FACTORS AFFECTING FLOWERING IN LETTUCE

By

Lawrence Rappaport

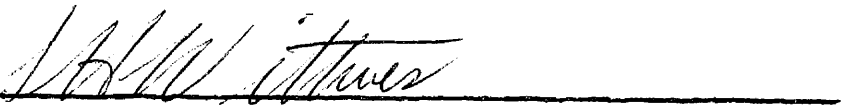
AN ABSTRACT

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

DOCTOR OF PHILOSOPHY

Department of Horticulture

1956

Approved 

ABSTRACT

Seed vernalization, plant growing temperatures, daylength and chemical growth regulators, separately and interacting, markedly influenced flowering and seedstalk development in Great Lakes head lettuce. The superiority of a "physiological index" of flowering such as leaf numbers preceding a distinct developmental stage, in contrast to a growth measurement such as stem elongation, was demonstrated. Photoperiod and air (but not soil) temperatures were shown to influence the number of leaves preceding the developing inflorescence. Flowering was accelerated in plants grown from moist seed germinated at 60 to 70°F for 24 to 48 hours and subjected to 40°F for a minimum of 13 days or more. Seed germinated 3 days at 60 to 70°F were not effectively vernalized at 40°F for 16 days. Periodic temperature interruptions at 60 or 90°F for two hours daily or at three day intervals did not nullify the vernalizing effect of cold exposure. Plants from lettuce seed which had been soaked at 60 to 70°F and air dried 6 or 12 hours prior to chilling at 32 or 40°F were delayed in flowering as compared with those from vernalized seed. When Maleic Hydrazide (20 or 40 ppm), was applied during vernalization, plants produced visible flower parts at a lower node than those from seed vernalized in water alone. Growing lettuce plants at night

temperatures above 65°F subsequent to vernalization accelerated seedstalk development without preceding head formation. Below 65°F vernalized plants first produced a high percentage of firm, vegetative heads and then flowered. Non-vernalized plants flowered only at night temperatures above 65°F. Earlier flowering resulted in plants exposed continuously to warm temperatures as compared to alternating cool (50°F) and warm (70°F) night temperatures. Apparently, the potential for flowering is greater following seed vernalization, but expression is subsequently delayed by continuous night temperatures of 50°F. Soil temperatures influenced the rate of seedstalk development, but not the vernalization stimulus per se. At high soil temperatures (64 and 70°F) flowering (days to anthesis) was promoted, while at low temperatures (50 and 57°F) flowering was delayed. A decrease in percent seedstalks occurred as soil temperatures declined from 71 to 50°F. Photoperiod markedly affected flowering in vernalized head lettuce. The minimum night temperature following vernalization which favored early flowering was reduced from 65 to 60°F when plants were grown at a daylength of 16 hours. At 60°F with a nine-hour day, both vernalized and non-vernalized plants formed firm, vegetative heads which flowered simultaneously, thus the effects of seed vernalization were nullified by a short daylength. The delaying effect

of cool temperatures (both soil and air) and relatively short days on seedstalk development demonstrated in the greenhouse, was verified in field studies. Plants grown in the early Spring formed heads irrespective of previous seed vernalization and treatment with chemical growth substances. Similarly, endive (variety, Full Heart Batavian) displayed marked sensitivity to cool (below 60°F) temperatures during early growth. Overwintered plants and seedlings, plants from seed sown out of doors April 1, or transplanted to the field May 21, after vernalization flowered earlier than those from seed sown after May 1 or from non-vernalized transplants.

Possible biochemical differences induced in lettuce seed by vernalization were studied by subjecting the ether extracts from vernalized and non-vernalized seedlings to paper partition chromatography. Fluorescent spots detected with ultra-violet light and occasional colored spots detected with Salkowski and Ehrlich's reagents were thus isolated. R_f values possibly comparable to those of 3-Indoleacetic Acid, Tryptophan, Indoleacetonitrile, and Ethyl Ester of Indoleacetic Acid developed both with water and Isopropanol-ammonia water-water (8:1:1 V/V) appeared on chromatograms of extracts of both vernalized and non-vernalized seedlings. No consistent differences were evident. Extracts developed with the 8:1:1 solution on paper yielded spots which fluoresced in

ultra-violet light at 0.52, an R_f value at which no biologically active indole compound has been identified insofar as the author is aware. Bioassays for L-tryptophan revealed that .00127 and .00293 per cent occurred in vernalized and non-vernalized seedling extracts, respectively. The low value of L-tryptophan in vernalized seedlings suggests direct utilization of L-tryptophan or conversion during cold exposure to another indole compound, conceivably 3-Indoleacetic Acid.

FACTORS AFFECTING FLOWERING IN LETTUCE

By

Lawrence Rappaport

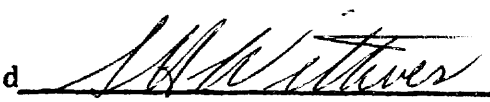
A THESIS

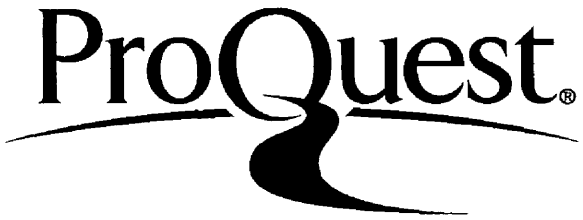
Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture
and Applied Science in partial
fulfillment of the requirements for
the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1956

Approved 



ACKNOWLEDGEMENT

The author is deeply indebted to Dr. S. H. Wittwer whose friendliness, guidance and criticism during the performance of the research and writing of this manuscript are genuinely appreciated.

Dr. H. N. Fukui is acknowledged for his suggestions and aid in the performance of the biochemical studies.

The interest and technical assistance of Drs. S. K. Ries, and D. H. Dewey, are gratefully acknowledged.

Lastly, appreciation is expressed to Drs. L. M. Turk, R. L. Carolus, S. T. Dexter, and H. M. Sell, members of the guidance committee, and to Dr. H. B. Tukey for their suggestions on the graduate program and the manuscript.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Seed production from non-bolting varieties of lettuce often necessitates slashing the mature heads to permit emergence of vigorous seedstalks, a practice which is costly, encourages disease transmission, and may be ineffective (18). A method to circumvent this operation would materially reduce production costs.

Vernalization (chilling partially germinated seed) as a technique for promoting flowering and seed production has been employed successfully in a variety of important crops (1, 15, 22, 23, 24, 25, 32, 33, 34, 35, 50, 72, 73, and 81). In lettuce, however, conflicting reports occur regarding the effectiveness of vernalization in accelerating seedstalk development without prior head formation (1, 24, 23, 42, 45 and 70).

Since vernalization may hold promise in seed production these apparent inconsistencies suggested a more critical evaluation of the internal and external conditions favoring flowering and seedstalk development in lettuce. Such a study might add not only to our knowledge of flower formation in head lettuce but in other crops as well.

REVIEW OF LITERATURE

Foreword: For centuries man has sought to control the growth and reproductive development of cultivated crops. However, it was not until Sachs published his classic treatise (55) that the scientific approach in plant physiology was adequately presented. By demonstrating that different species possess minimal, optimal and maximal temperature requirements, he provided the basis for later investigations on the temperature requirements for specific responses such as flowering in many crops.

Perhaps without realizing the enormity of his discoveries in "electro-horticulture" Bailey (4) described the response to day length of "spinage" which "ran to seed" when exposed to the electric arc light. Later Garner and Allard (21) discovered photoperiodism which has since been a source of intense scientific interest. Coincident with the study of daylength considerable attention has been focused on the intensity and quality of light in relation to physiological responses in plants (5,6,16). Investigators are now making a concentrated search for the specific portion of the spectrum and the pigments associated with such phenomena as related to photoperiodic flower induction and vegetative development (38).

The suggestion by Sachs (55) that transmissible flower-forming substances occur in plants, and the subsequent isolation of natural and synthetic compounds exhibiting biological activity, (3, 29, 30, 44, 50, 63, 77) have provided a thread which may eventually bind together the many factors responsible for vegetative and reproductive expression. Because flowering in many plants is so intimately related with temperature, day length, and chemical growth regulators, consideration of these factors in a comprehensive study of flowering is imperative.

The Effect of Temperature on Flowering: The acceleration of flowering by chilling or freezing wheat seed was reported by Klippart (32) in 1857:

> "To convert winter into spring wheat nothing more was 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."

Gassner (22) in 1918 found that when seed of cereal crops were planted on progressive dates, those exposed to near-freezing, as compared to higher temperatures, flowered earlier and developed most uniformly.

The publication by Lysenko (39,43) on the promotion of flowering by vernalization renewed interest in temperature studies among investigators who, for a time, had been diverted by the newly discovered phenomenon of photoperiodism. A classic series of papers by Purvis and Gregory

(23, 25, 26, 27, 47, 48, 49, 50) and their associates in England have helped to elucidate the factors related to vernalization in cereals and to dispel certain of the misconceptions introduced by Lysenko and his colleagues (39, 43). Principally, they found that the stimulus which is perceived by the embryo (25, 26) and, likely, the apical meristem (48) causes acceleration of flowering in winter rye (47). The response of vernalized grain was influenced by subsequent temperature and photoperiod (23, 47). The concept of "minimal leaf number" preceding the inflorescence is characteristic of the work of Purvis (47). McKinney and Sando (40), Leopold (35, 36), Wittwer et al (81), Lang (34), and recently Gott (23), have re-emphasized the reliability of leaf numbers as a specific physiological index of flower expression.

In the United States, McKinney and Sando (40) working with cereal crops found results comparable to those of Purvis and Gregory, but Sprague (62) showed that "jarovization" caused very little acceleration of flowering in corn.

Sen and Chakravarti (57) reported that only a minimum of pregermination was necessary to accelerate flowering in chilled mustard seed. Seedlings soaked long enough to initiate germination, but not to permit emergence of the

radicle, could be vernalized and then dried six years without loss of the stimulus.

Melchers (41) found that Hyoscyamous niger was induced to flower without customary cold treatment when grafted to a flowering plant.

Thompson and coworkers (67) investigated the response to cold temperatures of a series of horticultural crops. In experiments by Knott, et al (33) lettuce seedlings were exposed at various stages of development to a temperature of 40°F and -5°F. Varying with variety vernalization at 40°F for 10-20 days accelerated flowering, while in results comparable to those of Rudorf and Stelzner (54), freezing temperatures retarded germination and did not influence flowering. In contrast to seedlings not vernalized, seedlings grown at 70 to 80°F or at 50 to 60°F produced seedstalks earlier. At 60 to 70°F vernalized plants produced loose heads.

Reimers (79) in 1938 found a difference in varietal response of lettuce to vernalization. He concluded that only germinating seedlings, as compared to those with the first true leaf expanded, were receptive to cold exposure of 2.5 to 5°C and produced plants in which flowering was accelerated. In contrast, Warne (76) reported that lettuce seedlings germinated three days were not effectively vernalized. He found that compared with non-vernalized seed only

those not germinated or pre-germinated 24 hours before cold exposure, produced plants which flowered earlier.

In an experiment to determine the effects of duration of the chilling period on seedstalk development of lettuce Gray (24) exposed seedlings of Imperials D and 847 to a temperature of 40°C for periods of 28, 42, and 56 days. With the longer periods of chilling few seedlings developed normally, however, in those plants which survived seedstalk development was accelerated by two to three weeks. In a comparable study Simpson (61) found that vernalized plants remained in the hearted condition 4 to 5 days previous to shooting seedstalks. Interestingly, botrytis infection was reduced by 50 per cent in vernalized plants. This may have been due to the upright growth which is characteristic of vernalized plants.

Without further elaboration Milthorpe and Horowitz (42) reported that bolting was accelerated when vernalized lettuce was exposed to long days and high night temperatures. Like Knott (33) and Reimers (79) they found differences in varietal responses. In some varieties flowering was induced by low and in others by high temperatures.

Andrew (1) found that seedstalk development was promoted when germinated seedlings of the Great Lakes, Imperial 456, Imperial 847 and Slobolt varieties were

exposed to temperatures of 38 to 42°F for 16 to 28 days. Vernalization for 16 days was as effective as vernalization for periods up to 28 days. Vernalization in soil was even more effective than chilling seedlings in petri dishes. In disagreement with the results of Warne (76) Andrew found that vernalization was effective when plants two to three centimeters high were vernalized, however, such plants were delayed in flowering as compared to plants from seedlings vernalized at an earlier stage of development. In relation to vernalization of lettuce varieties, Andrews concluded:

> "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."

Recently Rappaport and Wittwer (51) reported that flowering in endive (variety Broad Leaf Batavian), a plant botanically related to lettuce, was hastened by two months when seedlings were vernalized at 40°F for 20 days or by planting outdoors in Central Michigan about April 1.

Pollard, et al (46) studied the effects of overwintering lettuce on subsequent head and seed production. Hawthorne (27) selected for higher seed production in firm headed lettuce varieties while Lewis (36) found that removal

of lettuce heads effected the release of seedstalks.

The Effect of Daylength on Flowering: The concept of photoperiodism, first crystallized by Garner and Allard (21), stimulated great interest in the effects of daylength on flowering.

In an investigation of the effects of light, carbon dioxide, and temperature on the flowering of lettuce, Arthur and Guthrie (2) reported that lettuce flowered when exposed to photoperiods longer than 12 hours.

A critical study of the effects of photoperiod on several varieties of lettuce was reported by Bremer in 1929 (7). When the varieties May King and Leppermann were subjected to daylengths of 17 to 18½ hours, seedstalks were produced without marketable heads. Excellent heads were produced at a 9 or 12-hour day, while only "loose knittings" were obtained with a 6-hour day. Because of the distinct differences in varietal response to day length and temperature Bremer (7) classified lettuce as either Winter lettuce (sensitive to long days), Late Spring lettuce (less responsive to daylength), and Summer lettuce (not responsive to daylength). He observed that cool night temperatures delayed flowering in lettuce. In a later report (8) Bremer confirmed his original findings and suggested that genetic factors were associated with the photoperiodic response of leaf lettuce.

Comparable results were obtained by Reimers (79) when Winter, Summer and Intermediate types of lettuce were exposed to varying daylengths. He concluded that length of day was more influential than temperature in inducing flowering of lettuce.

Tincker (71) using the "butterhead" variety Early Paris found that best vegetative development occurred with a 15-hour photoperiod while an 8 to 11-hour daylength induced earliest bolting.

Thompson and Knott (69) investigating the effects of temperature and photoperiod on premature seeding in White Boston lettuce, found that irrespective of daylength, temperatures of 70 to 80°F hastened seedstalk development. Most satisfactory vegetative development occurred at 60 to 70°F.

Recently, Rappaport and Wittwer (52) demonstrated a marked sensitivity of Bibb leaf lettuce to photoperiod and Grand Rapid leaf lettuce to night temperature. Tendergreen, the selection from a cross between Bibb and Grand Rapids, flowered more readily with long days and high night temperature than either parent.

In vernalization studies with head lettuce (variety Great Lakes) Andrew (1) found that vernalized, as compared to non-vernalized plants, produced seedstalks earlier if grown with a 15-hour than a 9-hour day:

> "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."

Flowering and Chemical Growth Regulators: The isolation from plants of substances exhibiting biological activity has encouraged research into the relationships between growth substances and flowering. Cholodny (11) postulated that an auxin moves from endosperm tissue into the embryo of vernalized cereal seed and is responsible for subsequent acceleration of flowering. This theory was examined by Purvis and Gregory (49) and finally discarded as a result of the experiments of Hatcher (27). The fact that seed can be devernalized by high temperatures subsequent to vernalization has suggested that a substance is produced during the chilling period which is responsible for acceleration of flowering.

Several reports (13, 14, 19, 35) suggest that synthetic growth substances may modify flowering. However, these substances have not been shown to coincide in activity with that proposed for the hypothetical "florigen".

A substance extracted from vernalized rye embryos which had an accelerating effect on flowering when applied to unchilled embryos was reported by Purvis and Gregory (50).

Highkin (29) reported that diffusates from various seeds accelerated flowering when applied to seed of plants which respond to vernalization.

Leopold (34, 35, 36) found that a variety of plants could be "chemically vernalized" by seed treatment with growth regulators during cold exposure. Chakravarti *et al* (10) showed that treatment with 3-Indoleacetic Acid (IAA), Indolebutyric Acid (IBA), and Naphthaleneacetic Acid (NAA) accelerated flowering of vernalized *Brassica campestris* plants but that 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Triiodobenzoic Acid (TIBA) delayed flowering. They concluded that the similar responses of 2,4-D and TIBA may contradict the auxin/anti-auxin concept of flowering. Chakravarti and Sen (9) reported that IAA, IBA, and NAA depressed flowering in *Linum* when applied during vernalization. Wittwer (80) found that 1 ppm of 2,4-D inhibited the response of lettuce seed to vernalization. Similarly Andrew (1) working with Great Lakes lettuce, found that the effect of vernalization was accentuated when in conjunction with seed treatment of 1 ppm of 2,4-D a subsequent plant spray of 5 ppm 2,4-D was employed.

In an attempt to effect the release of seedstalks or hasten their development in head lettuce without slashing the heads, Franklin (18) sprayed 2,4-D, NAA, and Parachlorophenoxyacetic Acid (PCPA) to induce epinasty of the leaves

in the rosette stage. Comparable results were obtained with 2,4-D by Clark and Wittwer (13) but not by Andrew (1). Apparently a critical developmental stage exists for the hastening of flowering in lettuce by growth regulators.

Crafts et al (14) reported severe bolting in lettuce from foliage sprays of 1000 ppm of Maleic Hydrazide (MH). When head lettuce plants (var. Wonderful) were sprayed with 0.05 per cent MH three weeks after transplanting, Choudri (12) likewise obtained flowering one to two weeks earlier. Bolting was prevented, however, if semi-mature heads (six weeks after transplanting) were sprayed with 0.05, 0.10, or 0.20 per cent of MH.

Stimulation and delay of seedstalk development in celery was similarly obtained by Wittwer et al (80) with varying concentrations of MH applied at different developmental stages.

The promotion of flowering by 2,3,5-Triiodobenzoic Acid has been known since 1942 when Zimmerman and Hitchcock (83) described the formative effects on tomato. Recently N-meta-Tolylphthalamic Acid and its derivatives have markedly increased flower numbers in tomato (67).

Biochemical Studies Related to Flowering: The resolution of the causes of developmental responses in plants to growth regulators and environmental treatments (vernalization and photoperiod) is often best effected by chemical

analysis immediately after completion of the treatment in addition to gross examination of the test plants at a later date. With the advent of quantitative tests for auxin activity (13, 27, 30, 65) and particularly chromatographic techniques for separation and characterization of growth substances (44, 59, 64, 65, 77), a number of auxins other than 3-Indoleacetic Acid (IAA) have been characterized. The relationships between free auxin (IAA) and free tryptophan, as well as the probability of compounds other than IAA in strawberry achenes have been demonstrated by Nitsch (44). Thus far only Hatcher (27) has provided any quantitative evidence for changes in auxin concentrations in vernalized embryos of cereal crops.

The recognized biological activity of several indole compounds isolated from plant tissues (3, 30, 44, 63, 65, 66, 77), suggests that such compounds may occur in lettuce possibly in association with the vernalization process.

THE PROBLEMS FOR INVESTIGATION

Those who have thus far studied vernalization of lettuce seed or small seedlings disagree as to its reliability as a technique for the promotion of flowering and seed production. Little is known concerning the process or the factors which control the expression of flowering in lettuce subsequent to vernalization. The nature of the stimulus has been studied, but thus far no chemical substances displaying biological activity similar to the vernalization stimulus have been isolated and characterized. The problems for investigation, therefore, include; (a) a study of the vernalization process, (b) an evaluation of the environmental factors which subsequent to chilling partially germinated lettuce seed are conducive to flowering, and (c) the characterization of substances in the vernalized plant possibly associated with earlier flowering.

GENERAL PROCEDURES

All experiments were conducted in the Horticultural Greenhouses and Field Plots of Michigan State University from December 1953 to September 1955.

Vernalizing Seed: Except where otherwise specified lettuce seed (variety Great Lakes, Stock No. 11447, Ferry Morse Seed Company, Detroit) was vernalized in soil, vermiculite, or in Petri dishes on two Whatman No. 1 filter papers moistened with three milliliters of water or water containing a chemical growth substance. The seed were germinated 24 to 48 hours at 60 to 70°F and the petri dishes were then stored in a room thermostatically controlled at 40 $\pm$ 2°F for 20 days. Non-vernalized (control) seedlings, comparable in size to those vernalized, were obtained by pre-germinating seed 24 hours at 60 to 70°F prior to shifting all seedlings to the greenhouse.

Growing Plants: In the greenhouse the seedlings were transferred to flats of vermiculite, grown to the 3-leaf stage and then transplanted into 2 to 4-inch pots. They were later transferred to 4 to 8 inch pots, a groundbed, or a coldframe prior to setting in the field. A high level of (soil) fertility was maintained in the greenhouse by supplemental feeding during irrigation with a high

analysis, (10 - 52 - 17) soluble fertilizer.

Control of Temperature: Greenhouse air temperatures were maintained at night by thermostatic controls but day temperatures were often higher. After March 1 a temperature of 50°F could not be consistently achieved at night within the greenhouse and transfer of the plants of low temperature treatments to an outdoor cold frame was necessary.

In the root temperature studies, in addition to thermographs, a Brown Electronik Recording Potentiometer was used to record both soil and air temperatures at 15-minute intervals.

Control of Daylength: Plants were grown on movable carts which were rolled into dark rooms at the designated time for short day exposure. Lighting providing for a 16-hour photoperiod was obtained from 300-watt photoflood lamps which gave an average of 55 to 80 foot candles of light at the plant surface. In other experiments the photoperiod was extended with mazda or fluorescent lamps which produced 10 to 90 foot-candles of light, respectively. Such lamps did not produce sufficient heat to alter the temperature at the plant level.

Measurements of Flowering Responses: Leaves, exclusive of leaf bracts, produced prior to flowering, days to the appearance of the visible flower parts (developing

inflorescence, Figure 1), and days to anthesis were used as specific indices of the effects of the various factors on reproductive development.

Statistical Methods: The experimental designs were either randomized blocks or split plots. The analysis of variance was used to evaluate statistical differences.

Figure 1. An Immature Inflorescence

EXPERIMENTAL

Limitations in the control of night temperatures within the greenhouse, imposed by seasons, of the year often necessitated conducting similar experiments at different times. Nevertheless, experiments related as to objective follow in sequential order.

I. FACTORS INFLUENCING VERNALIZATION

Duration of Chilling: To determine the duration of chilling necessary for promotion of flowering lettuce seed were sown in flats of moist vermiculite on successive dates from September 24, to October 21, 1954 so that separate lots were chilled 0, 6, 9, 13, 21, and 30 days at 40°F constant temperature. Following cold treatment the flats were moved to a 70°F night temperature greenhouse simultaneously with non-vernalized seedlings germinated 48 hours. After the appearance of three leaves the seedlings were transferred to 3-inch pots of soil and subsequently to 7-inch pots where they were grown to maturity.

A minimum cold exposure of 13 days was found essential for acceleration of flowering as indicated by decreased numbers of leaves preceding the appearance of visible flower parts (Table I).

Age of the Plant: The stage of development (age) at

TABLE I

THE DURATION OF VERNALIZATION NECESSARY
FOR ACCELERATION OF FLOWERING IN GREAT LAKES HEAD LETTUCE

Days of Cold Exposure	Leaves to Developing Inflorescence (Ave. of 3 Replicates)	Days to Visible Flower Parts (Ave. of 3 Replicates)
0	27.9	154
6	28.4	149
9	27.6	146
13	25.8	143
21	24.1	138
30	25.3	140
L.S.D. (5%)	1.8	
(1%)		5

TABLE II

EFFECT OF AGE OF THE PLANT PRIOR TO VERNALIZATION ON SUBSEQUENT FLOWERING OF GREAT LAKES LETTUCE

Days Seeded Prior to Vernalization	Leaves to Developing Inflorescence (Ave. of 3 Replicates)	Days to Visible Flower Parts (Ave. of 3 Replicates)
Not vernalized	27.9	153.2
3	27.3	144.4
6	31.4	138.8
9	31.0	134.4
12	34.3	136.4
15	33.9	131.3
L.S.D. (5%)	1.9	
(1%)		6.4

which seedlings are most receptive to vernalization was next studied. Lettuce seed were sown in flats of vermiculite in a 60°F greenhouse and at three-day intervals thereafter for a 15-day period. Eighteen days subsequent to the initial sowing, when seedlings ranged from those with the first leaf fully expanded to those seeded three days earlier, the flats were shifted to 40°F and held for 16 days. All seedlings were then transferred to a 70°F greenhouse.

The results (Table II) indicate that seedlings pregerminated three days or longer are not receptive to vernalization (16 days at 40°F).

Temperature: In preliminary vernalization studies partially germinated lettuce seed in Petri dishes interchanged among 35, 40 and 45° constant temperatures at 6-day intervals for a period of 18 days, produced firm heads when grown in a 50°F night temperature greenhouse. Similarly, seedlings exposed for 1 to 2 hours daily or at five day intervals to 75°F during vernalization formed heads at 50°F plant growing temperatures.

In a subsequent study the effects on flowering of exposing seedlings to high temperatures during vernalization were further evaluated. Lettuce seed were vernalized in the usual manner except that during vernalization some of the dishes were periodically exposed for two hours to temperatures of 60 and 90°F. The treatments are designated in Table III.

TABLE III

FLOWERING OF GREAT LAKES HEAD LETTUCE AS AFFECTED BY INTERRUPTIONS OF VERNALIZATION BY 2-HOUR EXPOSURES TO 60 OR 90°F TEMPERATURES DAILY AND AT 3-DAY INTERVALS

No.	Treatment	Leaves to Developing Inflorescence (Ave. of 4 Replicates)	Days to Visible Flower Parts (Ave. of 4 Replicates)
1.	Seed held at 40°F, exposed 2 hours daily at 60°F.	45	90
2.	Seed held at 40°F, exposed 2 hours daily at 90°F.	43	89
3.	Seed held at 40°F, exposed 2 hours every 3 days at 60°F.	44	89
4.	Seed held at 40°F, exposed 2 hours every 3 days at 90°F.	41	88
5.	Seed germinated at 40°F for 20 days (Control)	44	86
6.	Seed germinated at 60°F not vernalized (Control)	52	94
7.	Seed germinated at 90°F not vernalized (Control)	50	93
	L.S.D. (5%)	4.2	2.0

Following chilling the seedlings were transferred to three-inch pots in a 65°F night temperature greenhouse, and later to a ground-bed (April 27, 1955).

The results of this experiment were somewhat modified by a heavy incidence of root rot which occured in the greenhouse because of hot weather and high soil temperatures. The data (Table III) indicate, however, that two hour temperature interruptions of 60 and 90°F daily or at three day intervals during vernalization did not affect the numbers of leaves preceding the developing inflorescence. As compared to vernalized plants, the appearance of the developing inflorescence of those not vernalized (Treatments 6 and 7), was delayed.

Moisture: The effectiveness of vernalization as influenced by the moistness of the seed and temperatures of 32 and 40°F was next studied. An experiment comprising nine treatments (Table IV) was started September 24, 1954. After vernalization the seedlings were transferred to a 70°F night temperature greenhouse.

Moistening and drying the seed followed by exposure to either 32 or 40°F did not prevent germination and subsequent development. In contrast to seedlings vernalized at 40°F for 20 days (Treatment 7) no other treatment proved effective in accelerating flowering (Table IV).

TABLE IV

THE INFLUENCE OF MOISTURE BEFORE AND DURING SEED VERNALIZATION ON SUBSEQUENT FLOWERING OF GREAT LAKES LETTUCE

No.	Treatment	Leaves to Developing Inflorescence	Days to Visible Flower Parts	Days to Anthesis
		(Ave. of 3 Replicates)		
1.	Seed soaked in water 6 hours, air dried 4 hours; chilled 20 days, 40°F	33.5	134	143
2.	Seed soaked in water 12 hours, air dried 4 hours; chilled 20 days, 40°F	32.1	132	146
3.	Seed soaked in water 6 hours, blotted dry; chilled 20 days, 40°F	32.7	129	143
4.	Seed soaked in water 12 hours, blotted dry; chilled 20 days, 40°F	34.2	132	145
5.	Seed soaked in water 24 hours, blotted dry, chilled 20 days, 40°F	33.8	133	145
6.	Seed soaked in water 24 hours, chilled in water 20 days, 32°F	32.4	129	145
7.	Seed soaked in water 24 hours, chilled in water 20 days, 40°F (Control)	29.4	120	137
8.	Seed soaked in water 24 hours, not chilled (Control)	34.3	130	144
9.	Seed planted directly, not chilled (Control)	33.7	132	145
	L.S.D. (5%)	1.9	1.4	3.8

Light Quality and Duration: In an experiment to determine the effects on flowering of ultra-violet light radiation during vernalization, lettuce seed was placed in Petri dishes on moist filter paper July 4, 1954. Twenty-two hours and again 10 days later during vernalization some of the seedlings were exposed to ultra-violet light (2537A) at a distance of three feet for periods of 10, 30, and 60 seconds. On July 23 the vernalized and non-vernalized seedlings were planted in flats of soil in a 65°F greenhouse and on August 16 were transplanted to a prepared field plot.

Ultra-violet light treatment for 60 seconds delayed growth slightly. However, within three weeks after transplanting the plants were equal in size. Irrespective of previous light or vernalization treatment all plants formed firm heads. By October 20, there was no indication of seedstalk development.

In a preliminary study of the effects on flowering of photoperiod during 18 days of vernalization, lettuce seed at 40°F were subjected to (a) 7-hours of light quality provided by a 10-foot candle lamp source, (b) continuous light, and (c) continuous darkness. Exposure of seedlings to continuous light, compared to a 7-hour photoperiod or continuous darkness during vernalization, reduced germination by 40 per cent. At a growing temperature of 50°F plants formed firm heads irrespective of vernalization or light treatment.

Chemical Growth Regulators: In an initial series of experiments with chemical growth substances, lettuce seed treated during vernalization with Naphthaleneacetic Acid (NAA), 2,3,5-Triiodobenzoic Acid (TIBA), Maleic Hydrazide (MH), and 2,4-Dichlorophenoxyacetic Acid (2,4-D) formed heads when grown subsequently at 50°F night temperatures, thus no means of evaluating possible flowering responses was possible.

The influence of 2,4-D and MH applications during and subsequent to seed vernalization was further evaluated in a replicated field study initiated March 17, 1954. Seed of two lettuce varieties, Great Lakes and Cornell 456, were rolled in filter paper and inserted in test tubes containing 1.5 milliliters of the following water solutions: 1 or 0.5 ppm of 2,4-D, 11 or 56 ppm of MH, and no chemical regulator. The tubes containing the seeds were held at 60°F for 24 hours and then at 40°F for 16 days. On March 31 seedlings of the two varieties were started at 60°F with the same chemical treatments. On April 2 and 3 the seedlings were transferred to flats of soil in a 60°F greenhouse. The plants were placed in a coldframe May 11 and one week later were transplanted into prepared field plots.

Subsequent to transplanting some plants were sprayed with 10 ppm of 2,4-D, 5 ppm of 2,4-D, 560 ppm of MH or water containing no growth regulator at either the appearance of

the 6th or 7th leaves, or at the rosette stage, just prior to heading.

Germination of both varieties was inhibited by high concentrations of MH and 2,4-D. This inhibition was reflected in the field by uneven plant development. Lettuce plants irrespective of vernalization, growth regulator treatment, or variety formed firm heads. Thermograph records indicated that night temperatures remained below 60°F during the heading period.

The lack of response to growth regulator treatment and to vernalization itself suggested a study of the effects of subsequent temperature on flowering of plants from "chemically vernalized" (36) lettuce seed. On February 14, two lots of seed were germinated on filter paper moistened with water solutions of 0, 20 and 40 ppm of Triiodobenzoic Acid (TIBA), Maleic Hydrazide (MH), and N-meta-Tolylphthalamic Acid (7R5) in Petri dishes. Twenty-four hours later they were stored at 40°F for 17 days. Eighteen days after the initial sowing two additional lots of seed were germinated at 60°F. One day later, one lot of vernalized and one of non-vernalized seedlings were placed in a 60°F night temperature greenhouse and the remaining two lots were placed at 70°F.

Plants grown at 70°F produced seedstalks while those

TABLE V

FLOWERING OF GREAT LAKES HEAD LETTUCE AS AFFECTED BY N-META-TOLYLPHTHALAMIC ACID (7R5), MALEIC HYDRAZIDE (MH), AND 2,3,5-TRIIODOBENZOIC ACID (TIBA) APPLIED DURING SEED VERNALIZATION

Chemical	Treatment (ppm)	Leaves to Developing Inflorescence (Ave. of 3 Replicates)	Days to Visible Flower Parts (Ave. of 3 Replicates)
7R5	20	30.3	96.0
MH	20	29.0	104.0
TIBA	20	30.5	97.0
7R5	40	30.5	99.0
MH	40	29.0	103.0
TIBA	40	30.6	96.0
Water only	Not vernalized	37.6	106.0
Water only	Vernalized	31.7	98.6
	L.S.D. (1%)	1.96	4.7

at 60°F remained vegetative despite previous vernalization. Vernalization accelerated flowering at 70°F as indicated by a reduction in leaf numbers preceding the developing inflorescense, (Table V). In combination with 20 or 40 ppm of MH vernalization was even more effective in hastening flowering. However, MH inhibited growth of the developing inflorescence. Neither TIBA nor 7R5 influenced reproductive development when used in conjunction with seed vernalization.

II. FLOWERING AS AFFECTED BY THE PLANT GROWING ENVIRONMENT SUBSEQUENT TO VERNALIZATION

Night Temperature:

1954 Tests: To determine the effects of night temperature on developmental responses lettuce seedlings were vernalized beginning December 24, 1953 and together with those not vernalized, were subsequently maintained in 50, 65, and 70°F night temperature greenhouses. Plants grown continuously at 65°F produced seedstalks without preceding head formation while plants grown at 50°F remained vegetative irrespective of vernalization (Table VI). Both vernalized and non-vernalized plants produced seedstalks at 70°F although non-vernalized plants formed loose heads prior to the emergence of comparatively weak seedstalks. At 70°F plant growing temperature seedstalk initiation and elongation were most rapid in vernalized plants. However, as the season progressed, seedstalk elongation of non-vernalized plants surpassed that of the plants grown from vernalized seed. Vernalization of seedlings followed by growth at 65°F resulted in plants with 7.2 more leaves than those grown at 70°F. In contrast, those not vernalized flowered at 70°F but only after producing 14.4 more leaves than the vernalized plants. Temperatures of 65 and 70°F following vernalization of seedlings did not affect

TABLE VI

THE EFFECTS OF NIGHT TEMPERATURE ON GROWTH, FLOWERING, AND SEED MATURITY OF VERNALIZED AND NON-VERNALIZED GREAT LAKES LETTUCE (1954)

Variable Measured		Vernalized					Not Vernalized			(c)
		50°F	65°F	70°F	(a) (b) 50 - 65	(a) (b) 65 - 70	50°F	65°F	70°F	L.S.D (1%)
						(Ave. of 3 Replicates)				
Seedstalk elongation: (cm)										
March 31, 1954			2.0	31.3	1.9	29.6			5.4	4.5
April 29, 1954			90.5	---	81.1	121.1			30.4	13.1
Harvest			117.1	133.9	120.6	130.5			117.4	13.8
Leaves to developing inflorescense	No Seedstalks		34.8	27.6	28.0	28.0	No Seedstalks		42.0	6.1
Days to visible Flower parts			109.0	107.0	109.0	106.0			129.0	14.1
Days to anthesis			135.4	124.3	134.6	123.5			144.1	5.6
Days to seed maturity			157.0	151.2	155.4	151.9			164.2	5.9

(a) Temperature for 20 days after vernalization
(b) Temperature for remainder of the growing period
(c) L.S.D. may be used to compare any pair of measurements

the number of days preceding the visible flower parts. Non-vernalized plants, however, produced visible flower parts three weeks later. A 70° (as compared to a 65°F) temperature hastened anthesis in vernalized plants by 11.1 days. In comparison to those not vernalized, seed maturity of vernalized plants was hastened.

The effects of differential temperatures at progressive developmental stages on flowering of lettuce were determined by growing plants immediatly after vernalization at night temperatures of 50 and 65°F and shifting after 20 days to 65 and 70°F, respectively. Flowering in plants subjected to these temperature patterns, as compared to those grown continuously at 65 and 70°F, was not influenced significantly.

1955 Tests: In a more detailed study initiated December 1954 plants from vernalized and non-vernalized seedlings were grown at night temperatures of 50, 60, 65, and 70°F. Vernalized plants produced no seedstalks when grown at 50°F, 25 per cent seedstalks at 60°F and at 65 and 70°F 100 per cent seedstalks without prior head formation (Table VII and Figure 2). No seedstalks nor flowers developed from non-vernalized plants maintained at 50 or 60°F. As in 1954 (Table VI) fewer leaves preceded the appearance of the developing inflorescence in vernalized plants as compared to those not vernalized. Leaf numbers in vernalized

Figure 2. The Effects of Night Temperature on Seedstalk Development in Great Lakes Lettuce. Left to Right: 70, 65, 60, 50°F. Above: Vernalized. Below: Not Vernalized

TABLE VII

FLOWERING OF GREAT LAKES LETTUCE AS INFLUENCED BY NIGHT TEMPERATURE FOLLOWING VERNALIZATION (1955)

Variable Measured	Not Vernalized				Vernalized				(a) L.S.D.	
	50°F	60°F	65°F	70°F	50°F	60°F	65°F	70°F	(5%)	(1%)
	(Ave. of 4 Replicates)									
Leaves to developing inflorescence	No flowering	--	47	45	No flowering	35	31.3	27.7	2.89	
Days to visible flower parts		--	139	139		139	115	119		8.3
Days to anthesis		187	167	165		167	139	138		9.6
% Seedstalks without prior head formation	0	0	17	33	0	25	100	100		

(a) The L.S.D may be used to compare any pair of measurements.

plants decreased significantly with each 5°F rise in temperature. Plants grown at a 65°F night temperature subsequent to vernalization, although partially covered with black cloth were, nevertheless, exposed to light from an adjacent experiment. It is interesting, therefore, that compared with similar treatments (Table VI) in the 1954 experiment, leaf numbers preceding the visible flower parts and days to anthesis were reduced. At 70°F vernalized plants produced 3.6 fewer leaves than those at 65°F. The effect of 60 as compared to 65°F in delaying flowering despite previous vernalization was indicated by the greater number of leaves and days preceding the appearance of visible flower parts and days to anthesis. Plants not vernalized and grown at 65°F and those vernalized and grown at 60°F flowered simultaneously and approximately the same percentage of plants produced seedstalks without prior head formation.

The influence of cool temperatures suppressing and warm temperatures promoting flowering following vernalization of Great Lakes lettuce seed was further studied by growing some vernalized plants continuously at 50 or 70°F night temperatures while others were maintained at either 50 or 70°F for 66 days and then interchanged. Plants grown continuously at 50°F were strongly vegetative producing heads despite previous vernalization, while at 70°F vernalized plants produced 100 per cent seedstalks. However, only six days

TABLE VIII

FLOWERING OF GREAT LAKES LETTUCE AS AFFECTED BY
50 AND 70°F NIGHT TEMPERATURES FOLLOWING VERNALIZATION

Variable Measured	Not Vernalized				Vernalized				
	50°F	70°F	(a) (b) 50-70°	(a)(b) 70-50°	50°F	70°F	(a) (b) 50-70°	(a) (b) 70-50°	L.S.D.* (1%)
	(Ave. of 4 Replicates)								
Leaves to developing inflorescence	No Seedstalks	45	43	No Seedstalks	No Seedstalks	27.7	34.3	38.1	3.06
Days to visible flower parts		139	135			119	125	147	4.8

(a) Temperature for 66 days
(b) Temperature to flowering
* L.S.D. may be used to compare any pair of measurements.

separated the appearance of the developing inflorescence of vernalized plants grown continuously at 70°F and those held for 66 days at 50°F and then shifted to 70°F (Table VIII). Flowering was greatly delayed when high preceded low night temperature following vernalization of the seed.

Soil Temperature: The influence of soil temperature on flowering of lettuce was studied by growing vernalized (40°F for 20 days) and non-vernalized plants in 2-gallon glazed crocks of soil in controlled temperature tanks (52) maintained at 50, 57, 64, and 71°F (Figure 3). Thus variable temperatures were provided within the same greenhouse air temperature of 70°F. Only at the highest temperature did non-vernalized plants produce seedstalks without prior head formation. Although leaf numbers preceding the developing inflorescence (primordia) of vernalized plants were not affected significantly by soil temperatures, the occurrence of this stage and anthesis of the first flower were accelerated above and delayed below a soil temperature of 64°F. The percent seedstalks produced without prior head formation from vernalized plants decreased as soil temperatures declined from 71 to 50°F (Table IX and Figure 4).

Photoperiod: The separate and interacting effects of photoperiod, night temperature and vernalization on flowering of head lettuce were evaluated in a series of experiments.

Figure 3. The Appearance of the Soil Temperature Experiment Showing the Comparative Differences in Development of Great Lakes Lettuce Growing in the Various Temperature Tanks (A - 71°F; B - 64°F; C - 57°F; D - 50°F)

TABLE IX

EFFECT OF SOIL TEMPERATURE ON FLOWERING OF VERNALIZED GREAT LAKES LETTUCE GROWN AT AN AIR TEMPERATURE OF 70°F

	Soil Temperature				
	71°F	64°F	57°F	50°F	L.S.D. 1%
	(Ave. of 6 Replicates)				
Leaves to developing inflorescence	32	33	34	31	No Significance
Days to visible flower parts	89	93	109	116	6.4
Days to first anthesis	108	112	135	146	13.2
% seedstalks without prior heading	100	83	33	33	

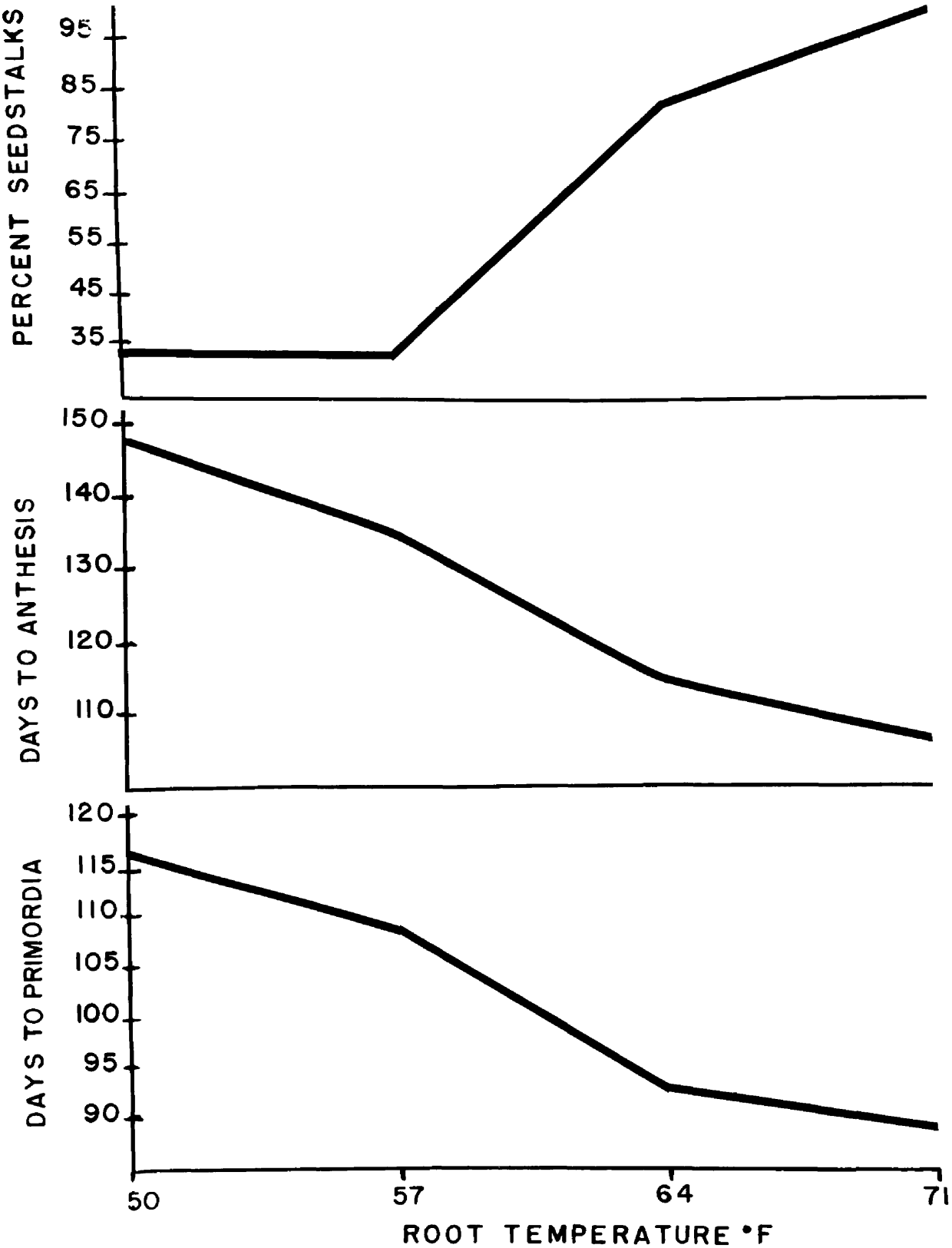


Figure 4. The Influence of Soil Temperature on Days to Primordia and Anthesis, and Per Cent Seedstalks of Vernalized Great Lakes Lettuce Grown at 70°F Night Air Temperature

1954 Tests: Beginning December 29, 1953 lettuce seedlings were exposed to 0, 7, and 24 hours of light during vernalization. On January 18, with seedlings germinated 24 hours, they were transferred to a 50°F greenhouse where half the plants were placed under continuous lighting (10 foot-candles from mazda lamps), and half under normal daylength.

It was observed that vernalized plants, as contrasted to those not vernalized, irrespective of previous light conditions produced a high percentage of seedstalks without prior heading when grown with a continuous light. In contrast, both vernalized and non-vernalized plants grown under natural day-length produced heads.

1955 Tests: Vernalized and non-vernalized seedlings were shifted to two night temperatures (60 and 70°F) and two photoperiods (9 and 16-hours).

The developmental responses are shown in Table X and Figure 5. In non-vernalized plants seedstalk development occurred only under a 16-hour day and a 70°F night temperature. With all other photoperiod and temperature combinations plants not vernalized produced firm vegetative heads. Flowering of vernalized lettuce was markedly promoted by a 16-hour day and 70°F temperature. No seedstalks developed without prior head formation when lettuce, vernalized or not, was grown at a 9-hour photoperiod.

Figure 5. The Effects of Temperature and Photoperiod on Seedstalk Development in Great Lakes Lettuce: A - 70°F, 16-Hours; B - 70°F, 9-Hours; C - 60°F, 16-Hours; D - 60°F, 9-Hours. Above: Vernalized. Below: Not Vernalized

TABLE X

THE EFFECT OF PHOTOPERIOD, NIGHT TEMPERATURE, AND VERNALIZATION ON THE FLOWERING OF GREAT LAKES LETTUCE

Variable Measured	70 F				60 F				(a) L.S.D. (1%)
	9 hours		16 hours		9 hours		16 hours		
	V*	NV*	V	NV	V	NV	V	NV	
	(Ave. of 4 Replicates)								
Leaves to developing inflorescence	–	–	30	56	–	–	32	–	1.3
Days to visible flower parts	–	–	112	138	–	–	126	–	2.0
Days to first anthesis	166	173	135	163	187	188	161	183	11.8
% seedstalks without prior heading	0	0	100	17	0	0	100	0	

(a) L.S.D. may be used to compare any pair of measurements
* V and NV indicate Vernalized and Non-vernalized

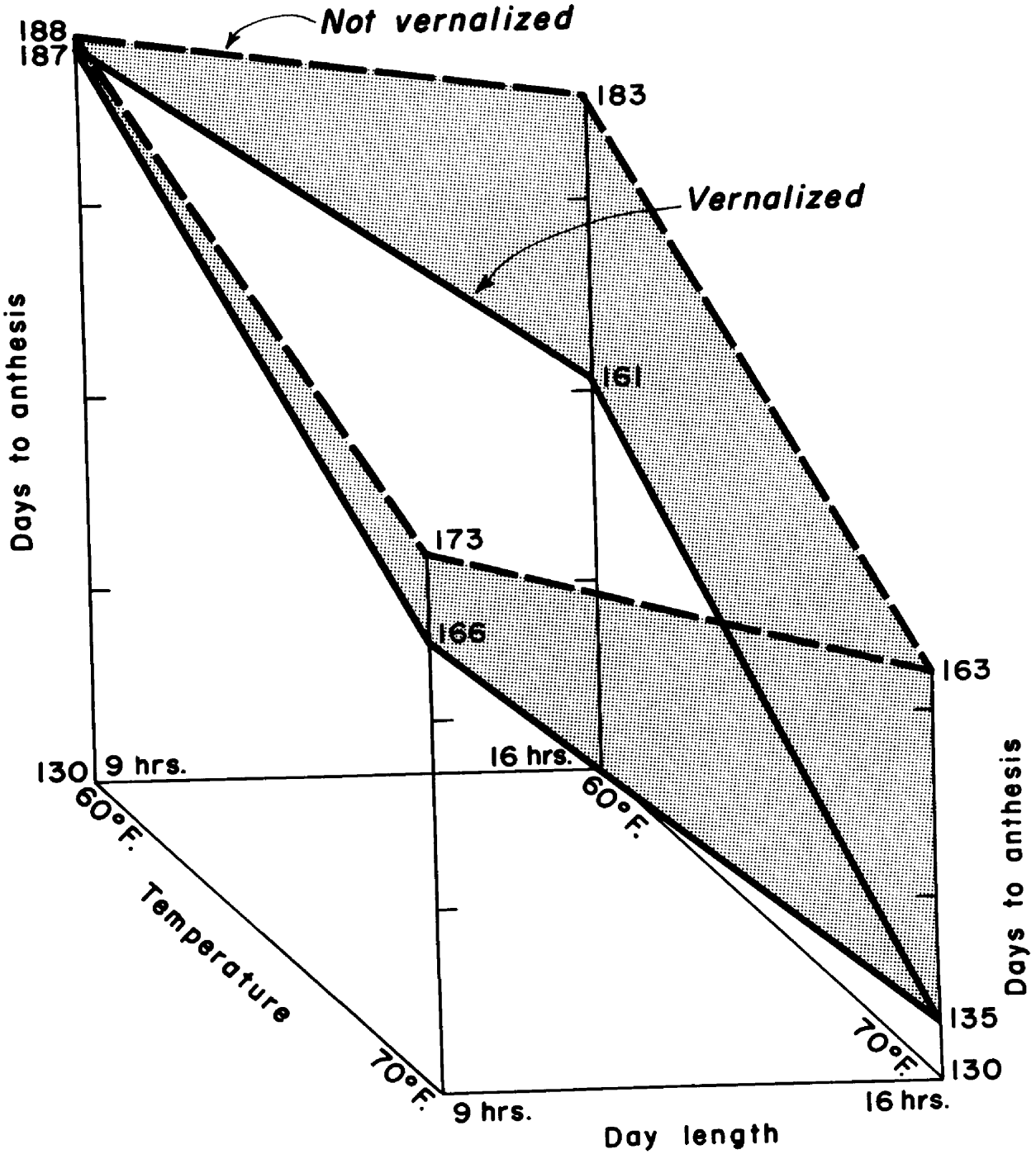


Figure 6. Interaction of Daylength (9 and 16-Hours) and Temperature (60 and 70°F) on the Number of Days to Anthesis (Vertical Axes). Shaded Area Indicate Differences Resulting from Seed Vernalization

Leaves produced prior to flowering of vernalized plants grown at 60 and 70°F with long days varied only slightly. However, at 70°F and a long day, vernalized plants, as compared with those not vernalized, produced 26 fewer leaves preceding the developing inflorescence. The combined effects of long days and high temperatures in promoting flowering were further indicated by the fewer numbers of days preceding visible flower parts and anthesis of the first flower in vernalized as compared to non-vernalized plants. Interestingly, vernalization also accelerated flowering at 60°F without previous head formation under a 16-hour photoperiod, thus nullifying the effect of the cool temperature in preventing seedstalk development. This is in accordance with the results of Andrew (1). Irrespective of temperature vernalized plants and those not vernalized, when grown at a 9-hour photoperiod, initially formed firm heads and then flowered (Table X). The number of days preceding anthesis, however, were not significantly different. A 16-hour day especially favored flowering of vernalized lettuce in that all plants produced seedstalks without prior head formation both at 60 and 70°F although the 60°F temperature delayed flowering and seedstalk development. Figure 6 shows diagramatically the interaction of temperature, photoperiod and vernalization on flowering of Great Lake lettuce.

Field Experiments: To determine whether seed over-

wintered outdoors in Michigan is effectively vernalized and induced to flower without preceding head formation, and to evaluate the effects of outdoor temperatures on flowering of vernalized salad crops, a series of field experiments were designed. In December 1953, seed of Great Lakes and Grand Rapids lettuce, and Full Heart Batavian endive were placed in three-inch pots of soil sunk in a coldframe groundbed. The developing seedlings were transplanted to a field plot May 29, 1954. Other seedlings, not exposed to natural outdoor cold, were started May 8 in a 60^{o} greenhouse and on June 6 were transplanted to a field plot.

Great Lakes lettuce grown from over-wintered transplants formed loose heads and flowered about two weeks earlier than greenhouse-grown plants. Grand Rapids leaf lettuce was not affected by over-wintering the seed in the coldframe. However, a remarkable sensitivity to cold-exposure was exhibited by endive. Overwintered plants produced seedstalks directly and flowered two months earlier than greenhouse-grown transplants which first produced a characteristic rosette, (Figure 7). Thermograph records indicated that night temperatures remained below 60^{o}F during the heading period.

To further evaluate the effects on flowering of over-wintering seed and seedlings of salad crops, a field plot was designed on August 1, 1954 as a randomized block consist-

Figure 7. Effect of Vernalization on Flowering in Endive (Variety Full Heart Batavian). (Left) A Flowering Coldframe-grown Plant, Seeded December 29, 1953. (Right) A Vegetative Plant, Started in the Greenhouse April 12, 1954. Photographed August 11, 1954

ing of three replicates. Four rows of oats were seeded August 5 between each of the plots for the purpose of holding the winter snow cover. Eight salad crop varieties were seeded monthly in each plot from August 1 to December 1, 1954, and on April 2, 1955. For control comparisons seedlings of the same crops were vernalized in the usual manner and grown in a 60°F greenhouse to the three-leaf stage, when they were shifted to a cold-frame (May 14). A week later they were transplanted to the field simultaneously with non-vernalized plants. The following varieties with their corresponding stock numbers and sources were utilized in the experiment:

Variety	Stock Number	Source
Great Lakes	11447	Ferry Morse Seed Company, Detroit, Michigan
Cornell 456	15329	do
Bibb	15490	do
Grand Rapids	15057	do
Pennlake	15043	do
Full Heart Batavian (Endive)	15540	do
No. 407	352-3-2	Dessert Seed Company, El Centro, California
Great Lakes	535504	Rohnert Seed Company, Gilroy, California

Without exception, irrespective of planting date or ver-

nalization under controlled conditions (40°F for 20 days), the head lettuce varieties produced firm marketable heads and later weak seedstalks. Flowering in the leaf lettuce varieties was not significantly accelerated by overwintering or vernalization at 40°F. In results comparable to those of the previous year (Figure 7) endive plants from overwintered seed, from seed sown April 2, or from seedlings vernalized at 40°F, flowered from June 27 to July 10. In contrast, non-vernalized transplants or seed sown after May 1 in the field produced large vegetative heads and did not flower until September 1. Records showed that average night temperatures remained below 60°F during the heading period.

III. BIOCHEMICAL ASPECTS OF LETTUCE SEED VERNALIZATION

The purpose of this study was the possible isolation and characterization of indole compounds exhibiting biological activity from vernalized and non-vernalized seedlings. Essentially the same techniques as described by Nitsch (44) for the isolation of free auxin from strawberries were utilized.

First Seed Lot:

Vernalizing Seed: Several one hundred gram lots of lettuce seed were spread evenly over moistened filter paper placed in plastic serving trays on May 13, 1955. An additional layer of wet filter paper was spread over the seed and the covered trays of seed were exposed to 60°F for 48 hours prior to storage at 40°F for 20 days. On May 29 additional trays of seed were prepared and maintained at 60°F. Four days later half the seed were frozen at -5°C.

Extraction of Fresh Seedlings: The fresh seedlings were extracted immediately by grinding with sand in the presence of peroxide-free ether for two to four hours in the dark in a water bath maintained at 0 to 10°C. Extracts of the vernalized and non-vernalized seedlings were decanted and concentrated in an atmosphere of nitrogen to prevent

oxidation of possible indole compounds. These concentrates were then made up to a volume of 50 milliliters with ethanol. The subsequent concentration, separation and chromatographic procedures utilized were as follows:

Extract 1: Ten milliliter aliquots of the 50 milliliter ethanol extracts were concentrated on a steam bath to one milliliter and chromatographed as Extract 1 (Table XI).

Extract 2: Although seedlings were grown in the dark and little or no chlorophyll was extracted a water soluble pigment, probably a carotene, was obtained which often caused streaking on the filter paper. To separate pigments from the desired compounds, portions of Extract 1 were spotted heavily on filter paper sheets which were then developed with water. The pigmented spots were then cut off and the remainder of the sheets extracted with ether for 10 hours. These ether extracts were concentrated to 1 milliliter and chromatographed (44).

Extraction of Frozen Seedlings: The seedlings placed at -5°C were next thawed and extracted with peroxide-free ether and sand in the manner previously described for the extraction of fresh seedlings. These ether extracts were treated as follows to provide Extracts 3 to 8 (Table XI):

Extract 3: Portions of the original ether extracts from frozen seedlings were chromatographed.

Extract 4: The remainder of the original ether extracts were concentrated to 50 milliliters and chromatographed.

Extract 5: Ten milliliters of Extract 4 were concentrated to 1 milliliter and chromatographed.

Extract 6: Another 10 milliliter aliquot from Extract 4 was spotted heavily on filter paper, developed with water, dried and the pigmented spots cut off. The remaining portion of the paper was then extracted 10 hours with ether. The resulting extracts were concentrated and chromatographed.

Extract 7: Pigments in Extract 5 were separated in the manner described for Extract 6. The resulting ether extracts were concentrated and chromatographed.

Extract 8: Twenty milliliters of Extract 3 were shaken with sodium sulfate to remove water and to salt-out adsorbed compounds. The clear solutions were concentrated and chromatographed.

Chromatographic Techniques: Standard ascending and descending chromatographic techniques were used for the separation of indole compounds. Seed extracts, together with Indoleacetic Acid and Tryptamine at known concentrations, were spotted on filter paper sheets, air dried and developed. The developing agents used were those described by Nitsch (44) and included (a) Isopropanol - 28 per cent ammonia water - water, (8:1:1 V/V), and (b) water. Salkowski's ($FeCl_3$) and

Ehrlich's (p-Dimethylaminobenzaldehyde or PBZ) reagents, and a 2537 A ultra-violet light source were used as detecting agents after the filter paper was air dried. Relatively few colored spots were obtained from ether extracts of vernalized lettuce seedlings perhaps indicating (1) an extremely low concentration of indole compounds in the seedlings, or (2) too low a concentration of indole compounds in the extracts for detection by the methods employed.

R_f values of the IAA and Tryptamine controls approximated 0.36 and 0.73 when developed with Isopropanol-ammonia water-water and 0.23 and 0.86, respectively when developed with water. Possible naturally occurring indole compounds are indicated in Table XI. Colored spots were found at R_f 0.63 on chromatograms of Extract 4 of vernalized seedlings developed with isopropanol-ammonia water-water (detected with Salkowski's reagent), and at 0.83 on chromatograms of Extracts 6 and 7 (vernalized seedlings) developed with water and detected with PBZ. All other R_f values were obtained with ultra-violet light. In Extract 1, while no R_f values were obtained by chromatographing ether extracts of vernalized seedlings, some comparable to those of 3-Indoleacetic Acid, and Tryptophan occurred consistently (ultra-violet light) in the non-vernalized seedling extract developed both with isopropanol-ammonia water-water and with water (Table XI). R_f values approximating those for Indoleacetonitrile and the

Ethyl Ester of Indoleacetic Acid were also found on chromatograms of some extracts. Significantly, several of the extracts of non-vernalized seedlings developed with Isopropanol-ammonia water-water provided R_f values around 0.52, at which no naturally occurring indole compounds have been detected insofar as the author is aware. Only in Extract 6 was an R_f of 0.52 obtained on a chromatogram from a vernalized seedling extract.

Second Seed Lot:

A further study of indole compounds was initiated utilizing a new lot of vernalized and non-vernalized seed.

Vernalizing Seed: The paucity of colored spots on chromatograms in the previous study suggested that a higher concentration of indole compounds in the original extract was necessary. Therefore, additional trays of seedlings were vernalized. To circumvent the lack of uniform germination obtained previously, only 75 grams of lettuce seed were germinated per tray. A layer of wax paper was placed between the tray and the filter paper to facilitate removal of the seedlings. Vernalization was started June 23 and control seedlings were germinated July 12. All seedlings were frozen at $-5^{o}C$ on July 15. About three kilograms of seedlings (fresh weight) were obtained after thawing.

Extraction of Frozen Seedlings: Methods similar to

TABLE XI

R_f VALUES OF POSSIBLE NATURALLY OCCURRING INDOLE COMPOUNDS SEPARATED BY PAPER CHROMATOGRAPHY FROM ETHER EXTRACTS OF VERNALIZED AND NON-VERNALIZED GREAT LAKES LETTUCE SEEDLINGS (DETECTION BY ULTRA-VIOLET LIGHT)

Extract No.	Separation Procedure (First Seed Lot)	Seed Treatment	Developer: Isopropanol-ammonia water-water (8:1:1 V/V) (R_f Values)	Developer: water (R_f Values)	Likely Indole Compound
1	Ether extraction of Fresh Seedlings: 1 ml. ethanol extr. from original ether extr.	V*	None	None	--
		NV*	0.24 0.34	0.56 0.85	Tryp.** IAA
2	Ether extr. of Extr. 1 pigments removed.	V	None	None	--
		NV	0.50 0.55	None	?
3	Ether extr. of Frozen seedlings: Original extr.	V	None	0.52	Tryp.
		NV	0.49 to 0.56	None	?
4	Extr. 3 conc. to 50mls.	V	0.34 0.63+	do	IAA
		NV	None	do	
5	10 ml. aliquots of Extr. 4 conc. to 1 ml.	V	0.34	0.90	IAA
		NV	0.28	0.58	Tryp.
6	10 ml. aliquots of Extr. 4, pigments removed	V	0.52 0.90	0.63++	? IAA
		NV	None	None	--
7	Extr. 5, pigments removed	V	do	0.83+++	IAA
		NV	do	do	do

TABLE XI (Continued)

Extract No.	Separation Procedure (First Seed Lot)	Seed Treatment	Developer: Isopropanol-ammonia water-water (8:1:1 V/V) (R_f Values)	Developer: water (R_f Values)	Likely Indole Compound
8	20 ml. aliquots of Extr. 4 dried with Na_2SO_4 and conc.	V	0.77	None	IAN
			0.80		ETIAA
		NV	0.37	0.91	IAA
			0.52		?

*V and NV indicate Vernalized and Non-vernalized

**Tryp., IAA, IAN and ETIAA are tryptophan, 3-Indoleacetic Acid, 3-Indoleacetonitrile, and Ethyl Ester of Indoleacetic Acid, respectively.

+Brown - Detected with Salkowski's Reagent

++Purple - Detected with Ehrlich's Reagent

+++Gray - Detected with Ehrlich's Reagent

those previously described were utilized in the extraction of indole compounds from the second lot of seedlings. The following concentrates and separations were made:

Extract 1: Portions of the original ether extracts were chromatographed (Table XII).

Extract 2: The remainder of the original extracts were next concentrated and made up to volumes of 100 milliliters with water and chromatographed.

Extract 3: The water layer from Extract 2 was separated, acidified to pH 2.85 (59), re-extracted with ether, and the ether extract concentrated to 1 milliliter. This was chromatographed.

Extract 4: Extract 3 was next spotted on filter paper sheets which were then developed with water. The pigmented spots were cut off and the remaining portions of the sheets extracted with ether. These ether extracts were concentrated and chromatographed.

Extract 5: Another method for the removal of pigments suggested by Nitsch (44) was employed. Ten milliliter aliquots of Extract 2 were extracted 24 hours by liquid:liquid partition with hexane to remove lipids and pigments and with acetonitrile to dissolve auxins. The hexane fraction was then concentrated and chromatographed.

Extract 6: The acetonitrile fraction was heated to dryness on a steam bath and the residue dissolved in a small quantity

TABLE XII

R_f VALUES OF POSSIBLE NATURALLY OCCURRING INDOLE COMPOUNDS SEPARATED BY PAPER CHROMATOGRAPHY FROM ETHER EXTRACTS OF VERNALIZED AND NON-VERNALIZED GREAT LAKES LETTUCE SEEDLINGS (DETECTION BY ULTRA-VIOLET LIGHT)

Extract No.	Separation Procedure (Second Seed Lot)	Seed Treatment	Rf*** Values	Likely Compound
1	Original ether extracts of frozen seedlings	V**	None	--
		NV**	do	--
2	Extr. 1 conc. and made to a volume of 100 mls. with water	V	0.23	?
			0.42	IAN*
		NV	0.24	?
			0.57	Tryp.
			0.64	IAA
3	Water layer from Extr. 2 separated, acidified to pH 2.85, extr. with ether. Ether extr. conc. to 1 ml.	V	0.26++	?
			0.39	IAN
			0.48 to 0.58	ETIAA to Tryp.
		NV	0.25	?
			0.39	IAN
			0.51 to 0.63	Tryp. or ETIAA
4	Extr. 3, pigments removed	V	0.78++	?
			0.89	IAA
		NV	0.78++	?
5	Extr. 2, pigments removed by liquid:liquid partition Hexane fraction	V	0.55	Trp.
		NV	None	--
6	Extr. 2, pigments removed by liquid:liquid partition Acetonitrile fraction:	V	0.37	IAN
			0.52	Tryp.
		NV	0.23	?
			0.42	IAN
			0.57	ETIAA

*IAN, Tryp. IAA, ETIAA are 3-Indoleacetonitrile, Tryptophan, 3-Indoleacetic Acid, and Ethyl Ester of Indoleacetic Acid, respectively.
**V and NV indicate Vernalized and Non-Vernalized
***Developed with water
++Blue - detected with Ehrlich's Reagent

of ether. These ether solutions were then chromatographed.

Chromatographic techniques were identical with those already described. Values from extracts of lettuce seedlings comparable to compounds occurring naturally are indicated in Table XII. With water as developer colored spots appeared at R_f values of 0.26 in Extract 3 (vernalized seedlings), and at 0.77 and 0.78 of Extract 4, both from vernalized and non-vernalized seedling extracts. R_f values possibly indicative of Indoleacetonitrile, Tryptophan, Indoleacetic Acid, and Ethyl Ester of Indoleacetic Acid were obtained. However, no specific differences in biochemical substances resulting from vernalization were discerned.

Determination of L-Tryptophan:

Vernalizing Seed: Four one-hundred gram lots of lettuce seed were spread evenly on layers of moist filter paper in two large stainless steel trays. These were held at 60°F for 48 hours and then shifted to a 40°F temperature for 18 days. On July 4 two additional trays of seed were prepared and held at 60°F until July 7 when all seedlings were frozen at -5°C.

Extraction and Determination of Free L-Tryptophan: According to the method described by Nitsch (44) free tryptophan in lettuce seedlings was determined quantitatively by precipitating proteins with boiling ethanol for three minutes, evaporating the alcohol on a steam bath, extracting

tryptophan twice with hot water, and shaking the aqueous extract with ether to purify it of indole and anthranilic acid which give positive tests with the bioassay. The pH of the aqueous extract was adjusted to 6.0. Tryptophan was measured by employing Lactobacillus arabinosis[1] as a bioassay.

Quantitative determination of the free tryptophan in the water extracts revealed that vernalized seedlings yielded .00127 per cent and non-vernalized seedlings .00293 per cent L-tryptophan on a fresh weight basis.

---

[1]The author wishes to thank Dr. Erwin J. Benne and Dr. Richard W. Luecke, Department of Agricultural Chemistry, Michigan State University, for determining total nitrogen (necessary to establish standard curves for the bioassy), and for performing the bioassay, respectively.

DISCUSSION

ENVIRONMENTAL FACTORS AFFECTING FLOWERING OF LETTUCE

Indices of Flowering: The number of leaves or nodes preceding the developing inflorescence is recognized as a specific index of flowering (23, 34, 36, 37, 40, 47, 80, 81). It is especially useful in vernalization studies with lettuce because it is associated with floral initiation in response to various environmental conditions. The value of a "physiological index" like leaf numbers is best demonstrated in the growth regulator studies (Table V) and in the 1954 and 1955 night temperature studies (Tables VI and VII). The application of Maleic Hydrazide to seedlings during vernalization inhibited vegetative growth during early plant development at 70°F and resulted in a significant delay in days to flowering. However, the fact that leaf numbers were reduced preceding a distinct developmental stage (e.g. the developing inflorescence) indicates a specific acceleration of flowering by the chemicals. In Tables VI and VII, while 27.6 and 34.8 leaves preceded the appearance of the developing inflorescence at 70 and 65°F, respectively, 109 and 107 days preceded the same stage. The acceleration of flowering at the higher temperature, as indicated by leaf numbers, is not supported by the number of

days to the appearance of the visible flower parts. Vernalized plants grown at 70°F under similar conditions in 1954 and 1955 produced 27.6 and 27.7 leaves during the two years, respectively. In contrast, plants at 65°F in 1955 produced 3.5 fewer leaves than those grown in 1954. It is suggested that the difference probably resulted from additional light received by those plants grown in the 1955 experiments. Further evidence for the reduction in leaf numbers preceding the developing inflorescence (vernalized plants) by extended photoperiod occurs in Table X. With a 16-hour day a difference of only two leaves preceded the developing inflorescence of plants grown at 60 and 70°F night temperatures.

Leaf numbers of plants grown at the same temperature varied between experiments. This was probably due to differences in season of planting (naturally occurring photoperiods), the effects of additional lighting, and even growing plants in different pot sizes or in a ground-bed. Despite the variation between experiments similar imposed factors, such as temperature, increased or decreased leaf numbers relatively within each experiment.

In the 1954 night temperature experiment (Table VI) seedstalk elongation was utilized as an index of flowering (1). Although the initial effects of temperature on seedstalk development were clearly indicative, as growth progressed the

differences between vernalized and non-vernalized plants decreased until, finally, they no longer existed. Andrew (1) in comparable observations, found that the effects of chemical growth regulators on elongation and branching of the inflorescence were not indicated by measurements of seedstalk heights. Except where growth regulators delayed plant development, coupled with such complementary indices as the production of seedstalks without previous head formation and the number of days preceding visible flower parts and flowering, leaf numbers serve as a reliable index of reproductive expression.

The results suggest that the major factors affecting flowering of vernalized lettuce are temperature and photoperiod. A discussion of these factors, therefore, may explain many of the negative results obtained in the present as well as in other studies.

Night Temperature: Flowering in a variety of crops (15, 22, 23, 24, 33, 35, 36, 39, 43, 47, 51, 57, 68, 72, 73) has been hastened by cold-exposure of seedlings or young plants. The results of the night temperature experiment indicate a critical thermoperiodic specificity for flowering in lettuce grown from vernalized seedlings. The acceleration of flowering in lettuce with high, and the delay with low night temperatures following vernalization is both of fundamental and practical interest. Under greenhouse tempera-

tures favorable for normal vegetative development (50 to 60°F), regardless of vernalization, lettuce plants formed firm heads. In contrast, with high night temperatures seedstalk development was promoted in vernalized lettuce plants. In the 1954 night temperature experiment no differences in flowering were found in vernalized plants grown at 65 and 70°F after transfer from 50 and 65°F, respectively, suggesting that temperature conditions during early plant growth do not affect subsequent flower expression of vernalized lettuce plants.

To determine the specific effects of cool and warm temperatures in delaying and accelerating flowering, vernalized and non-vernalized plants were grown at 50 and 70°F for 66 days and then interchanged. Exposure to 70°F night temperature induced seedstalks following 66 days at 50°F while flowering was delayed when warm preceded cool temperatures (Table VIII). In comparison to non-vernalized plants shifted to 50°F from 70°F, vernalized plants produced seedstalks directly. Seemingly, in lettuce the potential for flowering is greater following vernalization, but expression is delayed by subsequent 50°F night temperatures.

Photoperiod: Photoperiod, separately and interacting with night temperature, markedly affected flowering in vernalized head lettuce. A 16-hour day promoted and a 9-hour day delayed seedstalk development without prior heading

irrespective of 60 or 70°F night temperature. Although plants grown with a short day flowered after initially forming firm heads, the number of days preceding anthesis of vernalized, as compared to non-vernalized plants, were not significantly different.

There are several reports (1, 2, 4, 7, 8, 53, 68, 70), many of them contradictory, on the effects of photoperiod and temperature on flowering of lettuce.

In a brief note on photoperiod and temperature studies with vernalized lettuce, Milthorpe and Horowitz (42) reported that high temperatures and long days promoted flowering. However, this report included no experimental data nor did it describe the treatments used. Andrew (1), using 9 and 15-hour photoperiods and 50 and 60°F night temperatures found that long days and high temperatures affected early seedstalk development. He obtained accelerated flowering, however, under conditions of 50°F and short days and concluded that "neither factor - (temperature or photoperiod)- appeared to be the limiting or controlling influence in determining vegetative or reproductive development". It was observed in the present study that with 50°F night temperatures and continuous 24-hour photoperiod, seedstalks in vernalized, as compared to non-vernalized plants, were induced. Analysis of Andrew's methods indicated that the experimental plants were "pricked out" March 1, and potted April 8.

Measurements of seedstalks were recorded from June 4 to July 14. Thermograph records in 1954 and 1955 indicated that although 60°F night temperatures could be maintained until mid-June, after March 1 maintenance of 50°F night temperatures within the greenhouse was not possible.

As indicated by leaf numbers preceding the developing inflorescence, and days to anthesis (Table IX), only with long days were 60°F temperatures effective in promoting seedstalk development. With short days lettuce plants at 60 and 70°F formed firm vegetative heads irrespective of vernalization. To determine the specific effects of short days on flowering of lettuce, heads of vernalized plants grown at 70°F and a nine hour day were cut open and forty-five leaves were found to precede the developing inflorescence of the enclosed seedstalks. This indicates that short days actually delay expression of flowering of vernalized plants grown at high temperatures. That vernalized and non-vernalized lettuce plants flowered simultaneously when grown with a short day and a cool temperature suggests that the effects of vernalization were nullified (Figure 6).

The critical temperature and photoperiod requirements subsequent to chilling of lettuce seed for acceleration of flowering in the greenhouse, were verified in field studies. Overwintered seed and seedlings and vernalized and non-vernalized transplants placed in the field in the early Spring

all formed marketable heads. Thermograph records revealed that average night temperatures in the vicinity of the experimental plots remained below 60°F.

The remarkable sensitivity to cool temperatures of Full Heart Batavian endive seed is of economic significance since vernalized endive plants flowered two months earlier than those not vernalized. It would behoove a market grower therefore, to delay planting endive at least until May 1 to prevent cold-induction. On the other hand, by early transplanting or direct seeding, or late transplanting of vernalized plants, endive seed, normally produced in the West, could perhaps be produced in Michigan.

Soil Temperature: It has been reported that the vernalization stimulus is perceived by the embryo (25, 26) and likely the apical meristem (48). A change from the vegetative to the reproductive condition in the apical meristem, after chilling Matthiola plants four days, was reported by Emsweller and Borthwick (15). It is especially interesting, therefore, that subsequent to vernalization different air temperatures significantly altered the number of leaves preceding the developing inflorescence, but that leaf numbers were not affected by root (soil) temperature. This may support the view that the apical meristem is the site of perception of the vernalization stimulus.

Since leaf numbers were not influenced by root

temperature (e.g. "physiological" as contrasted to "chronological" reproduction was not accelerated) high soil temperatures seemingly affect the rate of seedstalk development rather than the response to vernalization *per se*.

THE VERNALIZATION PROCESS

The previous discussion of environmental factors affecting flowering of lettuce contains the information necessary for evaluation of the experiments concerned with the vernalization process.

Duration of Chilling: The duration of cold exposure necessary for acceleration of flowering in lettuce has been previously investigated (1, 24, 33, 61). Knott (33) found that 10 to 20 days at 40°F was sufficient to accelerate seedstalk development; the longer period was more effective. Simpson (61) and Andrew (1) concluded that 16 days chilling effectively vernalized the varieties Ideal, Imperial 847, Great Lakes, and Slobolt. Exposure at 40°F for 28, 42 and 56 days was shown by Gray (24) to promote flowering in lettuce equally although the longer periods of chilling weakened the seedlings. The present results indicated that a minimum of 13 days of vernalization is necessary for Great Lakes lettuce.

Age of the plant: Another factor influencing the response of lettuce to cold exposure is the age of the plant

or the stage of development. Reimers (79) found that lettuce exposed to temperatures of 2 to 5°C at the first leaf, in contrast to germinating seeds, flowered later. With seed germinated 24 and 72 hours prior to vernalization Warne (76) found that seed with radicles emerged (72 hours) was not receptive to vernalization. Andrew's results (1) were generally in accord with those of Reimers. He concluded that there was no appreciable difference between cold exposure of seed at the swollen stage and after the radicle emerged. Andrew further reported that seedlings two to three centimeters in height were set back, in comparison to smaller seedlings, by subsequent cold temperatures. Such seedlings, contrary to the results of Reimers (79) and Warne (76), produced plants which flowered earlier than non-vernalized plants.

In the present experiment, plants germinated 72 hours or more prior to cold exposure were delayed in flowering as indicated by leaf numbers prior to the appearance of the developing inflorescence. It is interesting that the number of days to anthesis tended to decrease in relation to a progressive increase in days of germination prior to chilling. Lacking in the experimental design were treatments with plants germinated 0, 24, and 48 hours. However, subsequent observations in the present study indicated that even seed germinated 48 hours were effectively vernalized.

The acceleration of flowering by vernalization without presoaking seed, described by Andrew (1), was also observed in this study.

Temperature Patterns During Vernalization: Discussing the results of Thompson and Kosar (69), Andrew (1) suggested that the lack of response of Slobolt and Cos lettuce may have resulted from (a) exposure of the seed to too cold a temperature (1 to 2°C), (b) exposure of the control seed to low temperatures for a "few hours" which may have had a slight vernalizing effect. The fact that chilling seed at 32°F did not stimulate flowering lends credence to Andrew's suggestion (a), above. The apparent lack of information concerning the temperature factors during vernalization which affect flowering suggested a series of detailed temperature studies. As indicated in the results, irrespective of 35, 40, or 45°F temperature patterns during vernalization, the plants formed firm heads when grown at 50°F temperatures. Since at this temperature no seedstalks developed the delaying effects on flowering of 50°F temperatures are further emphasized. The effects of high temperature interruptions of the chilling period are shown in Table III. In comparison with those plants vernalized in the usual manner or interrupted during vernalization, seed germinated at 60 or 90°F and grown subsequently at 65°F night temperature produced plants which were delayed in flowering. Lack of uniformity resulting from root

rots may have been responsible for the relatively small difference in days to anthesis between vernalized and non-vernalized plants. The results of this study suggest that a critical time period may exist in lettuce seed vernalization during which cold-exposure is effective in accelerating flowering. The detection of such a period would materially aid investigators in the biochemical isolation of substances associated with the vernalization stimulus.

Moisture During Vernalization: In the vernalization of rye (47) and mustard (57), prior to cold-exposure, seed was pre-soaked sufficiently to cause swelling but not emergence of the radicle. Mustard seed vernalized in this manner was then dried and stored as long as six years without loss of the accelerating stimulus. Since lettuce seed germinated at 40°F produces radicles it was necessary to modify the pre-soaking and drying techniques utilized. The results indicate that seedstalk development was accelerated only from seedlings pre-germinated 24 hours and vernalized in the usual manner. Soaking 6 and 12 hours and drying before exposure to 40°F, or soaking 24 hours at 70°F prior to chilling at 32°F did not accelerate flowering. