# Induction of Reproductive Development in Sugar Beets by Photothermal Treatment of Young Seedlings<sup>1</sup> JOHN O. GASKILL<sup>2</sup>

In many types of breeding jobs and genetic studies with sugar beets, Beta vulgaris L., a short life cycle is very desirable, provided it does not promote the development of annual genetic tendencies. Various techniques have been employed for that purpose by breeders in the United States.

One of the most efficient of these methods, from the standpoint of time required for the complete cycle, was developed a number of years ago by the Division of Sugar Plant Investigations<sup>3</sup>. Seed is planted in the greenhouse in the fall, and the young seedlings are photothermally induced (in the greenhouse) by prolonged cool temperature and continuous light. The induction process<sup>4</sup>, with average temperature of approximately 50° F., requires about two and one-half to three months, and the seed crop may be harvested within six to seven months from date of planting.

The method has proved satisfactory through experience at Fort Collins, Colorado; Beltsville, Maryland, and Salt Lake City, Utah, but its usefulness is limited by its dependence upon cold weather for induction.

As a result of a series of experiments conducted recently at Fort Collins, a new method has been developed by means of which two successive generations of sugar-beet seed can be produced in 12 months. Seed yields are relatively small. However, in breeding work and genetic studies a short life cycle frequently is far more important than a large quantity of seed per plant. Although further research undoubtedly will lead to improvements in technique and to a clearer understanding of the usefulness and limitations of this method, results obtained thus far have shown quite conclusively that it is practicable under conditions existing at Fort Collins.

A brief, tentative outline of the method has been presented in another report (1)<sup>5</sup>, and it is the purpose of this paper to summarize the experimental results.

# **Experimental Procedure and Results**

A preliminary experiment, begun in the fall of 1950, showed that induction can be accomplished satisfactorily in young sugar beet seedlings in the absence of sunlight by means of prolonged exposure to low temperature with continuous artificial light.

In subsequent experiments discussed in this paper all induction treatments were performed in that way, with light provided by means of one 150-watt, white-frosted, incandescent electric bulb in a medium depth reflector, 14 to 16 inches in diameter, suspended 20 inches above the sur-

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face of the soil. In cases where "continuous" illumination is referred to in connection with field and greenhouse conditions, sunlight was supplemented by means of the same type of incandescent unit, operated throughout each night, with the reflector about 30 inches above the soil at first and raised as seed stalks elongated. More than one light unit was used only for especially large groups of pots. Temperatures in greenhouse and field were recorded in the shade by means of thermographs. Thermometers were used for this purpose in the induction room with bulbs directly beneath the light, about one-half inch above the surface of the soil, and not shaded.

# Age of Seedlings

An experiment, designated as number two, was designed primarily to study the effect of age of seedlings on reproductive response to photothermal treatment. The wide-base, leaf-spot-resistant variety, U. S. 226, about average in bolting<sup>9</sup> tendencies, was chosen for this study. Seed was planted on four different dates in three-inch pots which were kept in a warm greenhouse with continuous light until March 9, 1951, when they were transferred to the induction room. Seedlings were thinned at random to two per pot not long after appearance of the first pair of true leaves on the majority of plants of any given age group. After 6 to 10 weeks' induction treatment at approximately 48° F., the plants in each pot were transferred to a sixinch pot and returned to moderately warm greenhouse conditions where continuous light was provided. The seed was harvested and final counts were made 13 weeks after the end of induction exposure.

The principal highlight in the summarized results of experiment 2 (Table 1) is in the relationship between seed yields and ages of plants at the beginning of induction—a strong trend toward higher yields for older plants. This trend is illustrated in Figure 1 which also shows a tendency for older seedlings to bolt and reach flowering stage more promptly.

From these results it was concluded that, for most practical purposes, seedlings should be allowed at least to emerge from the soil before being subjected to induction treatment. In this experiment, emergence in the greenhouse occurred largely between the fifth and tenth days after planting; none before the fifth day. A second highlight which may be noted in Table 1 is that 38 of the 40 plants receiving 10 weeks' induction exposure (series "C") developed seed balls by date of harvest—23 to 27 weeks after the seed had been planted. Germination for the four treatment numbers ranged from hourly readings on thermograph charts, for the first 6 weeks of the post-induction period for series "C" were 68, 67, 65, 64, 68 and 66 respectively; average 66. Determined from the same charts, the average daily maximum for the 6-weeks' period was  $84^\circ$ , and the average daily minimum was  $54^\circ$ .

From the seed-stalk counts given in Table 1 it is apparent that induction exposure of 8 weeks or less was insufficient to produce satisfactory bolting under the conditions of the post-induction period. However, it should be noted that the 3 groups of plants, representing the 3 lengths of induction, were returned to the greenhouse on different dates. Consequently they

<sup>6</sup> Seed-stalk production.

were not under identical conditions of light and temperature immediately following induction and therefore were not strictly comparable.

### **Induction Time and Temperature**

Experiment 3 was conducted for the purpose of studying effects of induction time and temperature, using a seed lot which had been produced by means of a representative mass increase of the backcross, multigerm x (multigerm x monogerm). The population dealt with was known to include a substantial proportion of slow-bolting individuals requiring more than the average length of induction treatment for satisfactory bolting-a characteristic inherited from the monogerm parent (2).

Seedlings were grown for two weeks in three-inch pots in a warm greenhouse, thinned to four plants per pot, and then subjected to the following induction treatments:

Table 1.—Reproductive R			Seedlings	of	Different	Ages	to	Photo-
thermal Induction Treatments;	U. S. 226	Variety.						

			Harvest results <sup>4</sup>							
Treatment No.	Pre- Induction period in greenhouset	Induction period*	Total plants living	Plants with normal seed stalks*	Plants having well formed seed balls	seed per	Germinations			
	Days	Weeks	Number	Number	Number	Grams	Percent			
1A	Ó	6	10	8	7	1.4	67			
2A	4	6	10	5	5	1.1	69			
3A	21	6	10	8	8	2.8	80			
44	28	6	10	8	8	2.8	74			
18	0	8	10	5	4	0.6	45			
2B	4	8	5	7	5	2.6	87			
<b>5B</b>	14	8	10	9	7	4.2	89			
4B	28	8	10	8	8	4.8	91			
IC	0	10	10	10	9	1.0	75			
2C	4	10	10	10	10	1.5	88			
3C	14	10	10	9	9	2.3	90			
4C	28	20	10	10	10	5.0	85			

<sup>1</sup> Time computed from date of seeding until beginning of induction period. <sup>3</sup> Induction period (low temperature and continuous artificial light) began on March 9, 1951, for all treatment numbers. <sup>4</sup> Seed Analysis (classed as abnormal) were excluded. <sup>4</sup> Receiptive type each stalks (classed as abnormal) were excluded.

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3C	14	10	10	9	9	2.3	90
4C	.28	10	10	10	10	5.0	85
<sup>1</sup> / <sub>2</sub> Time c	omputed from d	ate of see	eding until b	eginning o tinuous ar	f induction	period.	on March

<sup>2</sup> Induction period Clow temperature<sup>2</sup> and continuous artificial light) began on 1951, for all treatment numbers. <sup>4</sup> Seed harvested and final counts made 13 weeks after end of induction exposure. <sup>4</sup> Vegetative type seed stalks (classed as abnormal) were excluded. unciai iignt) began on March 9,

5 Based on plants having well-formed seed balls, only.

Three groups of plants were held for five weeks at temperatures of approximately 40°, 48°, and 57° F., respectively. Another set of three groups was given 10 weeks' exposure in a similar manner. Plantings were so timed that induction treatments for all six groups ended on July 27, 1951. On. that date the four seedlings in each pot were transplanted in an eight-inch pot and taken to the field together with a set of comparable controls which were four weeks old at that time. Since days were rather long when this experiment was begun, no direct artificial light was given to the controls before July 27 or to the other plants prior to induction treatment. However, as a precaution against reversal, continuous light was provided as usual during the post-induction period. The plants remained in the field under a lath shelter (partial shade) from July 27 until September 12, and then were returned to the relatively warm greenhouse. Average field temperatures (°F.) during the first six weeks following July 27 were 72, 67, 66, 62, 65

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The results (Table 2 and Figure 2) show that five weeks' induction treatment was decidedly inadequate at each temperature. Ten weeks' induction produced better results at each temperature—strikingly so in the case of treatments four and five—as judged by final percentages of normal





Figure 1. Top: Sugar beet seedlings of the U. S. 226 variety in three-inch pots at the end of 10 weeks' induction treatment, May 18, 1951; age of plants in the four pots at the beginning of induction, from left to right, was 0, 4, 14, and 28 days, respectively (time computed from date of seeding)-

Bottom: Same treatments as above, six and one-half weeks after induction; six-inch pots with two plants in each.

Considering these final figures, together with counts made earlier, it appears that, under conditions such as those maintained in this experiment, the optimum temperature for induction of young seedlings of the type used in this study is about or possibly a little below  $48^{\circ}$  F. It is of interest to note that this conclusion approximates that reported by Stout (3) for induction of sugar-beet roots stored in the dark.

In actual practice, normal seed-stalk production closely approaching 100 percent usually is desirable. Although the best treatment in this experiment (No. 5—intermediate induction temperature for 10 weeks) resulted in only 86 percent normal bolting, it should be noted in the results reported for experiment four that 11 weeks' induction produced nearly 100 percent normal bolting in plants of the same strain (Table 3).

