SEEDSTALK DEVELOPMENT OF LETTUCE AS AFFECTED BY GROWTH REGULATORS, VERNALIZATION, TEMPERATURE AND PHOTOPERIOD

Thesis for the Degree of Ph. D.

MICHIGAN STATE COLLEGE

William T. Andrew

1953

This is to certify that the

thesis entitled

SEEDSTALK DEVELOPMENT OF LETTUCE AS AFFECTED BY GROWTH REGULATORS, VERNALIZATION, TEMPERATURE AND PHOTOPERIOD

presented by

William T. Andrew

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Horticulture

Sylvan H. Wittwer
Major professor

Date October 12, 1953

N.V.

SEEDSTALK DEVELOPMENT OF LETTUCE AS AFFECTED BY GROWTH REGULATORS, VERNALIZATION, TEMPERATURE AND PHOTOPERIOD

By

William T. Andrew

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Horticulture

1953

ACKNOWLEDGEMENT

Appreciation is expressed to Professor S. H. Wittwer for his valuable suggestions and guidance during the investigations and preparation of this manuscript and to the American Cyanamid Company whose financial assistance enabled the writer to continue training on the graduate level.

DEDICATION

To Leone, my wife, who was patient, helpful and understanding.

To John and Steven who are about to become much better acquainted with their dad.

To Edith Andrew and the late John F. Andrew because the earlier efforts of parents are sometimes forgotten.

William T. Andrew

ABSTRACT

A series of field and greenhouse experiments was conducted over a period of two years to determine the effects of vernalization, certain growth regulators, plant growing temperatures and photoperiod on the seedstalk development of a number of varieties of lettuce.

2,4-Dichlorophenoxyacetic acid (2,4-D) at concentrations of 1, 5, 10, 20, and 40 ppm applied singly and in repeated applications to lettuce plants at several stages of development resulted in a significant hastening of seedstalk development when applied to Great Lakes lettuce plants 94 days old, in a significant retardation when applied to Great Lakes and Imperial 456 plants 65 days old and had no effect when applied to 65 day old plants of the Slobolt variety. The degree and nature of the response varied with the variety, the concentration of the solution, the time of application and the temperature at which the plants were grown. No significant differences were obtained by spraying with maleic hydrazide (50, 100, 500 ppm), n p. chlorophenylphthalamic acid (100, 500 ppm) or benzothiazol-2-oxyacetic acid (100, 500 ppm). Soaking seed in solutions of 2,4-D varying from 0.1 ppm to 10 ppm resulted in a decrease in the rate of and percentage germination as the concentration of the growth regulator was increased. Plants grown from seed soaked in 1 ppm 2,4-D before vernalization developed seedstalks more rapidly than plants from seed sown dry, but not as rapidly as plants soaked in water before vernalization. Soaking non-vernalized seed in 1 ppm 2,4-D or in water for 24 hours before seeding resulted in plants developing seedstalks more rapidly than control plants grown from seed sown dry in

the customary manner.

Seed of Great Lakes, Imperial 456, Imperial 847, and Slobolt lettuce vernalized at a temperature of $40 \pm 2^{\circ}F$ for 16 days resulted in significantly earlier seedstalk development and flowering compared with control plants grown from seed handled without cold treatment. Plants grown from seed subjected to low temperatures and moist conditions without previous soaking in water produced seedstalks as early as plants from seed allowed to soak before low temperature treatment. Plants of head lettuce varieties (Great Lakes, Imperial 456 and Imperial 847) grown from vernalized seed developed almost entirely without the customary head formation. Cold treatment of seeds sown in flats of moist soil was as effective as the laboratory technique involving the use of petri dishes provided treatment was applied no later than seedling emergence. Vernalization in seed flats offers a seemingly practical solution to obtaining desired seed production from hard headed and long standing lettuce varieties.

A night temperature of $60^{\circ}F$ was more effective than a night temperature of $50^{\circ}F$ in increasing the rate of seedstalk development of plants subjected to vernalization or sprayed with 2,4-D. In the case of plants receiving vernalization plus a 2,4-D spray and those receiving neither spray nor cold treatment, the effect of the higher temperature was not so pronounced. Plants subjected to a photoperiod of 15 hours did not produce seedstalks earlier than those grown under a nine hour day unless the germinating seed had previously been vernalized. Although time of flowering was influenced by both temperature and photoperiod, neither factor appeared to be the limiting or controlling influence in determining vegetative or reproductive growth. The responses to variations in photoperiod indicate that classification of lettuce as a long day plant may not be justified.

Sylvan H. Wittwer

TABLE OF CONTENTS

Table of Contents (continued). Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Coincident with the development of heat resistant varieties of lettuce there has arisen a seed production problem of considerable economic importance. Certain characteristics of these varieties, such as their ability to produce well at higher temperatures, and their tendency toward delayed seedstalk elongation, are greatly desired by truck gardeners but it is becoming increasingly difficult for the seed grower to produce seed. In some of the areas best adapted for lettuce seed production there is hardly sufficient time under normal growing conditions for completion of the seedstalk elongation, floral development, seed formation, and maturation sequence (9) (27). These new varieties not only develop seedstalks at a later date but in the case of crisphead types, the tightly formed heads offer a physical barrier to seedstalk development after its growth has been initiated (9) (27).

The combined influence of these two factors usually results in a major portion of the seed being harvested from the earlier bolting heavier seed producing plants. Thus a natural selection is set up defeating the original purpose for which these varieties were developed. Two possible solutions are suggested: (1) by some physiological or chemical means not transmittible to the progeny, to bring about an earlier differentiation of the floral primordia or seedstalk initials, and (2) to prevent the development of, or overcome, the effect of the tight head as a physical barrier.

REVIEW OF LITERATURE

Studies of the effects on plants of such factors as vernalization, temperature, photoperiod and growth regulators may be termed, in general, studies of the developmental physiology of plants. Investigations of such phenomena are said to have begun with the experiments of Klebs (24) (39) which were concerned with the control of growth and development by appropriate adjustments of two decisive factors of the environment, temperature and light. Since that time there have been periodic surges of interest in single factors or in the interactions of various factors influencing the development of plants. Advancements in the study of the flowering phase of development, however, have, in the opinion of Gregory (13) been determined by three recent researches: first, Gassner's work (11) concerning the exposure of germinating cereal seeds to low temperatures, secondly, the discovery by Garner and Allard (10) of the importance of the duration of alternate light and dark periods in controlling flowering and, thirdly, the isolation by Went (39) of plant hormones controlling extension growth and meristematic activity.

The first recorded studies of low temperature effects on floral initiation appear to pre-date the work of Klebs and Gassner. In 1857 J. H. Klippart (16) reported:

> "To convert winter into spring wheat nothing more is necessary than that the winter wheat should be allowed to germinate slightly in the fall or winter but kept from vegetation by a low temperature or freezing until it can be sown in the spring".

Later, Gassner, in 1918 (11), sowed seeds of cereals in sand at different dates and subjected them to temperatures of from 1° to 24° C during germination. Gassner's data indicate that plants germinated at a low temperature reached the bolting stage more rapidly and more regularly than those germinated at higher temperatures. The term "vernalization", however, in reference to the chilling of seed became known to the English speaking world only with the revived interest in temperature effects following the publication (in English) of the work of Lysenko and his associates at the Institute of Plant Breeding and Genetics at Odessa (24) (39).

Rudorf and Stelzner (30) in 1934 noted that lettuce plants grown from seedlings exposed immediately after germination to a temperature of $-5^{o}C$ for a period of 10 days showed a very perceptible retardation in growth rate as compared to plants exposed for 10 days to $+5^{o}C$. Investigations conducted at Cornell University from 1935 to 1937 (17) by Knott et al, indicated that exposure of germinating lettuce seed to a temperature of $40^{o}F$ for a period of 20 days resulted in more rapid seedstalk development than any of the lower temperatures used. Plants from seed so treated failed to produce as many good heads as plants subjected to other treatments, and the tendency to form an open rosette of leaves indicated that 10 to 20 days at $40^{o}F$ had vernalized the lettuce seed and stimulated the reproductive process. Twenty days at $40^{o}F$ also appeared to be more effective than 10 days at $40^{o}F$.

In studying the possibilities of applying phasic development to plant breeding, Reimers in 1938 (39), working in the laboratory of Physiology of the U.S.S.R. Research Institute of Vegetable Production, subjected germinating seeds of four varieties of lettuce to a temperature range of from 2.5 to $5^{o}C$ for periods of 10, 20 and 30 days, respectively. Vernalization for 10 days was apparently sufficient to accelerate bolting of one of the four varieties but the other three were not able to complete what Reimers termed their "thermo-phase" in ten days. He concluded that the variety "Ideal" was able to pass its thermo-phase in 10 days but the three other varieties required about 20 days. When green plants, at the stage of formation of the first true leaf were subjected to temperatures of from 2.5 to 5°C, both the growth and shooting of the lettuce plants were slowed down. Reimers concluded that lettuce can be vernalized only at the stage of germinating seeds.

More recently two crisphead types of head lettuce, Imperial D and Imperial 847, were vernalized by Gray (12). Seeds previously soaked for one day were placed in a 4°C refrigerator for 28, 42, and 56 days respectively. There were no important differences in flowering responses among treatments. Some difficulty was encountered, however, in keeping the material alive for the longer periods. Plants from treated seed produced seedstalks 2 to 3 weeks earlier than the controls.

Similar results were obtained by Simpson (31) who vernalized the variety "Ideal" at 2 to 8°C for 16 days and obtained flower stalks 20 days earlier than from the controls. Simpson mentions the affect on heading similar to the observations made by Knott et al (17). Plants from the vernalized seed remained in the hearted condition only 4 or 5 days while the controls remained in the hearted condition approximately 26 days.

Warne in 1947 (38) varied the soaking period prior to cold treatment. Those seeds subjected to his treatment "V" were soaked 24 hours before being held at 0 to 4°C for 24 days; those subjected to treatment "VS" were soaked for 72 hours before being given the same cold treatment. Plants of treatment "V" bolted approximately 10 days before plants from treatment "VS". Warne (38) concluded that vernalization treatment after the radicle appears is much less effective than treatment commenced after the seed had swollen for only 24 hours and before germination had begun. Thompson and Kosar (35) investigating the possibilities of vernalizing the variety Slobolt to facilitate seed production obtained no significant differences between vernalized and non-vernalized plants. They concluded that,

"At no time in the development of seed stems was it possible to visually detect a definite difference in bolting between vernalized and check plots."

Milthorpe and Horowitz reviewing effects of environmental conditions on a number of crops (23) state that there are differences in the behavior of varieties of lettuce as regards head and seed formation. Flowering in some varieties of lettuce is initiated by low temperatures while in other varieties it is brought about by high temperatures. Exposure to low temperature for 10 to 20 days at 40°F during germination and early growth stimulates seed stalk production if such conditions are followed by high temperatures and long photoperiods.

Photoperiodic effects on lettuce have also been studied by other workers, as a single factor and in conjunction with growing temperatures. Arthur and Guthrie (1) in 1926 studying the effects of carbon-dioxide, light, and temperature on flower and fruit production concluded that lettuce flowers under long days (exceeding 12 hours). In 1931 Bremer (3) reported that winter and spring lettuce varieties are unable to form heads in summer because of the long days. Forcing lettuce "Golden Queen" and spring lettuce "May King" grown under 17 - 18½ hours of daylight bolted in 50 to 56 days without forming marketable heads. When given a twelve hour day, fine, firm heads were formed and the plants bolted in 75 to 80 days. Under a 9-hour day satisfactory heads were formed and bolting occurred in 85 to 90 days. Plants subjected to only 6 hours of light produced some loose "knittings", others only leaves, and 107 to 117 days were required to reach the bolting stage. The summer types of lettuce, e.g. Tom Thumb, Rudolph's Favorite, were not influenced in time of bolting by length of day. Bremer and Grana (4) in attempts to apply the variation in daylength to study of genetic differences between winter, spring, and summer forms of lettuce selected plants from the variety "Kaiser Treib" which produced seedstalks during a short day or long day but formed rosettes only during a short day.

