seeds from the seedballs in the first classification and all unsprouted seeds from the seedballs in the second classification.

The data in table 4 show that the extent of sprouting of the seed during the vernalization treatment may be the most important factor considered. The bolting in plants grown from sprouted seed was 25.4 percent as compared with 4.7 percent bolting in plants grown from unsprouted seed at the first date of count (table 4). This highly significant difference continued throughout the experiment. Since the improvement in bolting resulting from all treatments was correlated with the degree of sprouting and the healthy appearance of the sprouts, it appears that any vernalization treatment that will improve sprouting may be expected to affect subsequent bolting.

Table 3.—Greenhouse planting of vernalized sugar-beet seed (S. L,. C. 5638). The effect of different chemical treatments of seed on subsequent bolting of plants. All lots stored 40 days at approximately 40° F. with 40 percent added moisture. Planted October 12, 1938, under continuous illumination.

Soaking solution	Number of plants	Bolters at respective days after planting 59	73	97	118	134
		Percentage	Percentage	Percentage	Percentage	Percentage
Untreated	68	1.5	8.8	25.0	35.3	38.2
Water	104	17.1	25.4	37.8	49.2	59.0
0.15 percent thiourea	131	22.9	54.0	62.0	70.8	79.9
0.5 percent hydrogen peroxide	75	11.7	35.0	42.5	48.3	66.7
0.5 percent taka diastase	85	10.0	23.9	35.0	46.4	54.4
0.02 percent potassium permanganate	89	7.8	26.1	34.2	46.4	58.1

Table 4.—Effect of sprouting before or during cold treatment on bolting. All lots stored 40 days at approximately 40° F. with 40 percent added moisture. Planted October 12, 1938, under continuous illumination.

Condition of seed when planted	Number of plants	Bolters at respective days after planting 59	73	97	118	134	146
		Percentage	Percentage	Percentage	Percentage	Percentage	Percentage
Untreated*	68	1.5	8.8	25.0	35.3	38.2	57.4
Not sprouted	318	4.7	17.3	26.2	38.7	55.0	68.6
Sprouted	166	25.4	48.4	58.3	65.7	72.2	84.9
Difference for	Odds 19:1	10.28	14.42	14.40	15.87	13.11	11.73
significance	Odds 99:1	14.97	21.00	20.97	23.11	19.09	17.08

*Data on plants from untreated seed not statistically analyzed, but included for comparison.

Field Experiments.—Until recent years the commercial reproduction of sugar beets was induced by storing the roots grown in one season over winter, until time for replanting the following spring. Thus seed was produced during the second summer. Following the discovery of the method of seed growing by overwintering plants in the field (5). this method has been used almost exclusively in the commercial production of American-grown sugar-beet seed.

Tests have shown (8) that the short, mild winters in the areas where the sugar-beet seed industry was first established tended to reproduce predominantly the easy-bolting types and failed to reproduce non-bolting types satisfactorily. It was evident that supplementary thermal induction would be desirable in these areas and vernalization of seed was suggested as having helpful possibilities.

Field plantings of vernalized and untreated sugar-beet seed were made over a period of 2 years in 3 western states. A summary of some of the data are presented in table 5. In tests at St. George, Utah, and Logandale, Nevada, vernalized seed produced a small increase in the percentage of plants classified as seed producers.

Winter and early spring plantings at Davis, California, and Salt Lake City, Utah, respectively, resulted in increased bolting of plants grown from vernalized seed. However, the total percentages of bolting in these plantings were lower than were obtained in the same areas from untreated seed planted earlier in the season.

Table 5.—Field plantings of vernalized and untreated sugar-beet seed. The influence of date of planting' and eliniate on subsequent bolting- and maturity of plants.*

Planting date	Location of test	Variety and treatment	Plants counted	Bolters	Seed producers
			Nurn-ber	Per-centage	Per-centage
August 26, 1936	St. George, Utah	550 untreated	100	97.0	
		550 vernalized	400	96.5	
Sept, 15, 1936	St. George, Utah	5(538 untreated	100	88.0	32.0
		5638 vernalized	300	89.7	41.0
		550 untreated	600	97.0	74.3
		550 vernalized	400	99.5	90.0
Sept. 20, 1936	Logandale, Nev.	5638 untreated	100	86.0	35.0
		5638 vernalized	500	89.0	48.2
		550 untreated	100	97.0	65.0
		550 vernalized	200	97.5	77.5
Oct. 24, 1936	Riverside, Calif.	5638 untreated	2550	3.58	
		5638 vernalized	3071	6,98	
		550 untreated	787	11.20	
		550 vernalized	1923	6.90	
Sept. 15, 1937	St. George, Utah	017 untreated	200	98.5	87.5
		617 vernalized	1400	98.7	91.0
Dec. 23, 1937	Davis, Calif.	5638 untreated	1018	18.0	
		5638 vernalized	2723	29.3	
March 16, 1937	Salt Lake City, Utah	618 untreated	95	1.0	
		618 vernalized	162	12.9	
May 5, 1937	Salt Lake City, Utah	5651 untreated	291	0.0	
		5651 vernalized	139	0.0	
		Misc. vernalized	1591	0.0	

*Tests at St. George, Utah, and at Logandale, Nevada, were conducted in cooperation with Bion Tolman, Assistant Agronomist; the test at Riverside, California, by Eubanks Carsner, Senior Pathologist; and the test at Davis, California, by IP. G. Larmer, Assistant Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture.

A May 5 planting at Salt Lake City failed to produce any bolting in plants grown from either vernalized or untreated seed.

Tests in commercial seed fields planted during August and September indicated little or no increased bolting resulting from the use of vernalized seed. The warm weather following planting evidently caused reversal of the thermal induction effected during vernalization treatment, so that little residual effect was left by the time temperatures in the field became cool enough for the resumption of thermal induction.

Discussion and Conclusions

That some measure of thermal induction may be accomplished in sugar-beet seed during germination was shown by successive tests conducted over a period of several years. The extent of the induction was highly correlated with the extent of sprouting. Without some visible sprouting previous to or during the vernalization process, the amount or degree of induction was small or insignificant.

Although greater thermal induction can be accomplished in the more advanced stages of germination, it is not practical to handle vernalized seedlings with long sprouts which become detached from the seedball. If the seed is to be hand-planted while moist, sprouts up to ½ inch in length are not objectionable, and for specialized greenhouse work this extent of sprouting is probably optimum. For field plantings, however, sprouting of seed should not be allowed to advance to the point where radicles are injured during planting.

The extent of sprouting during vernalization is dependent upon the moisture content of the seed, the temperature, and length of the cold-storage period. Most of the experimental vernalization treatments were conducted at 33° to 36° F., and some of the periods of storage were as long as 175 days, but there was no apparent advantage in extending the period longer than 60 days. To limit the extent of sprouting, the moisture content of the seed was found to be highly important. Good results were obtained by drying the previously soaked seed to 135 percent to 140 percent of the original weight of the air-dried seed.

The increased bolting of plants from seed previously soaked in dilute solutions of thiourea was probably due to more uniform and complete sprouting of the seed during storage and a healthier condition of the sprouts rather than any specific influence on thermal induction. The action of thiourea is not known, but it may have helped to reduce injury from ammonia. This improvement in germination resulting from the use of thiourea is in agreement with the improved germination of lettuce seed as observed by Thompson and Kosar (11).

Plants from vernalized seed are usually from 2 to 4 days earlier in emerging from the soil than plants from untreated seed. It has been suggested that this factor may be of sufficient importance under some conditions to warrant vernalization of seed for field planting. Earlier emergence of plants has also been observed (10) when the seed was washed, slightly pregerminated, and dried before planting. This simpler method of hastening seedling emergence is preferable, because the vernalization treatment is more complicated and more difficult to control.

The practical application of vernalization to sugar-beet seed production is dependent upon two principal factors. The first of these factors is the extent to which the thermal induction process can be completed. The second and probably more important factor is the question of the reversibility of the process. The tests conducted by the writers showed that the extent of thermal induction by vernalization was limited and bolting was always dependent upon further photothermal induction after planting. The question of the reversibility of developmental processes in plants has been considered by several writers. The theory of phasic development in plants as formulated by Lysenko (4) considered that the steps or stages through which plants must pass before reaching sexual maturity were irreversible, but his opinion does not agree with the results of other workers. Ljubimenko and other Russian workers (4) cite instances of the evident reversibility of phasic development. Efeikin (2) demonstrated that vernalized winter wheat was rapidly devernalized at a temperature of 34° to 35° C. Chroboczek (1), Voss (13), and Owen (6) pointed out several examples of reversal in the developmental processes in sugar beets. The results obtained with plants grown from vernalized sugar-beet seed indicate that thermal induction acquired during vernalization was not expressed unless the seed was planted in an environment favorable to the continuation of the thermal induction process.

Vernalization of sugar-beet seed is useful to breeders because it affords a method of hastening reproductive development under controlled greenhouse conditions. Beets grown from vernalized seed have frequently flowered 1 month earlier than beets grown from untreated seed. By the use of vernalized seed the reproduction of lines of special interest has been hastened sufficiently to save the time of an entire season.

Field plantings for sugar-beet seed production in mild climates such as southern Utah are made during late August or early September when maximum temperatures above 90° F. are prevalent. It is probable that at these temperatures the induction process is reversed. This would account for the fact that little or no increase in reproductive development was obtained by using vernalized seed in this environment.

Summary

Vernalization or exposure of slightly germinating sugar-beet seed to temperatures between 33° and 40° F. hastened the reproductive development of plants when the seed was planted in an environment favorable for the continuation of photothermal induction with cool temperatures and long photoperiods. The success of vernalization treatments was found to depend upon securing a visible amount of sprouting before or during cold storage and upon a healthy condition of the sprouts at the time of planting. Thorough washing of the seed and air drying, followed by soaking in 0.15 to 0.2-percent solution of thiourea appeared to aid uniform germination and reduce injury to seed during storage. Frequent stirring or aeration further reduced storage injury. As the seed was placed in cold storage the addition of 35 percent to 40 percent of moisture to the original weight of air-dried seed appeared to be optimum.

Vernalization is a practical means of hastening reproduction under controlled greenhouse conditions. It is probably impractical as a means of increasing bolting in seed fields because, under the warm temperatures usually prevailing for some weeks after planting time, the thermal induction acquired by seed vernalization appears to be lost because of the reversible nature of the process.

Literature Cited

1. Chroboczek, Emil. A Study of Some Ecological Factors Influencing Seedstalk Development in Beets *(Beta vulgaris* L.). N. Y.'Agr. Exp. Sta. Mem. 154, 84 pp., illus. 1922.
2. Efeikin, A. K. Devernalization of Vernalized Winter Wheats. Compt. Bend. (Doklacly) Acad. Sci. U.S.S.R, **30:661-663,** illus. 1941.
3. Fisher, R. A. Statistical Methods for Research Workers. Ed. 4, rev. and enl. 307 pp., illus. Edinburgh and London. 1932.
4. Imperial Bureau of Plant Genetics. Vernalization and Phasic Development of Plants. Imp. Bur. Plant Genet. Bul. 17, 151 pp. 1935.
5. Overpeek, John C. Seed Production From Sugar Beets Overwintered in the Field. U. S. D. A. Circ 20, 8 pp., illus. 1928.
6. Owen, F. V. Asexual Propagation of Sugar Beets. Jour. Heredity 32:187-192. 1941.
7. Owen, F. V. Carsner, Eubanks, and Stout, Myron. Photothermal Induction of Flowering in Sugar Beets. Jour. Agr. Res. 61:101-124. 1940.
8. Owen, F. V., et al. Curly-top-resistant Sugar-beet Varieties in 1938. U. S. D. A. Circ. 513, 9 pp., illus. 1939.

9. Stout, Myron, and Tolman, Bion. Factors Affecting the Germination of Sugar-beet and Other Seeds, with Special Reference lo the Toxic Effects of Ammonia. Jour. Agr. Res. 63: 687-713, illus. 1941.

10. Stout, Myron and Tolman, Bion. Interference of Ammonia, Released from Sugar-beet Seedballs, with Laboratory Germination Tests, flour. Amor. Soc. Agron. 33:65-69, illus. 1941.

11. Thompson, R. *C*, and Kosar, W. F. Stimulation of Germination of .Dormant Lettuce Seed by Sulfur Compounds. Plant Physiol. 14 :567-573, illus. 1939.

12. Tolman, Bion, and Stout, Myron. Toxic Effect on Germinating Sugar-beet Seed of Water-soluble Substances in the Seedball. Jour. Agr. Res. 61:817-830, illus. 1940.

13. Voss, J. Experimentell auslosung des schossens und prufung der schossneigung der rubensorten *(Beta vulgaris* L.). Angew. Bot. 18 :370-407, illus. 1936.

Winter Stecklings[1]

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In a sugar-beet breeding program such as ours it is highly desirable to go ahead rapidly with the current year's seed increase of many promising lines. Under our conditions this year's seed would normally be planted next spring or summer for the production of roots or stecklings which in turn would be stored during the following winter and then used for seed production in isolations the spring of the second year. Obviously, by producing stecklings during the winter immediately following the harvest of the new seed, a full year in this cycle can be saved.

The growing season in northern Wyoming, where the major portion of our breeding work is being done, is very short and sugar-beet seed does not mature until after the first of August and at times maturity extends well into September, depending upon varietal differences and other conditions. The period between harvest of the seed and killing frosts is too short for field production of stecklings of a usable size as is possible in southern Colorado where the total frost-

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