Supplemental Light in the Post-Induction Period

When fully induced sugar-beet roots (mother beets or stecklings) are used for growing seed in the usual way, with transplanting in the field taking place fairly early in the spring, artificial light is not necessary. However, it was suspected that small seedlings might require supplemental light in the post-induction period, especially when that period begins in warm weather. In order to study the importance of extra light at that time, a simple comparison was made between two sets of plants. For convenience this will be called experiment 4.

Table 2.—Results of a Time and Temperature Study Pertaining to Photothermal Induction of Young Sugar-beet Seedlings of a Relatively Slow-bolting Strain; 28 Plants in Each of 7 treatments.

	Induci	lon treatment		nts-total	Final counts—planta		
Treatment		Average		i seed stalks	with normal seed stalks		
No.	Weeks	temperature	I week	2 weeks	Total	Flowering	
	Number	°F.	Percent	Percent	Percent	Percent	
1	5	40	0	0	21	14	
2	5	48	0	4	18	18	
5	5	57	11	14	22*	22ª	
	10	40	7	54	711	68	
s.	iõ	48	50	71	861	79	
6	10	57	4	29	364	52	
Ť	ō	(Control)	õ	0	4	4	

<sup>1</sup> All plants with seed stalks large enough to detect, one week and two weeks after the end of multition treatment. A shortmain were exclusied. <sup>3</sup> Based on 27 living plants. <sup>4</sup> Analysis of variance, performed for treatments 4, 5, and 6, only showed that 4 and 5 did not differ significantly in percentage of plants with normal seed stalks, but each was significantly superior to number 6.

1 pi a of induction treatment. Counts made 10 weeks after end of induction period; vegetative type seed stalks (classed a bnormal) were excluded. Based on 27 living plants. \* Analysis of variance, performed for treatments 4, 5, and 6, only, showed that 4 and 5 did not differ significantly in percentage of plants with normal seed stalks, but each was significantly superior to number 6.

Seedlings of the same strain as that used in experiment three were grown in flats for 13 weeks-the first two weeks in the greenhouse and the remaining 11 weeks in the induction room, with conditions of light and temperature in each case being approximately the same as those described for treatment five (experiment 3).

At the end of the induction treatment (August 2, 1951) 80 plants were transplanted singly in six-inch pots and divided at random into two groups of 40 each which were placed in the field, in the open, a short distance apart. Comparable control plants, five weeks old, were included in each location. Conditions of light, temperature, etc., were identical for the two groups, except that one (treatment 1) received no supplemental light and the other was lighted continuously. On September 12 all plants were returned to the relatively warm greenhouse where the field lighting system was continued, except that very weak, indirect artificial light reached the plants of treatment 1. While in the field, the two groups were near the plants of experiment 3, and consequently field temperatures listed for that study apply also to experiment 4, omitting the first week.

The results given in Table 3 show that supplemental light was essential for development of normal seed stalks under the conditions of this experiment. It is postulated that the beneficial effect of the artificial light in this case was due to prevention or retarding of the reversal process which is known to be promoted by moderate, to high temperatures (3). Reversal

seed stalks. On November 2, 1951, 26 weeks after date of planting, all seed was harvest from the 38 plants of treatment 2 listed as flowering 10 weeks after induction (Table 3). Average yield per plant was 2.3 grams, and germination of the pooled seed was 77 percent.



Figure 2. Top—Sugar beet seedlings in three-inch pots (four plants per pot) on July 25, 1951, two days before the end of induction period. Induction treatments, from left to right: low, medium, and high temperature, flowesks; low, medium and high temperature, 10 weeks; and control (no induction treatment). Plants of the first six treatments were two weeks old at the beginning of induction; the control plants were approximately four weeks of whether was taken.

Bottom-Same treatments as above, seven weeks after the end of induction period; four plants per pot.

#### **Post-Induction Temperature**

Since it was known that induction tends to be reversed in moderate to high temperatures (3), and since such temperatures would be encountered frequently in general use of the new seed production method described in this paper, an experiment (number 5) was conducted to study the question of temperature tolerance in the post-induction period. Seedlings 25 days old, of a relatively slow-bolting strain similar to that used in experiment 3, were given 14 weeks' induction treatment under conditions of light and temperature approximating those of treatment 5 of that study. At the end of induction (November 5, 1951) the plants were transferred singly to fourinch pots and divided into four comparable groups. All four groups were placed on one bench in the greenhouse, one group in the open and the other three in transparent chambers. One chamber was allowed to follow the normal greenhouse temperatures, and the other two were electrically heated. Each chamber was provided with continuous forced ventilation, and each of the four groups was given continuous light. At the end of four weeks all pots were placed on a greenhouse bench, under identical conditions, with supplemental illumination about the same as before.

This experiment is still in progress as this paper is being written and consequently a full report cannot be made at this time. Table 4 gives a summary of seed-stalk counts made eight weeks after the end of induction, together with temperature data. Since each of the 40 plants of treatment 1 (not in chamber) had produced a normal, flowering seed stalk by that time, it was assumed that essentially all of the plants in the experiment had been fully induced.

Table 3.—Effect of	Supplemental	Light, i	in the	Post-induction	Period, or	1 Bolting of
Photothermally Induced	Sugar-beet See	dlings.				

				Plants producing seed stalks <sup>1</sup>				
Treatment	Supplemental			Vegetative	Nor	mal type		
No.	light	Induction	plants	type	Total	Flowering		
		Weeks	Number	Number	Number	Number		
1	Negligible	(11	40	7	5	3		
		¥ 0	10	0	0	Û		
2	All night,	ίn.	40	0	39	58		
	150 W.	1 0	11	0	0	0		

<sup>1</sup> Stalks one inch or more in length; counts made 10 weeks after the end of induction.

Comparisons of treatments for temperature effects are valid only for numbers 2, 3, and 4-i.e., the three sets of plants which were inside the chambers. The highlight of these results is the strong tendency toward increased reversal with high temperature. For mean temperatures of 65°, 74°, and 83° F., the total number of plants lacking normal seed stalks was two, seven, and 12, respectively. Expressed as percentages, these figures became five, 18, and 30. This significant trend shows the need for caution with respect to high temperatures during the early part of the post-induction period. It should be noted, however, that the strain used in this study is a slow-bolting type. The reversal problem may be less serious where strains with average bolting tendencies are used. A preliminary experiment has indicated that the reversal tendency expected when the induction period ends during hot weather may be at least partially circumvented by holding the plants in a refrigerated room for several weeks, under artificial light, at an intermediate temperature (e.g., about 60° F.) before transferring them to the field or greenhouse.

## Discussion

The results presented in this paper have shown conclusively that the seed production method described is feasible under conditions at Fort

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Collins, Colorado. However, exploration of the various phases of the problem worthy of study has only begun. Among the unexplored questions meriting attention is that of light effects—intensity, type, day length, etc.— before, during and after induction. Until more is known about these factors, the use of continuous illumination throughout the entire life cycle is suggested. From various observations—not planned studies—it appears that low light intensity following induction tends to increase reversal action, especially in slow-bolting material.

The need for research on this phase of the problem is particularly urgent because of the relative absence of sunshine during the winter months in some sugar-beet regions.

Another avenue of light study which is especially inviting concerns the production of seed indoors without sunlight. Evidence that such a procedure may be feasible has been obtained at Fort Collins where a small

Table 4.—Effect o	f Temperature Upon	Seed-stalk Development	in	Photothermally	In-
duced Sugar-beet Seedli	ngs; 40 Plants in Eac	h of 4 Treatments.		-	

					Plants	Plants producing seed stalks*			
Treatment Enclo- No. sure <sup>3</sup>	Average temperatures <sup>a</sup>			without		Normal type			
		Daily maximum	Daily minimum	Mcau <sup>4</sup>	sced stalks	Vegetative type	Total	Flowering	
		°F.	۰۲.	°F.	Number	Number	Number	Number	
1	None	81	57	66	0	0	40	40	
2	Trans.								
	chambe	r 77	56	65	0	2	38	38	
.9	do	87	67	74	4	3	33	32	
4	do	54	76	83	4	8	28	27	

 $^1$  The plants of iteration 1 (in the open on a greenhouse beach) served merely as controls for appraisal of the behavior of the treatment 2 population. Plants of treatments 2, 3, and 4 all were in transparent temperature chambers.

<sup>4</sup> Recorded in the shade, by means of thermographs, during the first four weeks following induction. Thereafter, all plants in the experiment were held under identical conditions, with temperatures not greatly different from those shown above for treatment 3.

\* Stalks one inch or more in length; counts made eight weeks after the end of induction period.

<sup>4</sup> Estimated from thermograph charts-not averages of maximum and minimum temperatures.

NOTE: According to chi-square test, the apparent association between temperatures and numbers of plants with normal seed stalks (treatments 2, 5, and 4) is significant (P < .02).

group of seedlings, induced by low temperature and artificial light, subsequently was brought to seed in strong incandescent light without any sunlight whatsoever.

#### Summary

Through a series of experiments a method has been developed which permits the growing of two successive generations of sugar-beet seed in one year. Induction is accomplished in young seedlings by prolonged exposure to low temperature and continuous artificial light. Moderate to warm growing conditions in field or greenhouse, with continuous light, are maintained before and after the induction period. The complete life cyclefrom seed to mature seed—has been produced in six months, using a relatively slow-bolting strain, and in less than six months with a commercial variety having about average bolting tendencies. Flowering was near 100 percent in each case.

## Literature Cited

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