Using the White Boston variety of butterhead type lettuce Thompson and Knott (34) obtained data which showed most satisfactory head formation at a 60 to 70°F range in temperature. Under greenhouse conditions a temperature range of 70 to 80°F prohibited head formation even with a short daylight period (10 to 12 hours). Only open rosettes were formed at the high (70 to 80°F) temperature and plants bolted one month earlier than those grown at 60 to 70°F. Increasing the length of day did not appear to hasten the initiation of seedstalks but seedstalks elongated more rapidly under a long photoperiod than under a normal day.

Reimers (39) analyzed light requirement in correlating photo-phase and types of lettuce. One winter type, one summer type, and two intermediate types of lettuce were exposed to light periods of (a) 10 hours, (b) 14 hours, (c) sunrise to sunset, (d) during whole day plus continuous illimination at night. Shortening the time of exposure to light greatly retarded shooting in the early (winter) varieties but, as was also observed by Bremer (3), had little effect on the late (summer) varieties. Reimers concludes that length of day exerts the strongest influence upon the development of lettuce.

Tincker (36) studying the influence of length of day on lettuce concluded that although a higher temperature causes a more rapid growth it does not cause the length of day factor to be ineffective in controlling the growth made, either vegetative or reproductive, and in controlling the preliminary stem elongation.

"The general principal, however, is established that varieties of lettuce, as of numerous other plants already tested, show characteristic responses to different periods of light which control the nature of the growth made — vegetative and reproductive."

The relationship of hormones to flowering of plants and the interrelationships among hormones, vernalization, temperature and photoperiod are reviewed by Hamner (24) and by Whyte (39). Hamner credits Cailahjan and Moskov for recognition of green leaves as the organs for the perception of the photoperiodic stimulus which brings about flowering. Hamner also states that so far as he is aware no one has yet substituted the application of pure substances or extracts of plant material for vernalization. Work by Melchers (22), however, indicates Hyoscyamous niger, which is ordinarily assumed to require vernalization, may be induced to flower as the result of a stimulus received (by grafting) from other flowering plants without the necessity of vernalization treatment.

A number of workers have also reported recently on the influence of synthetic growth regulators on flowering (8, 9, 20, 29, 37). Clark and Wittwer (5) investigating the effect of certain growth regulators on seedstalk development in lettuce and celery make reference to a number of papers concerning the hastening or retardation of flowering and to two papers in which synthetic growth regulators failed to affect the date of flowering. The work of Clark and Wittwer will be more fully examined later, in the discussion. Franklin (9) has reported data on the use of certain growth regulators in an attempt to overcome the seed production difficulties in some varieties of lettuce.

With the exception of investigations by Franklin (9) and Thompson and Kosar (35), the work of those investigating the flowering of lettuce appears to have stressed two points of view. First, that of the market lettuce producer in an attempt to determine the optimum requirements for a good market crop, and second, that of the plant physiologist investigating the basic or fundamental processes of plant growth and development. The investigations herein described constitute a further study of the fundamentals of the flowering process and an attempt to ascertain procedures and methods which would facilitate seedstalk development and lettuce seed production.

Since the inception of these investigations, reports have been published of two experiments in which increased seed production of Great Lakes lettuce plants constituted the objective. Hawthorn and Pollard (15) have investigated the possibility of selecting plants of good market type on the basis of their capacity to produce seed and M. T. Lewis (21) has removed the heads to promote seed production from lateral branches arising from adventitious buds. The influence of various concentrations of maleic hydrazide on the bolting of lettuce has recently been reported by Crafts _et al_ (8).

THE PROBLEMS FOR INVESTIGATION

1. To determine whether lettuce plants will bolt, flower and produce seed in a shorter time than usual if sprayed with varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D).
2. To investigate the effect of soaking lettuce seed in 2,4-D on the bolting and flowering of plants grown from the treated seed.
3. To check varying results by other workers on the effect of vernalization on bolting and flowering of certain varieties of lettuce.
4. To ascertain whether crisp head varieties, such as Great Lakes and Imperial 456, may be induced to set seed earlier by the process of vernalization.
5. To study the comparative effectiveness of varying periods of vernalization.
6. To determine the combined effects and interactions of growth regulator treatments, vernalization, temperature and photoperiod on the bolting and flowering of lettuce.

GENERAL PROCEDURES

Growing plants:

Lettuce plants used in these tests were grown from seeds sown in vermiculite placed in standard wooden, plant-growing flats. Seeds were sown in rows 2" apart. In addition to regular watering, at the time the first primary leaves appeared, seedlings were given an application of 1 ounce in 2 gallons of water of a soluble fertilizer composed of equal parts by weight of mono-potassium and di-ammonium phosphate. Seedlings were "pricked out" into flats containing a mixture of two parts mineral to one part muck soil. Plants were placed with 2" spacing each way. From the flats, final transplanting was to 8-inch clay pots, a greenhouse ground bed, or field plots as required.

Vernalizing seed:

Seeds for the vernalization treatments were placed in standard petri dishes on two No. 429.0 c.m. Whatman filter papers previously soaked in water or various solutions of growth-regulators. After various periods of soaking at room temperature under natural light conditions, the dishes were placed in the dark in a cold storage room with temperatures thermostatically maintained at $40^{\circ} \pm 2$ F. While in storage seeds were occasionally subjected to artificial light of low intensity as it was necessary to place in, or remove other material from the same storage.

Application of sprays:

Spray applications were made with quart size sprayers operating at about 50 lbs. air pressure. No spreaders were used. In the greenhouse cardboard guards were used on each row to prevent the drift of spray. In the wider spacing in the fields, no guards were used.

Accumulation and analysis of data:

Wherever possible, results were obtained on a basis of measurement rather than observation. Most of the data represent actual counts or height measurements expressed in centimeters. The analysis of variance method was used on both the randomized blocks and split plot designs for determining differences.

EXPERIMENTAL

I The Influence of Applications of 2,4-Dichlorophenoxyacetic acid (2,4-D) Sprays on the Seedstalk Development of 3 Varieties of Lettuce

Seeds of Great Lakes, Slobolt, and Grand Rapids lettuce were sown March 3, "pricked-out" March 25, and transplanted to a greenhouse ground bed on April 16. The three treatments comprising the test were:

1. 5 ppm 2,4-D applied once.
2. 1 ppm 2,4-D applied twice.
3. Control plots to which no treatment was applied.

The three varieties were randomized within each of three replications, and the three treatments were randomized within each variety. Single plots consisted of five plants. Spacing was 12" between plants and 15" between rows.

Five ppm of 2,4-D (treatment 1) was applied May 19 when the plants were 77 days old. The first application of treatment 2 was also applied on May 19 and the second application one week later, on May 26. Weekly measurements of seedstalk elongation were initiated on May 28.

Results are presented in Table I. Very hot weather resulting in a number of diseased plants forced the abandonment of the test before any appreciable results could be obtained from the Slobolt and Great Lakes plants. Treatments made at a comparatively advanced stage of development in the case of Grand Rapids, apparently had little effect on seedstalk elongation. On the basis of this limited information and the experience of Franklin (9), it was determined that the application

Table I

Seedstalk Development in Three Varieties of Lettuce
as Influenced by 2,4-D Sprays

Treatment	Date of Measurement	Seedstalk Lengths (Centimeters) Great Lakes	Slobolt	Grand Rapids	Treatment Mean
	5/28	0.0	0.0	30.1	10.0
5 ppm 2,4-D	6/4	0.0	0.0	68.4	22.8
1 application	6/11	0.0	8.0	89.1	33.3
	5/28	0.0	0.0	30.1	10.0
1 ppm 2,4-D	6/4	0.0	5.8	65.7	23.8
2 applications	6/11	0.0	9.4	89.3	32.9
	5/28	0.0	0.0	29.0	9.7
Control (No	6/4	0.0	3.0	61.6	21.5
treatment)	6/11	0.0	9.8	85.0	31.6
Variety Mean	6/11	0.0	9.2	87.8	

of fugure sprays should be on the basis of growth development rather than on the basis of time subsequent to seeding.

II The Effects of Various Concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) Sprays Used in Single and Double Applications on the Seedstalk Elongation of Three Varieties of Lettuce

This experiment was designed to supplement or add to the results of investigations reported by Franklin (9) as to the possibility of improving lettuce seed production by the application of growth regulators and to obtain seed for a progeny test of the possible carry over affect of growth regulators applied to the plant.

The seven treatments used in the experiment consisted of spray applications as follows:

1. 10 ppm 2,4-D at large rosette stage.
2. 20 ppm 2,4-D at large rosette stage.
3. 40 ppm 2,4-D at large rosette stage.
4. 2 applications 1 week apart of 5 ppm 2,4-D.
5. 2 applications 1 week apart of 10 ppm 2,4-D.
6. 2 applications 1 week apart of 20 ppm 2,4-D.
7. Control (not sprayed).

Three varieties of lettuce were used, Slobolt, Great Lakes and Imperial 456. Seeds for all treatments were seeded May 24. Plants were set in the field July 7 and 8. The field design was a split plot type with five replications. Within each replication three main variety plots were randomized. Each varietal plot or main plot was comprised of eight single row sub-plots of twelve plants each, providing a one row plot for each treatment. Plots were three feet apart with 12" between plants. Guard rows were set out on both sides of each replication. Three rows of Bibb lettuce were direct seeded around the plot to serve as a trap crop for six spotted leaf hoppers (Macrosteles divisa). As an additional precaution against leaf hoppers and the dissemination of aster yellows disease, the entire area was sprayed on

July 8, 11 and 20 and August 10, with 15% parathion wettable powder (o-diethyl-p-nitrophenyl thionophosphate) at the rate of one half pound per 100 gallons of water. Spray solutions of treatments 1 to 6 were applied July 28 when a majority of the plants were judged to be in the large rosette stage. Treatments 4, 5 and 6 were applied 1 week later on August 4.

Data on Slobolt plants were recorded at later dates than those of the two head lettuces and were not included in the main analysis. In Table II are presented data showing a retardation of seedstalk development as a result of the application of 2,4-D sprays. On August 8, 11 days after the first sprays were applied and 4 days after the second spray application, there were apparently no differences between the treated plants and the control. By August 14, however, highly significant differences had developed and these differences increased until observations were concluded on August 30.

The results with Slobolt variety were somewhat different as shown in Table III. At no time during the period of observation (Aug. 17 to Sept. 20) were there significant differences among treatments. Treatment 3 (40 ppm 2,4-D) apparently was too strong for the Slobolt variety. Although several plants survived in replications 1 and 2, planted two days earlier than the other three replications, all plants succumbed in replications 3, 4 and 5.

Table II

Effect of Various Applications of 2,4-D on the Seedstalk Elongation of Great Lakes and Imperial 456 Lettuce.

Treatment	Seedstalk Length (Centimeters)			
	Aug. 8	Aug. 14	Aug. 23	Aug. 30
1. 10 ppm 2,4-D	12.9	30.7	56.3	68.4
2. 20 ppm 2,4-D	10.2	30.6	56.9	73.4
3. 40 ppm 2,4-D	9.5	33.4	64.0	78.0
4. 2 applications of 5 ppm 2,4-D	9.2	30.1	57.9	72.7
5. 2 applications of 10 ppm 2,4-D	6.3	23.3	45.6	59.7
6. 2 applications of 20 ppm 2,4-D	8.5	28.6	57.4	69.3
7. Control	9.8	41.6	79.1	94.6
Difference Required for Significance 5%	n.s.	9.2	13.9	15.9
Difference Required for Significance 1%	n.s.	11.9	18.6	20.0

Table III

Effect of Various Applications of 2,4-D on the Seedstalk Elongation of Slobolt Lettuce.

Treatment	Seedstalk Length (Centimeters)			
	Aug. 17	Aug. 26	Sept. 12	Sept. 20
1. 10 ppm 2,4-D	14.5	38.6	73.9	78.2
2. 20 ppm 2,4-D	8.6	31.3	60.7	74.5
4. 2 applications of 5 ppm 2,4-D	11.5	29.4	58.4	69.6
5. 2 applications of 10 ppm 2,4-D	12.9	36.5	69.2	79.1
6. 2 applications of 20 ppm 2,4-D	13.5	39.7	78.2	80.8
7. Control	7.4	27.8	60.7	70.9
Difference Required for Significance 5%		n.s.	n.s.	n.s.

III Seedstalk Development of Grand Rapids Lettuce as Affected by Various Spray Applications of Three Growth Regulators.

The effects of a number of growth regulators on the bolting of lettuce have recently been studied (5) (8) (9). To obtain additional information concerning the effects of three of the newer growth regulators, the following experiment was conducted. Seeds of Grand Rapids lettuce were sown January 25, pricked out March 3 and transplanted April 10 directly to the groundbed of a greenhouse maintained at 50°F night temperature. On May 13 when the plants were at a marketable stage but had shown no symptoms of bolting, the sprays listed in Table IV were applied. The eight treatments were replicated four times in a randomized block design. Single plots consisted of 3 plants.

When all plants had bolted, height notes of the seedstalks were recorded. These records of seedstalk height are presented in Table IV.

The figures may indicate a tendency to more rapid elongation of the plants to which growth regulators were applied but an analysis of variance of the data showed no significant differences.

Table IV

Effect of Various Concentrations of Three Growth Regulators on Seedstalk Development of Grand Rapids Lettuce.

Treatment	Average Seedstalk Length (Centimeters)
1. 50 ppm Maleic Hydrazide	67
2. 100 ppm " "	62
3. 500 ppm " "	63
4. 100 ppm n. p. Chlorophenylphthalamic acid	68
5. 500 ppm "	65
6. 100 ppm Benzothiazol-2-oxyacetic acid	68
7. 500 ppm " " " "	66
8. Control (No spray applied)	57

IV Percentage Germination of Three Varieties of Lettuce as Affected by Soaking the Seed in Solutions of 2,4-Dichlorophenoxyacetic acid (2,4-D)

Prior to investigations involving the soaking of seed in various solutions a preliminary study was made to determine whether the percentage germination of certain varieties of lettuce would be affected by soaking the seed in various concentrations of 2,4-D. Seeds of Grand Rapids, Slobolt, and Great Lakes varieties were placed in petri dishes as described under General Procedures. The five treatments applied are listed in Table V. The varieties were randomized within each of four replications; treatments were randomized within each variety; and each petri dish contained 50 seeds. Seeds began soaking at 4:00 p.m., March 23. Germination counts were made 42, 114, and 239 hours respectively after soaking had begun.

In Table V data are presented which indicate a decreased rate and percentage germination with an increased concentration of 2,4-D. On the basis of these results it was concluded that soaking in 10 ppm 2,4-D would be impractical in subsequent experiments. Also shown in Table V is the variation in response among varieties. Solutions of 5 and 10 ppm apparently had a greater retarding effect on Grand Rapids than on Slobolt and the greatest effect on Great Lakes.

Table V

The Percentage Germination of Three Varieties of Lettuce as Affected by Soaking the Seed in Different Concentrations of 2,4-D.

Treatment	Hours of Soaking	Percentage Germination			Treatment Mean
		Great Lakes	Slobolt	Grand Rapids	
10 ppm 2,4-D	42	0.0	0.0	0.0	0.0
	114	0.0	0.0	0.0	0.0
	239	3.5	30.0	17.5	17.0
5 ppm 2,4-D	42	0.0	0.0	0.0	0.0
	114	0.0	0.0	0.0	0.0
	239	4.0	63.5	55.5	41.0
1 ppm 2,4-D	42	1.0	0.0	13.0	4.7
	114	15.5	53.0	71.0	46.5
	239	33.0	83.0	95.5	70.5
0.1 ppm 2,4-D	42	59.5	75.5	93.5	76.2
	114	74.0	79.5	98.5	84.0
	239	79.0	82.0	99.0	86.7
Distilled H_2O Check	42	72.5	81.5	91.5	81.8
	114	83.5	85.5	98.0	89.0
	239	83.5	90.5	98.5	90.8
Variety Mean	239	40.6	69.8	73.2	

V Some Effects of Vernalization and Various Applications of 2,4-Dichlorophenoxyacetic acid (2,4-D) on the Bolting, Flowering and Seed Ripening Dates of Three Varieties of Lettuce.

A series of treatments was designed (1) to check varying results by other workers on the effect of vernalization on bolting, flowering and seed ripening dates, (2) to determine whether hard headed or long standing varieties, such as Great Lakes and Slobolt, may be induced to set seed earlier by vernalization of the seed, (3) to ascertain whether combined treatments of a growth regulator and vernalization would have an accumulative effect upon hastening flowering, (4) to check the comparative effectiveness of vernalization for periods of two weeks and four weeks, and (5) to determine the effect of soaking the seed in a growth regulator on bolting and flowering.

The eight treatments used in the experiment were as follows:

1. Soaked in H_2O 24 hours and vernalized for 28 days.
2. Soaked in H_2O 24 hours and vernalized for 16 days.
3. Soaked in 2,4-D (1 ppm) 24 hours and vernalized for 28 days.
4. Soaked in 2,4-D (5 ppm) 24 hours and vernalized for 28 days.
5. Soaked in 2,4-D (1 ppm) 24 hours and not vernalized.
6. Soaked in H_2O 24 hours and vernalized for 28 days, plus 2,4-D (10 ppm) in the field.
7. Soaked in H_2O 24 hours. Control 1.
8. Sown dry. Control 2.

Three varieties of lettuce were used — Slobolt, Great Lakes and Imperial 847. Approximately 150 seeds were started for each treatment, using two petri dishes with 75 seeds per dish. Seeds for treatments 1, 3, 4 and 6 began soaking at 1:30 p.m., April 23, and were removed at 1:45 p.m., April 24, to a 40°F storage room. Seeds for treatment 2 began soaking May 5 and were placed in the cold room May 6. Treatments 5 and 7 were initiated May 20.

Seeding and field setting dates were three days earlier than in Experiment II.

Field plot design and plant growing procedures were the same as in the second experiment.

Weather conditions prevented the spraying of treatment 6 at the proper stage. As a result, treatments 1 and 6 thus were identical. Rather than discard one of them, observations and analyses were made on both treatments to serve as a check on technique and design. Analyses showed no significant differences between treatments 1 and 6.

Recordings of seedstalk elongation were made from August 8 to August 30 on the Great Lakes and Imperial 847 plants and from August 23 to September 20 on the Slobolt plants. Measurements were made from ground level to the growing tip in the early stages of the experiment. As flower buds began to form, the measurements were made from ground level to the highest bud or flower. Data were also recorded as to the time of appearance of flower buds, flowers, and the feathering-out stage.

As in Experiment II, data on the Slobolt plants were not included in the main analyses. Also omitted from the main analyses were treatments 3 and 4. Too few plants survived the 5 ppm 2,4-D plus 28 days vernalization of treatment 4 to justify field setting. The combined or additive retarding effects of these two treatments on early vegetative growth apparently was too strong for the seedlings to survive. Slobolt and Imperial 847 also failed to grow after the soaking in 1 ppm 2,4-D and 28 days vernalization of treatment 3. Comparisons of the seedstalk elongation of the remaining six treatments at various dates are presented in Table VI.

Table VI

The Progressive Elongation of Seedstalks of Great Lakes and Imperial 847 Lettuce as Influenced by Vernalization and Treatment with 2,4-D.

Treatment	Seedstalk Lengths (Centimeters)					
	Aug. 8	Aug. 14	Aug. 17	Aug. 23	Aug. 26	Aug. 30
1. H_2O 24 hrs. ≁ Vern. 28 days	45.6	76.5	86.4	94.5	97.3	100.4
2. H_2O 24 hrs. ≁ Vern. 16 days	44.4	74.3	84.6	94.0	98.2	100.6
5. 2,4-D 1 ppm 24 hrs.	22.3	56.1	69.2	85.9	95.0	100.9
6. H_2O 24 hrs. ≁ Vern. 28 days	51.1	81.0	89.9	99.2	103.5	105.7
7. H_2O 24 hrs. Control 1	22.8	54.6	66.9	83.8	92.8	98.8
8. Control 2	14.2	48.7	62.0	78.5	89.8	97.0
Difference Required for Significance 5%	6.3	6.1	6.8	14.0	n.s.	n.s.
" " " " 1%	8.5	8.2	9.1	18.9	n.s.	n.s.

On August 8, thirty-five days after field setting, plants from the vernalized seed (treatments 1, 2 and 6) had developed seedstalks approximately three times the height of the control plants. Plants from seed soaked overnight (treatment 7) had elongated only half as rapidly as the others grown from vernalized seed but still showed a highly significant increase over the control plants grown from dry seed in the customary manner. There was no significant difference between the 1 ppm 2,4-D soaking of treatment 5 and the soaking in water of treatment 7. The vernalized plants maintained their significant margin in seedstalk length between the period August 23 to August 26. Those soaked overnight maintained their height advantage until August 17.

The data in Table VI indicate no apparent advantage for 28 days vernalization over 16 days vernalization. There was actually more variation between the two sets of supposedly identical plants of treatments 1 and 6 than between treatments 1 and 2. Figures 1 and 2 show that the differences between the vernalized plants of treatments 1 and 2 and the non-vernalized plants of treatments 6 and 7 were plainly visible in the field.

Data in Table VI also appear to indicate a leveling-off among treatments at later dates. This was not the case. As the plants approached their maximum height the length of seedstalk ceased to be a justifiable method of comparison. In later stages of reproduction the plants developed more laterally as their vertical development slowed down. The stage of floral development thus became a more accurate basis for comparison.

As the flowering and "feathering out" data in Table VII show, treatments 1, 2 and 6 maintained a decided margin in stage of development

Figure 1. Field grown plants of Experiment V. Left, seed vernalized in water 28 days (treatment 1); right, seed vernalized in water 16 days (treatment 2).

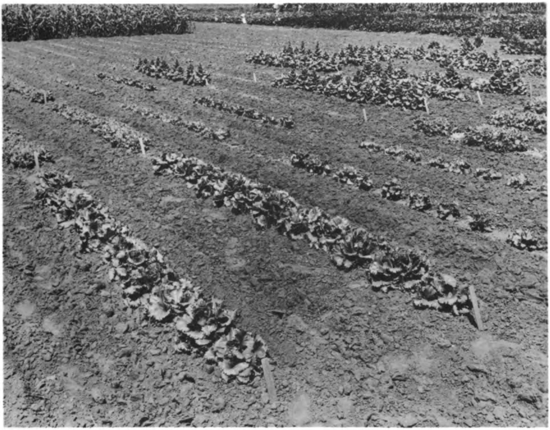

Figure 2. Field grown plants of Experiment V. Left, control plants (treatment 8); right, seed soaked overnight in water (treatment 7).

Table VII

The Influence of Vernalization and 2,4-D Treatments on the Flowering and Feathering-out Dates of Great Lakes and Imperial 847 Lettuce.

Treatment	Average Number of Plants			
	Flowering			Feathering-out
	Aug. 23	Aug. 26	Aug. 30.	Sept. 16.
1. H_2O 24 hrs. ≠ Vern. 28 days	8.7	9.3	9.8	7.1
2. H_2O 24 hrs. ≠ Vern. 16 days	7.2	9.6	10.0	6.0
5. 2,4-D 1 ppm 24 hrs.	3.1	6.1	8.8	2.6
6. H_2O 24 hrs. ≠ Vern. 28 days	8.9	9.0	9.5	6.8
7. H_2O 24 hrs.	3.2	5.9	8.5	2.1
8. Control	.5	3.7	7.2	.6
Difference Required for Significance 5%	1.9	2.1	n.s.	2.4
" " " " 1%	2.6	2.8		4.6

when recording of data was terminated on September 16.

Variations in varietal response are shown in Table VIII. Vernalizing the seed apparently had a greater effect on the slow bolting Great Lakes than on Imperial 847, while soaking without the addition of cold treatment was more effective on Imperial 847. Vernalization of Great Lakes brought the reproductive phase of the variety to approximately the same level as the earlier bolting Imperial 847. A considerably greater difference existed between the non-vernalized plants of the two varieties than between the vernalized plants.

Analysis of observations made on the performance of the Slobolt plants coincides very closely with the results obtained with Great Lakes and Imperial 847. The results are presented in Table IX.

Table VIII

Varietal Responses in Seedstalk Elongation as Induced by Vernalization and 2,4-D Treatment of Lettuce Seed.

Treatment	Seedstalk Lengths (Centimeters)					
	August 8		August 14		August 17	
	Gr.Lakes	Imper.847	Gr.Lakes	Imper.847	Gr.Lakes	Imper.847
1. H_2O 24 hrs. + Vern. 28 days	20.6	24.9	35.8	40.6	41.0	45.4
2. H_2O 24 hrs. + Vern. 16 days	21.3	23.1	36.9	37.4	41.4	43.2
5. 2,4-D 1 ppm 24 hrs.	6.2	16.1	22.0	34.1	29.2	40.0
6. H_2O 24 hrs. + Vern. 28 days	24.2	27.0	39.0	42.0	43.5	46.4
7. H_2O 24 hrs.	5.4	17.4	20.9	33.8	26.9	39.9
8. Control	4.0	10.2	20.5	28.1	27.3	34.7

Table IX

Seedstalk Elongation of Slobolt Lettuce as Affected by Various Vernalization and 2,4-D Treatments.

Treatment	Seedstalk Lengths (Centimeters)			
	Aug. 23	Aug. 30	Sept. 12	Sept. 20
1. H_2O 24 hrs. + Vern. 28 days	29.9	52.5	76.8	86.2
2. H_2O 24 hrs. + Vern. 16 days	31.7	50.7	75.8	85.9
5. 2,4-D 1 ppm 24 hrs.	20.0	41.4	68.4	76.4
6. H_2O 24 hrs. + Vern. 28 days	29.7	52.3	73.4	83.3
7. H_2O 24 hrs.	19.2	35.6	64.2	76.5
8. Control	8.8	28.5	53.5	63.7
Difference Required for Significance 5%	7.7	8.7	9.8	9.3
" " " " 1%	10.5	11.9	13.5	12.7

VI The Influence of Various Seed and Foliage Treatment on the Seedstalk Elongation of Great Lakes Lettuce.

As a further test of treatments similar to those in Experiment V, seven seed treatments were applied to Great Lakes lettuce as follows:

1. Seed soaked in H_2O until radicles just emerged then vernalized for 18 days.
2. Seed soaked in 1 ppm 2,4-D to same stage as treatment 1 then vernalized 17 days.
3. Seed soaked in 1 ppm 2,4-D only.
4. Seed soaked in H_2O, vernalized 18 days plus plant foliage spray of 5 ppm 2,4-D.
5. Seed soaked in 1 ppm 2,4-D, vernalized 17 days plus plant foliage spray of 5 ppm 2,4-D.
6. Seed soaked in 1 ppm 2,4-D plus plant foliage spray of 5 ppm 2,4-D.
7. Control plants, seed sown dry and plants grown in the customary manner.

On the basis of results obtained by Thompson and Knott (34) plants were grown in the greenhouse at night temperatures of 60° to 70°F. Five eight inch pots were sunk in the greenhouse ground bed for each treatment. Seeds for all treatments were sown October 15 and the plants requiring spraying were sprayed January 11th when they were in the early rosette stage. Treatments were randomized within each of four replications. Measurable seedstalks were in evidence on plants of treatments 1, 2 and 4 as early as February 12 but it was almost one month later before sufficient stalks were visible to justify recording measurements in treatments 3, 6 and 7.

In Table X data are recorded which indicate significant differences in seedstalk development. As was the case in Experiment V, a number of the seedlings did not survive the 1 ppm 2,4-D plus vernalization of treatments 2 and 5. To obtain a sufficient number of seedlings for one complete treatment, treatments 2 and 5 were combined and continued as treatment 2.

Only those seedstalks from the treatments involving vernalization elongated at an earlier date than those of the control. No significant differences were apparent among the vernalized treatments on March 12 but by March 31 treatment 4, sprayed with 5 ppm 2,4-D, had developed seedstalks significantly higher than treatments 1 and 2 and by April 15 the difference was highly significant. Soaking the seed in 2,4-D appeared to counteract the effect of vernalization. A comparison of the effects of the 5 ppm 2,4-D spray on treatments 4 and 6 is interesting. Treatment 4 soaked in water and vernalized, developed more rapidly after seeding than treatment 6 which had been soaked in 1 ppm 2,4-D and not vernalized. On January 19 when the spray was applied, plants in treatment 4 were further developed than those of treatment 6. (It might be noted that as early as January 9, before any data were taken, plants in treatments 1 and 4 were noticeably larger than those in any other treatment.) The data in Table X suggest that 5 ppm 2,4-D applied at the later stage of growth of plants (treatment 4) had an accelerating effect on seedstalk elongation or initiation while the same treatment applied at an earlier stage of growth (treatment 6) had a retarding effect. The accelerating effect of treatment 4 and the retarding effect of treatment 6 are discernable when each is compared with its non sprayed counterpart — treatments 1 and 3, respectively (Table X).

Figures 3 to 7 demonstrate pictorially some of the differences

Table X

Seedstalk Elongation of Great Lakes Lettuce as Affected by Vernalization and Treatments with 2,4-D.

Treatment	Seedstalk Lengths (Centimeters)		
	Mar. 12	Mar. 31	Apr. 15
1. H_2O + Vern.	21.7	56.9	83.8
2. 1 ppm 2,4-D + Vern.	16.7	37.1	70.0
3. 1 ppm 2,4-D	6.2	14.4	32.7
4. H_2O + Vern. + 5 ppm 2,4-D spray	24.7	71.7	117.2
6. 1 ppm 2,4-D + 5 ppm 2,4-D spray	3.4	8.4	24.1
7. Control	0.8	3.4	17.1
Difference Required for Significance 5%	7.6	13.3	21.6
" " " " 1%	10.4	17.9	29.5

presented in the data of Table X. In Figure 3 may be seen the characteristic loose, open type of growth of the vernalized plants compared with the close, well formed, more typically Great Lakes, head of the check plant. At the time the photographs were taken (March 8) it was thought that the slower development of plants in treatment 2 compared to treatment 1 was visible. Although the data in Table X bear out this observation, subsequent analysis indicates no significant difference in seedstalk elongation between the two treatments. There appeared to be no modification in form of leaf or plant characterizing the 2,4-D soaking of treatment 2 as compared with treatment 1. When 2,4-D was applied in the form of a 5 ppm spray, however, modifications of both the leaf and plant enabled observers to distinguish sprayed plants from those not sprayed. A reduction in vegetative growth accompanied by curling and twisting of leaves and petioles is evident in the middle plant of Figure 4 and the plant on the left in Figure 6. The malformation was most noticeable on plants of treatment 6, which received a "double dose" of 2,4-D (soaking of seed plus the application of spray to plants). An example of this extreme effect on form is seen in the middle plant of Figures 5 and 6. Figure 7 taken almost five weeks later, on April 13, shows the advanced stage of growth reached by vernalized plants while seedstalks of many of the check plants had not yet escaped the tightly formed heads. The forms of the two inflorescences in Figure 7 were thought to be somewhat characteristic of treatments 1 and 4 but recent work by Lewis (21) and Hawthorn and Pollard (15) indicates there is considerable variation in type and quantity of inflorescence within the variety.

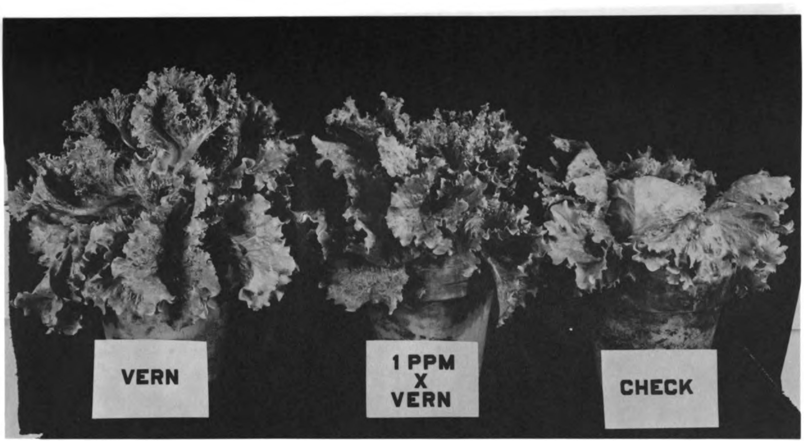

Figure 3. Greenhouse grown Great Lakes lettuce plants of Experiment VI. Left, seed vernalized in water (treatment 1); center, seed vernalized in 2,4-D (treatment 2); right, check plants - no seed treatment, no spray (treatment 7).

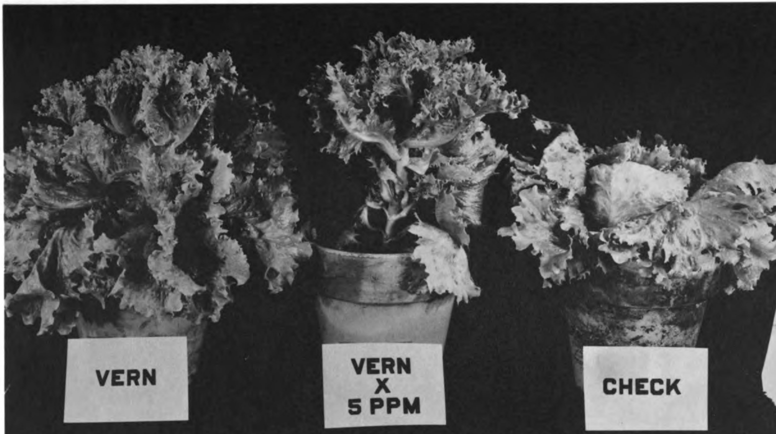

Figure 4. Greenhouse grown Great Lakes lettuce plants of Experiment VI. Left, seed vernalized in water (treatment 1); center, seed vernalized in water plus foliage spray of 5 ppm 2,4-D (treatment 4); right, check plants - no seed treatment, no spray (treatment 7).

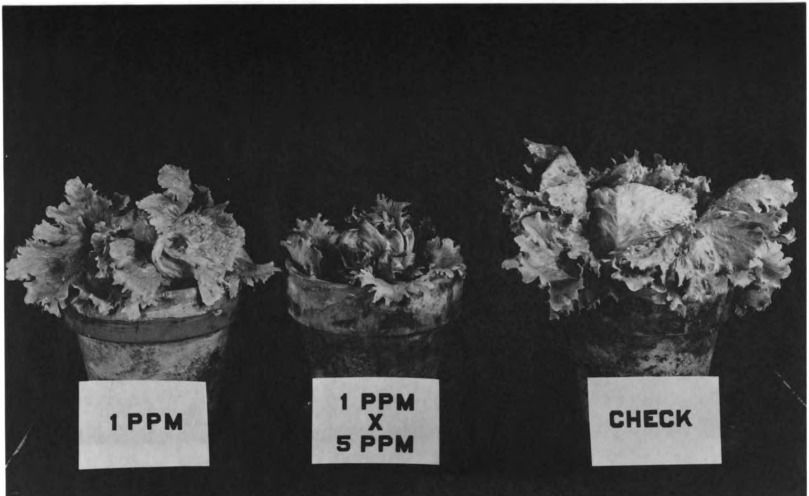

Figure 5. Greenhouse grown Great Lakes lettuce plants of Experiment VI. Left, seed soaked overnight in 1 ppm 2,4-D (treatment 3); center, seed soaked overnight in 1 ppm 2,4-D plus 5 ppm 2,4-D applied to plant (treatment 6); right, check plants - no seed treatment, no spray (treatment 7).

Figure 6. Greenhouse grown Great Lakes lettuce plants of Experiment VI. Left, seed vernalized in water plus foliage spray of 5 ppm 2,4-D (treatment 4); center, seed soaked overnight in 1 ppm 2,4-D plus foliage spray of 5 ppm 2,4-D (treatment 6) right, check plant - no seed treatment, no spray (treatment 7).

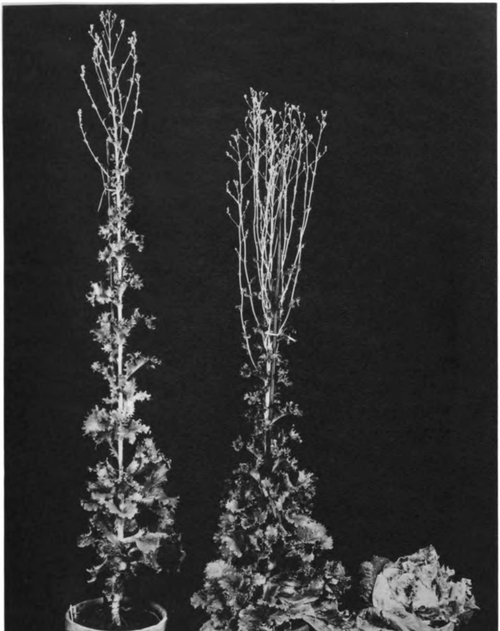

Figure 7. Greenhouse grown Great Lakes lettuce plants of Experiment VI. Left, seed vernalized in water, plant sprayed with 5 ppm 2,4-D (treatment 4); center, plant subjected to vernalization only (treatment 1); right, check plants - no seed treatment, no spray (treatment 7).

VII A Comparison of the Influence of Certain Vernalization Methods and the Application of Maleic Hydrazide on the Seedstalk Elongation of Two Varieties of Lettuce.

During the course of the preceding experiments a problem of significant practical importance arose in the sowing of vernalized seed. In each of the experiments involving vernalization a certain amount of seedling growth occurred during the cold treatment period. This growth made sowing of the vernalized seed very difficult. To provide a somewhat more practical method of vernalizing seed, therefore, and to verify the work of Reimers (39) and Warne (38) relating to stage of development at time of treatment, an experiment was designed to compare several possible methods of vernalization with the one used in the earlier experiments. In addition to the cold treatments and as a result of the current interest in the effect of maleic hydrazide on plant development (8, 26, 28, 41), concentrations of maleic hydrazide were applied in the form of sprays to a number of the lettuce plants. Nine treatments were applied to each of two varieties of lettuce. Plots were laid out in a randomized block design with 5 replications. Spacing and single plot size were the same as for Experiments II and V the previous year. Treatments 1 to 9 were applied to the variety Great Lakes. Treatments 10 to 18 designate the same treatments applied to Imperial 456. Treatments were as follows:

1. Seed placed in petri dishes as in General Procedures and removed to 40°F storage immediately.

2. Seeds remained in petri dishes at room temperature until seeds had swollen but radicle had not yet emerged -- a period of 18 hours (3 p.m. to 9 a.m.) -- and were then removed to 40°F storage.

3. Seeds were removed to storage just after radicles had emerged — a period of 22 hours (3 p.m. to 1 p.m.).

4. Seed sown directly into flats of 2 parts mineral to 1 part muck soil. The flats remained in a greenhouse with a night temperature of 60°F for approximately 48 hours and were then removed to the 40°F room. No seedlings had emerged from the soil at the time.

5. As in 4, but the flats remained in the greenhouse until seedlings were just breaking through the surface of the soil — a period of approximately 68 hours — before being removed to the 40°F room.

6. Flats removed to 40°F room when seedlings were 2 to 3 cm. high — $5\frac{1}{2}$ days after seeding.

7. Seed sown dry in the usual manner and plants sprayed with 50 ppm maleic hydrazide when they reached the early cluster stage.

8. Plants sprayed with 25 ppm maleic hydrazide when they reached the early cluster stage.

9. Control plants raised from seed sown in the customary manner.

Vernalization treatments were begun April 25. Seeds vernalized in the soil were watered once during cold treatment, on May 17. All vernalized seeds were removed from the 40°F room May 28. Seedlings in the petri dishes were transferred to flats containing soil and the non-vernalized seeds of treatments 7, 8, 9, 16, 17 and 18 also were seeded in soil. Plants were set in the field July 17 and the maleic hydrazide sprays were applied August 17.

A number of plants in the vernalized treatments had bolted by August 16. Records of seedstalk length were taken August 24 when most of the vernalized stocks had bolted. A further check on September 4 showed that plants sprayed with maleic hydrazide and the control plants still were showing no signs of bolting. In Figure 8 may be seen the typical firm heads of the check plants approaching the marketable stage while the vernalized plants had already produced considerable seedstalk growth with very little tendency to form heads. A comparison of the seedstalk lengths recorded August 26 is presented in Table XI.

As no seedstalks had developed on the control plants or on those sprayed with maleic hydrazide, their nil seedstalk lengths were not included in the analysis of variance. It would appear from the data in Table XI that vernalization of seed sown in soil is apparently not only as satisfactory as the laboratory technique but if the cold treatment is applied in the early stages of germination the results are even more desirable. Treatments 6 and 15 indicate a decrease of the vernalizing effect if seedlings developed past emergence rather than germinating seeds, or emerging seedlings, are exposed to low temperature treatment.

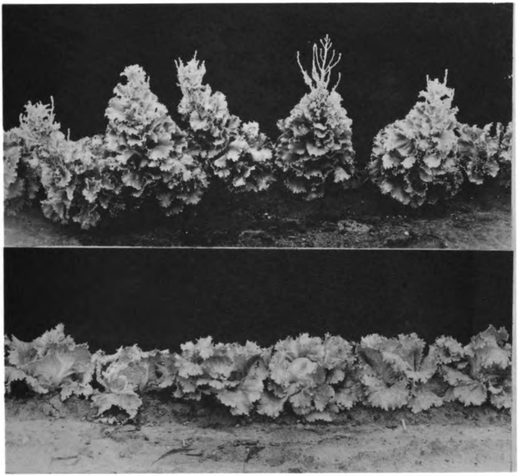

Figure 8. Field grown lettuce plants of Experiment VII. Above, plants from vernalized seed showing typical open leafy growth; below, control plants showing head formation more typical of the Great Lakes variety.

Table XI

The Effect of Various Vernalization Methods and Spray Application of Maleic Hydrazide on the Bolting of Imperial 456 and Great Lakes Lettuce.

Treatment	Average Seedstalk Length (Centimeters)
Great Lakes	
1. Petri dish, 40°F	34.4
2. Petri dish, swollen 40°F	34.8
3. Petri dish, radicles, 40°F	34.5
4. Soil, none emerged, 40°F	44.7
5. Soil, just emerged, 40°F	45.3
6. Soil, seedlings, 40°F	26.6
7. 50 ppm Maleic Hydrazide	0.0
8. 25 ppm Maleic Hydrazide	0.0
9. Control	0.0
Imperial 456	
10. Petri dish, 40°F	49.5
11. Petri dish, swollen 40°F	45.4
12. Petri dish, radicles, 40°F	55.7
13. Soil, none emerged, 40°F	58.4
14. Soil, just emerged, 40°F	57.9
15. Soil, seedlings, 40°F	27.2
16. 50 ppm Maleic Hydrazide	0.0
17. 25 ppm Maleic Hydrazide	0.0
18. Control	0.0
Difference Required for Significance 5%	7.8
" " " " 1%	10.4

VIII The Elongation of Seedstalks of Grand Rapid Lettuce as Affected by Soaking the Seed in 2,4-Dichlorophenoxy-acetic acid (2,4-D) Prior to Harvesting from the Parent Plant

That vernalization of the embryos of certain cereals can take place while the seed is still on the parent plant has been observed in nature by Kostjucenko and Zarubailo (18) and demonstrated experimentally by Gregory and Purvis (14). As demonstrated earlier in the present series of experiments treatment of germinating seed with 2,4-D has been shown to affect seedstalk elongation of lettuce. Accordingly an investigation of the possible effects of treating lettuce seed while still on the parent plant, was initiated. The seed bearing inflorescences of greenhouse-grown Grand Rapids lettuce plants were dipped momentarily into beakers of 2,4-D at concentrations of 1 ppm and 5 ppm. Plants dipped into beakers of distilled water served as controls for comparison.

Developing seeds were treated when approximately 50 per cent of the inflorescences had feathered out. Inflorescences of three plants were used for each treatment. Seeds were harvested at maturity and a composite sample from the three plants of each treatment was seeded January 25. Plants were pricked out March 3 and transplanted April 10 to the ground bed of a greenhouse held at 50°F night temperature. The three treatments were replicated five times in a randomized block design and single plots for each treatment consisted of four plants.

A comparison of the stalk lengths of plants from each treatment may be made from data presented in Table XII. Analysis of variance indicates a significant increase in seedstalk length of treatment with

Table XII

Seedstalk Elongation of Grand Rapids Lettuce Plants as Affected by Soaking the Seed in 2,4-D Prior to Removal from the Parent Plant.

Treatment	Seedstalk Lengths (Centimeters)					
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep.5	Mean
1 ppm 2,4-D	52.0	56.0	53.0	43.5	57.3	52.4
5 ppm 2,4-D	62.5	66.5	68.0	62.0	56.7	63.1
Control (Distilled water)	59.0	59.0	71.0	51.7	59.0	59.9
Difference Required for Significance 5%						6.7
Difference Required for Significance 1%						9.6

5 ppm 2,4-D over treatment with 1 ppm 2,4-D, but no significant difference between 5 ppm and the distilled water check. A significant difference is also indicated in the decreased seedstalk length of the 1 ppm 2,4-D treatment compared to the control.

IX The Flowering Response of Great Lakes Lettuce as Influenced by Vernalization, Growing Temperatures, Photoperiod and Chemical Treatment.

As indicated in the review of literature a number of studies have been made to determine the effects of temperature, photoperiod and vernalization on flowering of lettuce. There have been few investigations, however, concerned with these three factors singly, and interacting, and no work has been reviewed in which the interacting effect of growth regulators with temperature and photoperiodic responses has been included. A greenhouse experiment was designed, therefore, to determine some of the effects of temperature, photoperiod, vernalization, and growth regulators on the bolting and flowering of lettuce. Eight treatments were formulated as follows:

1. Plants from seed vernalized 26 days were grown at 50°F night temperature and 9 hour photoperiod.
2. Plants from seed vernalized 26 days were grown at 60°F night temperature and 9 hour photoperiod.
3. Plants from seed vernalized 26 days were grown at 50°F night temperature and 15 hour photoperiod.
4. Plants from seed vernalized 26 days were grown at 60°F night temperature and 15 hour photoperiod.
5. Plants grown from non-vernalized seed under conditions of treatment 1.
6. Plants grown from non-vernalized seed under conditions of treatment 2.
7. Plants grown from non-vernalized seed under conditions of treatment 3.
8. Plants grown from non-vernalized seed under conditions of treatment 4.

Plants grown under short day conditions were exposed to 9 hours of daylight (8 a.m. to 5 p.m.). Those grown under long days were subjected to fifteen hours of light (normal day length supplemented by artificial lights). Growing temperatures of 50° and 60°F were maintained only at night.

Seeds to be vernalized were soaked in water for 24 hours beginning December 29 and were removed to cold storage December 30. Seeds of all treatments were sown in vermiculite January 25, "pricked out" March 1, and potted April 8. The Great Lakes variety was used. To facilitate the transfer between the light and dark rooms, required in exposing plants to varying day lengths, pots were placed on portable tables.

Four replications of the eight main treatments were used. The main plots consisted of six plants each and were split into two subplots of three plants each. One complete set of subplots was sprayed with 5 ppm 2,4-D when a majority of the plants were at the early rosette stage (May 9). The remaining subplots served as the unsprayed checks. Lengths of seedstalks were recorded on June 4, June 23, and July 14. The test was then discontinued because high outdoor temperatures prevented proper control of indoor temperatures.

The effects of four interacting factors — vernalization, temperature, photoperiod and 2,4-D — on plant development are shown in Table XIII. Both vernalization and the application of 2,4-D spray increased the seedstalk length. The effect of each such treatment was more clearly evident in the absence of the other, however. Greater differences were apparent between vernalized and non-vernalized plants given no spray treatment than between vernalized and non-vernalized sprayed with 5 ppm 2,4-D. Greater differences were also apparent between sprayed and non-sprayed plants that had not been vernalized than

Table XIII

The Interactions of Daylength, Growing Temperatures, Vernalization and Application of 2,4-D as They Affect Seedstalk Elongation of Great Lakes Lettuce

Treatment	Seedstalk Lengths (Centimeters) June 23			July 14		
	5 ppm 2,4-D	Control	Mean	5 ppm 2,4-D	Control	Mean
1. Vern. Short Day 50°F	20.2	0.0	10.1	58.3	19.2	38.7
2. Vern. Short Day 60°F	9.1	12.4	11.0	38.8	47.4	43.1
3. Vern. Long Day 50°F	6.9	28.6	17.7	61.6	59.5	60.6
4. Vern. Long Day 60°F	41.0	36.1	38.6	68.7	76.9	73.8
5. Non-vern. Short Day 50°F	0.0	2.1	1.0	32.5	10.6	21.6
6. Non-vern. Short Day 60°F	19.8	0.0	9.9	62.2	13.7	38.0
7. Non-vern. Long Day 50°F	6.9	0.0	3.4	42.4	26.3	34.3
8. Non-vern. Long Day 60°F	20.2	0.0	10.1	59.0	17.8	38.4
Mean	15.5	9.9		52.9	33.9	
Difference Required for Significance 5%			11.8			16.3
" " " " 1%			15.9			22.0

between sprayed and non-sprayed that had been vernalized.

Although the differences were not all significant there is a strong suggestion that the 60°F night temperature favors earlier seedstalk elongation than does the 50°F night temperature. Exposure to 15 hours of light resulted in more rapid development of seedstalks than exposure to 9 hours of light. The advantages of a long day for seed production appear to be more pronounced on plants grown from vernalized seed than from plants grown from non-vernalized seed. The additive effect of high temperature, long days and vernalization are indicated in a comparison of treatment 4 with any of the other treatments. It would appear that the application of any one treatment promoting seedstalk development is not as effective as applying various combinations. The variable results obtained from spraying the vernalized plants with 2,4-D do not justify interpretation of the value of 2,4-D in effecting additive results in combination with vernalization, long days and high temperatures.

DISCUSSION

The effect of certain growth regulators on flowering of lettuce has received the recent attention of Clark and Wittwer (5), Crafts et al (8) and Franklin (9). In 1946 and 1947 Franklin (9) studied the effects of spray applications of a number of growth regulators. His objective was to induce sufficient epinasty of the lettuce leaves to prevent the usual compression of leaves or to cause headed plants to be less compressed thus permitting the release of the seedstalk. A number of growth regulators of varied concentrations were applied at three stages of development. In the 1946 experiment p-chlorophenoxyacetic acid at 100 ppm produced results closest to those desired. Where it was used, heading was almost entirely prevented and seedstalks, slightly dwarfed but otherwise normal, appeared and bore viable seed. In the 1947 experiment the ammonium salt of 2,4-D at 20 ppm produced the best results of both years. The concentrations of this substance in the previous year's experiment had been high enough (50 ppm and higher) to cause the death of many plants. Results of both the 1946 and 1947 experiments indicated that the most desirable stage for application of the sprays was that of a large rosette with enfolding of the leaves just starting. Although Franklin (9) reports promising results with 20 ppm of the ammonium salt of 2,4-D, results with 2,4-D in the present investigations were not conclusive.

In Experiment II where 2,4-D sprays were applied to Great Lakes and Imperial 456 at the rosette stage recommended by Franklin (9), a significant retardation of seedstalk development was obtained. With the leaf lettuce Slobolt, there were no significant differences in seedstalk development. In Experiment VI (Table X) a 5 ppm spray application of 2,4-D resulted in significantly earlier seedstalk elongation of treatment 4 as compared to treatment 1, but there was no significant difference between the sprayed plants of treatment 6 and the non-sprayed plants of treatment 3. Plants grown from non-vernalized seed in Experiment IX to which 5 ppm 2,4-D was applied produced seedstalks earlier than the plants which did not receive such treatment as indicated in Table XIII. Results of the preliminary greenhouse work described in Experiment I show no significant differences in seedstalk development between sprayed and non-sprayed plants. Clark and Wittwer (5) indicate that 2,4-D hastened seedstalk elongation if applied to 8 to 12 week old plants. They also report a greater effect for repeated than for single applications.

The lack of agreement in results as just outlined appears to justify consideration of why results do vary and what factors may be responsible for such variation. First, the concentrations of the growth regulators used will be considered. In 1946, Franklin (9) applied the ammonium salt of 2,4-D at concentrations of 50, 75, 100 and 150 ppm. Even at the lowest concentrations many of the treated plants died. In 1947 using 10, 20, 30 and 40 ppm of the same material, Franklin (9) obtained his best results with 20 ppm. Clark and Wittwer (5) in their first experiment, using 5 ppm 2,4-D, obtained seedstalks significantly longer than those of the control. In their second experiment, using 10 ppm 2,4-D, they obtained significant differences through to date of blossom bud appearance on Grand Rapids plants but with the Imperial 456 plants a significant difference in seedstalk length was recorded on one date only. In the present studies no significant differences were obtained using 5 ppm 2,4-D on the Grand Rapids variety (Table I). Significan retardation at all concentrations is indicated in Table II, Experiment II, and no significant differences are indicated in Table III. In Experiment VI, a 2,4-D spray of 5 ppm favored seedstalk elongation in one case (treatment 4 over treatment 1) and had no significant effect in another (treatment 6 not significantly different from treatment 3) while in Experiment IX (Table XIII) an application of 5 ppm 2,4-D increased the earlier seedstalk development of non-vernalized plants and had little or no effect on the vernalized plants. With such evidence as just outlined, we may be justified in assuming that the concentration of 2,4-D within a non toxic range is not the major factor determining its effectiveness in influencing seedstalk elongation of lettuce. Coincident with the experiments herein described Crafts and his co-workers (8) were investigating the effects of maleic hydrazide on a number of crops and weeds. In 1950 they reported (8) that severe bolting of lettuce plants resulted when 1000 ppm maleic hydrazide was applied and that 2000 ppm resulted in stunting of the plants.

Second, let us consider the effect of stage of growth or, as this writer would suggest, the "morphological age"* of the plant at time of 2,4-D application. In Clark and Wittwer's study (5), applications were made on the basis of days from seeding only, thus their results would have little significance in this phase of the discussion. Franklin (9) reports that in both his 1946 and 1947 experiments, most satisfactory

* In reference to the actual visible stage of growth of the plant in contrast to "physiological age", referring to physiological development within the plant not necessarily visible to the observer.

results were obtained by applying sprays when plants were in the large rosette stage, with enfolding of the leaves just starting. In the experiments of the present series involving spray application of 2,4-D, Experiment II, VI and IX, the plants were treated when a majority were at the rosette stage preferred by Franklin (9) and the remainder in a somewhat earlier stage. As was the case with the first factor considered, response varied among plants apparently treated at approximately the same visible stage of growth.

A third factor involved in the response to spray application of growth substance is the number of days from seeding. To present a comparison of the effects of the number of days from seeding to spraying in each group of experiments, a summary is presented in Table XIV.

A comparison of Experiment I (Andrew) with Experiment 2 (Clark and Wittwer) shows an increase in seedstalk development with 10 ppm 2,4-D applied at 77 days and no effect with 5 ppm at 77 days. The same variety, Grand Rapids, was used in both experiments. The variety Slobolt showed no response to 5 ppm 2,4-D applied at 77 days or to 10, 20 or 40 ppm applied at 65 days. Plants of Imperial 456 treated with the 10, 20, and 40 ppm applied at 65 days were significantly retarded in their seedstalk elongation while treatment with 10 ppm at 63 and 77 days may have given a slight increase. As with Imperial 456 and in contrast to results with Slobolt, the Great Lakes variety was retarded by sprays of 10, 20 and 40 ppm 2,4-D at 65 days. The work of Crafts _et al_ (8) indicated that when maleic hydrazide was applied, more mature plants were less severely affected. Great Lakes has given increases, however, when sprayed with:

5 ppm 2,4-D at 94 days,
50 ppm NH_4 Salt 2,4-D at 47 days
20 ppm NH_4 Salt 2,4-D at 77 days

Table XIV

A Summary of Results Obtained in Three Series of Investigations in which 2,4-D Sprays were Applied to Lettuce Plants of Varying Ages.

Source	Variety	Age of Plants	Effect on Seedstalk Elongation
Experiment I (Andrew)	Grand Rapids & Slobolt	77 days	No effect.
Experiment II (Andrew)	Slobolt	65 days	No effect.
	Great Lakes & Imperial 456	65 days	Significant retardation.
Experiment VI (Andrew)	Great Lakes	89 days	Significant increase on vernalized seed. Decrease, but not significant on non-vernalized seed.
Experiment IX (Andrew)	Great Lakes	94 days	Increase on non-vernalized seed, no increase on vernalized seed.
Franklin 1946	Great Lakes	47 days	Better development of seedstalks than on checks.
Franklin 1947	Great Lakes	77 days	Same results as in '46. (Same "morphological age" as in '46)
Experiment 1 Clark & Wittwer	Grand Rapids	61 days	Significant increase in seedstalk length first 3 weeks only.
Experiment 2 Clark & Wittwer	Grand Rapids	63 or 77 days	Significant increase.
	Imperial 456	63 or 77 days	Slight increase (Assuming that all 2,4-D treatments gave significant results as indicated by the 2,4-D average of Clark and Wittwer's Table III).

Such information may suggest a modification of Clark and Wittwer's statement (5),

"In general when any of these substances (including 2,4-D) was applied to 8 to 12-week-old lettuce plants, seedstalk elongation was hastened, the effect being greater for repeated than for single applications."

A hastening may occur when plants are treated at less than eight weeks (Franklin) or at more than 12 weeks (Andrew Experiment IX). There also may be no effect or a retardation where plants are treated within the 8 to 12 weeks range. The results of the present Experiment II are also not in agreement with the latter part of the quoted statement. Double applications of 5, 10 and 20 ppm 2,4-D applied to Imperial 456 and Great Lakes resulted in a significant retardation of seedstalk development rather than a hastening. With the variety Slobolt, the same repeated applications had no significant effect and in Experiment I, two applications of 1 ppm 2,4-D did not alter the seedstalk development of Great Lakes, Slobolt, or Grand Rapids. Three other growth-regulators applied at various concentrations to Grand Rapids lettuce had no significant effect on seedstalk development as evidenced by results presented in Table IV, Experiment III.

On the basis of the foregoing discussion involving the results of three series of experiments, it would appear that the factor or combination of factors affecting the degree and type of influence of growth regulators on the seedstalk development of lettuce is as yet uncertain.

In terminating the discussion of the effects of growth regulators on lettuce seedstalk development, we might refer briefly to the effect of soaking the seed in various concentrations of 2,4-D. Data in Table V, Experiment IV, show that soaking lettuce seed in a solution of 2,4-D causes a reduction in the rate and percentage of germination. When such treated seed was planted (Experiment V), however, plants from seed soaked overnight in 1 ppm 2,4-D developed seedstalks somewhat earlier than the control plants. On the other hand, as evidenced in treatment 2, Experiment VI, soaking in 1 ppm 2,4-D before subjecting it to the low temperature treatment somewhat counteracted the hastening effect of vernalization.

Results of the foregoing experiments, in which vernalization was the main source of variation, coincide to a large degree with results of earlier cold treatment investigations performed mainly on butterhead types of lettuce (4, 17, 31, 39). In the present experiments vernalization of germinating lettuce seed to promote earlier seedstalk elongation was effective on four varieties: Great Lakes, Imperial 847 and Imperial 456 of the crisphead group, and Slobolt of the leaf lettuce group. Such results are not in agreement, however, with those of Thompson and Kosar (35), who used the same Slobolt strain and a numbered strain of the Cos type. Thompson and Kosar (35) were unable to detect visually any definite difference in bolting between vernalized and check plots. They concluded that under the conditions of their experiments, vernalization did not offer any aid in hastening bolting of the two varieties of lettuce tested.

Papers by Rudorf and Stelzner (30) in 1934 and Knott _et al_ (17) in 1937 offer some explanation for the differences in results obtained in the previous and present investigations from those obtained by Thompson and Kosar (35). Rudorf and Stelzner found that plants grown from seedlings exposed immediately after germination to a temperature of $-5^{\circ}C$ showed a very perceptible retardation in growth rate as compared to plants exposed for 10 days to $\neq 5^{\circ}C$. Knott _et al_ (17) found that treatment of germinating seeds at 40°F resulted in more rapid seedstalk development than did treatment at 27°F. Following a description of their procedure Thompson and Kosar (35) stated,

"The methods used were believed to be comparable to those employed by Gray (12), Simpson (31), and Warne (38) and that comparable results could be expected."

This writer believes that in establishing comparable procedures closer attention might have been given to the holding temperature at which germinating seeds were stored. The temperatures at which four previous investigators held their seed were as follows: Gray (12), 4°C; Reimers (39), 2.5° to 5°C; Warne (38), 0° to 4°C; Simpson (31), 2° to 8°C; and Knott et al (17), 40°F. It is apparent that at some period during each of the previous experiments germinating seeds were exposed to temperatures from 2° to 6°C above the 1° to 2°C range used by Thompson and Kosar (35). If a relationship exists between temperature range used in these experiments, as one might surmise from the results of Rudorf and Stelzner (30) and Knott et al (17), such a relationship might be that of a gradual retardation of seedstalk appearance with a gradual decrease in temperature. Thus, a storage or holding temperature 2° to 3°C higher might have increased the differences obtained by Thompson and Kosar (35) to a level of significance.

Another possible source of variation in the tests involving vernalization of Slobolt was the difference in definition of control or check plants. In Experiment V of the present series the control plants were grown from dry seed sown in the usual manner. Thompson and Kosar's control plants (35) were soaked in water twenty-four hours, then kept in a low temperature chamber overnight before seeding. Such control plants would correspond more closely to the plants of treatment 7, Experiment V, which were soaked in water twenty-four hours before seeding. In Table IX of Experiment V may be seen the apparent highly significant difference between treatment 7 and treatment 1, corresponding more closely to the difference in the Thompson and Kosar test (35). Although highly significant it is not as great as the difference between treatment 8 and treatment 1 (Table IX). Thompson and Kosar (35) do not state what temperature their "low temperature" treatment of the controls was and there arises the possibility that even a few hours exposure to low temperature might have a slight vernalizing effect. Some varieties of lettuce are known to have been vernalized in as little as ten days (17, 39). If such were the case, the possibility of affecting a significant difference in Thompson and Kosar's (35) work might have been lessened by exposing germinating seeds of the control plants to low temperature overnight.

In addition to this possible influence of variations in holding temperature, the results of Experiment VII of this series and those of two earlier workers appear to indicate that the effectiveness of the vernalization procedure is also influenced by the stage of development at which the germinating seed or seedling is exposed to low temperatures. Reimers (39) found that lettuce seedlings exposed to from 2.5° to 5°C when they had reached the stage of formation of the first leaf, were slowed down in both growth and shooting to seed. He concluded that lettuce can be vernalized only at the stage of germinating seed. Warne (38) compared the effectiveness of vernalizing seeds soaked for 24 hours only with seeds soaked 72 hours before treating. The seeds soaked only 24 hours had swollen but radicles had not appeared. He concluded that vernalization treatment of lettuce after the radicle appears is much less effective than treatment commenced after the seed had swollen for 24 hours and before germination had begun. Results of treatment 6 and 15 in Table XI, Experiment VII, are in agreement with Reimers' results (39) but results of treatments 1, 2, 3, 10, 11 and 12 are not in accord with those of Warne (38). As indicated in Table XI there is apparently no appreciable difference between treating seed at the swollen stage and treating it after the radicles had emerged. So far as the Imperial 456 plants were concerned there appeared to be a slight advantage in treating seed after the radicles had emerged. A very interesting observation in Experiment VII was the performance of plants in treatment 1 in which seeds were removed to the cold room immediately after being placed to soak in the petri dishes. On the basis of results obtained in treatment 1, it would appear that at least in the case of the lettuce varieties tested, a presoaking period before subjecting the seed to low temperature treatment is not essential. Such a situation suggests the possibility of obtaining unsprouted vernalized seed which could be field sown in the usual manner, thus eliminating special field seeding procedures or the necessity of transplanting. Tolmacev (24) working with winter wheat had just such an objective in mind. He kept seeds in the form of seeds for as long a period as possible and at the same time broke their dormancy and started growth very slowly. This provided seeds, not seedlings, which might be sown in the normal manner.

Two further observations made during the course of Experiment VII are of interest. At the time the flats of treatments 4, 5, 6, 13, 14 and 15 were removed to the 40°F room, seedlings of treatments 6 and 15 were from two to three centimeters above the surface of the soil. Those of treatments 5 and 14 were just breaking through and those of treatments 4 and 13 were not showing. When the six treatments were removed from the cold room the order of height had been reversed. Such behavior may suggest that the seeds which had just germinated were subject to less shock or setback when exposed to the low temperature. The seedlings two to three centimeters high on the other hand, already developing rapidly and becoming adapted to 60°F plus, may have undergone sufficient setback to require considerable recovery time before growth was resumed again.

Another factor determining the effectiveness of vernalization is the duration of the low temperature treatment. Knott et al (17) found that exposure to 40°F for 20 days resulted in more rapid seedstalk development than 10 days at 40°F. Simpson (31) vernalized the variety "Ideal" for 16 days and obtained flower stalks 20 days earlier than from the controls. Reimers (39) in his tests concluded that the variety "Ideal" was vernalized in 10 days while for three other varieties he tested about 20 days was required. Gray (12) held seeds at 4°C for 28, 42 and 56 days, respectively, and found no important differences which could be ascribed to the duration of the treatments.

Data are presented in Tables VI and IX, Experiment V, which indicate no difference in seedstalk development between plants undergoing 16 and 28 days vernalization. We might therefore assume that for Great Lakes, Imperial 847 and Slobolt varieties of lettuce exposure of the germinating seed to a temperature of 40°F for 16 days is sufficient to bring about the vernalization effect.

From the point of view of the seed producer, one of the most practical results obtained from vernalization in the present investigations was that the vernalized plants of Great Lakes and Imperial 456 did not form tight heads. Indeed in a majority of cases no heads were formed at all. Such plants may be seen in Figures 3, 4, 6 and 8. Regardless of whether the vernalization process hastens initiation of seedstalks or whether it increases the rate of development after initiation, the fact that no hard, compact head is formed as a barrier to seedstalk development is of considerable practical advantage. Somewhat similar results were obtained by Knott et al (17) and Simpson (31). Knott et al (17), working with New York 728 and White Boston, referred to the failure of plants from vernalized seed to produce as many good heads and to a tendency to form an open rosette of leaves. Simpson (31), vernalizing the variety "Ideal", observed that plants from vernalized seed remained in the hearted condition only four or five days, while controls remained in the hearted condition approximately 26 days.

Photoperiod and growing temperature also exert influences on the seedstalk development of lettuce (1, 3, 4, 23, 34, 36, 39). Arthur and Guthrie (1) in 1927 concluded that lettuce flowers under long day conditions (longer than 12 hours). Milthrope and Horowitz (23) indicate that vernalization stimulates seedstalk production if followed by high temperatures and long photoperiods. Tincker (36) concluded that although a higher temperature causes a more rapid growth it does not cause the length of day factor to be ineffective in controlling the growth made, either vegetative or reproductive, and in controlling the preliminary stem elongation. In the light of the experiments herein described and the results obtained by Bremer (3), Bremer and Grana (4), Reimers (39) and Thompson and Knott (34), such conclusions appear subject to some revision.

Bremer (3) reported that the variety "May King", a butterhead type of lettuce, bolted in 50 to 56 days without forming heads fit for use under 17 - 18½ hours of daylight. When given a 12 hour day, fine firm heads were formed and the plants bolted in 75 to 80 days. Under a nine hour day (which would likely be generally classified as a "short" day) satisfactory heads were formed and bolting occurred in 85 to 90 days. Plants subjected to only six hours of light produced some loose "knittings", others only leaves and 107 to 117 days were required to reach the bolting stage. The summer varieties Tom Thumb and Rudolph's Favorite were not influenced in time of bolting by length of day. The writer wishes to add a further interpretation of Bremer's data (3) which may be of interest. Plants subjected to long days, of 17 - 18½ hours, required over 300 more hours of light to the bolting stage than did the plants subjected to six hour days. (56 x 18.5 = 692 hours; 1,036 - 692 = 344 hours). Bremer and Grana (4) were able to select plants from the variety "Kaiser Treib" which produced seedstalks during a short or long day. Reimers (39) also observed that shortening the time of exposure to light had little effect on the late (summer) varieties. In comparing the investigations of Thompson and Knott (34) to those of Tincker (36) we find that in contrast to Tincker's explanation (page 65) the Cornell workers report that increasing the length of day did not appear to hasten the initiation of seedstalks but seedstalks elongated somewhat more rapidly under long photoperiod than under a normal day. They also suggest that high temperature is an important factor involved in premature seeding of lettuce.

In Experiment IX of the present series it was demonstrated that the Great Lakes variety of lettuce will flower under short day conditions (nine hours) and at night temperatures as low as 50°F. It was also demonstrated that vernalization stimulated seedstalk production regardless of whether such treatment was followed by high temperatures and long photoperiods or low temperatures and short photoperiods. The data presented in Table XIII indicate that the length of day factor may not be effective in controlling the growth made (vegetative or reproductive) and in controlling the preliminary stem elongation. The effect of a number of interactions are also presented in Table XIII. Increasing the length of day appeared to affect plants from vernalized seed to a greater degree than plants constituting the control. An increase in temperature was more effective on plants subjected to vernalization or 2,4-D treatment than it was on plants receiving vernalization plus 2,4-D or neither vernalization nor 2,4-D. It would appear that as conditions vary, any one of the environmental factors observed in these experiments may become the deciding factor. Apparently temperature or photoperiod cannot in itself limit flowering if another factor or combination of factors is favorable.

SUMMARY AND CONCLUSIONS

To obtain a possible solution to the problem of producing seed from hardheaded and longstanding varieties of lettuce, a series of experiments was conducted to determine the effect of certain growth regulating substances, vernalization, temperature and photoperiod, on seedstalk development.

The application of certain growth regulators to Great Lakes, Imperial 456, Slobolt and Grand Rapids varieties of lettuce resulted in responses of considerable variability. Spray applications of maleic hydrazide, n. p. chlorophenylthalamic acid and benzothiazol-2-oxyacetic acid at the concentrations tested, produced no significant differences in seedstalk elongation of Grand Rapids lettuce. Concentrations of 2,4-D applied singly and in repeated applications to plants at several stages of development produced results varying from a hastening of seedstalk development, through no significant effect, to a retardation of seedstalk development. The degree and nature of response to spray application of 2,4-D was more pronounced with plants held at 60° night temperature than with plants held at 50° night temperature.

Soaking seed in concentrations of 2,4-D from 0.1 ppm to 10 ppm resulted in a decrease in the rate of and percentage germination as the concentration of growth regulator was increased. Plants grown from seed soaked in 1 ppm 2,4-D before vernalization did not develop seedstalks as early as plants soaked in water before low temperature treatment. They did, however, develop seedstalks more rapidly than control plants, and when soaked in a concentration of 1 ppm 2,4-D without vernalization, their performance was very similar to that of plants from seed soaked overnight in water before seeding.

When soaking the seed in 2,4-D and vernalizing were combined, seedstalk development was observed to be later than when the seeds were subjected to vernalization only. When spray application of 2,4-D to plants was combined with vernalization, seedstalk development was earlier than when subjected to vernalization only. The additive effect appeared to be somewhat more evident at night temperatures of 50°F than at night temperatures of 60°F. It would appear that the factors affecting response of lettuce plants to certain growth regulating substances are as yet not sufficiently well understood that substances could be recommended for field application to obtain earlier and/or better seedstalk development.

Subjecting the germinating seeds of Great Lakes, Imperial 456, Imperial 847 and Slobolt varieties to a temperature range of 38° to 42°F for periods of from 16 to 28 days, resulted in significantly earlier seedstalk development and flowering of plants from seed thus treated compared to control plants grown from seed handled in the customary manner. Exposure of seeds to low temperature for periods longer than 16 days was no more effective than exposure for 16 days. Soaking seed prior to low temperature exposure apparently was not necessary. Plants grown from seed subjected to low temperature without previous soaking produced seedstalks as early as plants grown from seed allowed to swell or those from which the radicle was allowed to emerge before low temperature treatments were applied.

Vernalizing seed on a larger scale, after seeding in flats containing a good loam soil was found to be even more effective in inducing earlier seedstalk development than the laboratory technique involving the germinating of seeds in petri dishes — provided, however, the germinating seeds were exposed to the low temperatures prior to or just at the stage of emergence. Subjecting emerged seedlings to the low temperature treatment when two to three centimeters high, resulted in significantly earlier seedstalk development than observed in control plants but was not as effective as treating at earlier stages of seed or seedling development.

Plants grown from vernalized seed of the varieties Great Lakes, Imperial 847 and Imperial 456 showed little tendency to form heads. While control plants formed tight solid heads, growth was loose and leafy on treated plants and seedstalks were able to develop practically free of any physical barrier. Vernalization was shown to favor earlier and stronger seedstalk development throughout the entire series of experiments. Such earlier development was also evident throughout all subsequent stages of flowering and seed production.

No significant differences in seedstalk development were observed between plants grown at 50°F night temperature and those grown at 60°F night temperature. There was an indication, however, that the higher temperature (60°F) was more effective in further increasing seedstalk development on plants subjected to vernalization or spraying with 2,4-D than on plants receiving both or neither of the treatments.

Plants subjected to a photoperiod of 15 hours did not produce earlier seedstalks than those grown under a nine hour day unless the plants had previously been vernalized. Increasing the length of day had considerably more effect on plants grown from vernalized seed than on plants grown from seed not vernalized.

The Great Lakes variety of lettuce bolted either under short days and lower temperature or long days and higher temperature. Although time of flowering was influenced by both temperature and photoperiod neither factor appeared to be the limiting or controlling influence in determining vegetative or reproductive development.

LITERATURE CITED

1. Arthur, John M., and Guthrie, John D. Effect of light, carbon dioxide and temperature on flower and fruit production. Mem. Hort. Soc. of New York. 3:73-74. 1926.

2. Bonner, James, and Bandurski, Robert S. Studies of the physiology, pharmacology, and biochemistry of the auxins. Ann. Rev. of Plant Physiology. 3:59-86. 1952.

3. Bremer, A.H. Einfluss der Tageslange auf die Wachstumsphasen des Salats. Gartenbauwis. 4:469-483. 1931.

4. Bremer, A.H., and Grana, J. Genetische Untersuchungen mit Salat. II Gartenbauwis. 9:231-245. 1935.

5. Clark, B.E., and Wittwer, S.H. Effect of certain growth regulators on seed stalk development in lettuce and celery. Plant Physiology. 24:555-576. 1949.

6. Clark, Harold E., and Kerns, Kenneth R. Control of flowering with phytohormones. Science 95:536-537. 1942.

7. Cooper, W.C. Effect of growth substances on flowering of the pineapple under Florida conditions. Proc. Amer. Soc. Hort. Sci. 41:93-98. 1942.

8. Crafts, A.S., Currier, H.B., and Day, B.E. Response of several crop plants and weeds to maleic hydrazide. Hilgardia 20:57-80. 1950.

9. Franklin, Delance F. Using hormone sprays to facilitate bolting and seed production of hard-headed lettuce varieties. Proc. Amer. Soc. Hort. Sci. 51:453-456. 1943.

10. Garner, W.W., and Allard, H.A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. 18:553-606. 1920.

11. Gassner, G. Beitrage zur physiologischen charakteristic sommer und winterannueller Gewachse, insbesondere der Getreidepflanzen. Zeitschr. Bot. 10:417-430. 1918.

12. Gray, S.S. Increased earliness of flowering of lettuce through vernalization. Jour. Australian Council Sci. and Indus. Res. 15:211. 1942.

13. Gregory, F.G. The control of flowering in plants. Sympos. Soc. Exp. Biol. #2 Growth 75-102. 1948.

14. Gregory, F.G., and Purvis, O.N. Studies in vernalization of cereals. II. The vernalization of excised mature embryos, and of developing ears. Ann. of Bot. N.S. 2:237-251. 1938.

15. Hawthorn, L.R., and Pollard, L.H. Selection of Great Lakes lettuce strains for higher seed yields. Proc. Amer. Soc. Hort. Sci. 57:323-328. 1951.

16. Klippart, J.H. An essay on the origin, growth, diseases, etc. of the wheat plant. Ohio State Board of Agr. Ann. Rpt. 12:562-816. 1857.

17. Knott, J.E., Terry, O.W., and Andersen, E.M. Vernalization of lettuce. Proc. Amer. Soc. Hort. Sci. 35:644-648. 1937.

18. Kostjucenko, I.A., and Zarubailo, T.Ja. Vernalization of seed during ripening and its significance in practice. Herbage Revs. 5:146-157. 1937.

19. Lang, Anton. Physiology of flowering. Ann. Rev. Plant Physiology. 3:265-306. 1952.

20. Leopold, A.C., and Guernsey, F.S. Flower initiation in Alaska pea; evidence as to the role of auxin. Amer. Jour. Bot. 40:46-50. 1953.

21. Lewis, M.T. Lettuce head removal results in more seed of choice lines, Penn. Agr. Exp. Sta. 64th Ann. Rpt. p. 56. 1951.

22. Melchers, G. Die Bluhhormone. Deutsche botanische gesellschaft Berlin. 57:29-48. 1939.

23. Milthorpe, F.L., and Horowitz, B. Effect of length of day and temperature on the flowering, seed production and growth of vegetables. Agr. Gaz. N.S.W. 54:53-57. 1943.

24. Murneek, A.E., and Whyte, R.O. "Vernalization and Photoperiodism", Chronica Botanica Co., Waltham, Mass. 1948.

25. Naylor, A.W. Physiology of reproduction in plants. Survey of Biol. Progress. 2:259-300. 1952.

26. Naylor, A.W., and Davis, E.A. Some effects of maleic hydrazide on growth and respiration of representative monocots and dicots (Abstract). Published in program of meetings of American Society of Plant Physiologists. A.A.A.S. Meetings Dec. 1949.

27. Pollard, L.H., and Hawthorn, L.R. Heads and seed from overwintered lettuce. Farm and Home Sci. (Utah) 7:6, 7, 17. 1946.

28. Schoene, D.L., and Hoffman, O.L. Maleic hydrazide, a unique growth regulant. Science. 109:588-590. 1949.

29. Rice, Elroy L. Effects of various plant growth-regulators on flowering in several crop plants. Bot. Gaz. 112:207-213. 1950.

30. Rudorf, W., and Stelzner, G. Untersuchungen uber lichtperiodische und Temperaturnachwirkung bie Sorten von Salat (Latuca sativa var Capitatal) und die Moglichkeit ihrer Ausnutzung im Gemusebau. Gartenbauwis. 9:143-153. 1934.

31. Simpson, A.C. Vernalization of lettuce. Nature 151 #3827:278-280. 1943.

32. Tang, Pei-Sung, and Loo, Shih-Wei. Tests on after affect of auxin seed treatment. Amer. Jour. Bot. 27:385-386. 1940.

33. Thimann, K.V., and Lane, R.H. After effects of the treatment of seed with auxin. Amer. Jour. Bot. 25:535-543. 1938.

34. Thompson, H.C., and Knott, J.E., The effect of temperature and photoperiod on the growth of lettuce. Proc. Amer. Soc. Hort. Sci. 30:507-509. 1933.

35. Thompson, Ross C., and Kosar, Wm. Vernalization and seed stem development in lettuce. Proc. Amer. Soc. Hort. Sci. 52:441. 1948.

36. Tincker, M.A.H. The influence of length of day on lettuce. Gard. Chron. 93:404-405. 1933.

37. Van Overbeek, J. Flower formation in the pineapple plant as controlled by 2,4-D and naphthaleneacetic acid. Science 102:621. 1945.

38. Warne, L.G.G. Vernalization of lettuce. Nature 159 #4027:31-32. 1947.

39. Whyte, R.O. "Crop Production and Environment", Faber and Faber, London, 1946.

40. Wittwer, S.H., and Sharma, R.C. The control of storage sprouting in onions by preharvest foliage sprays of maleic hydrazide. Science. 112:597-598. 1950.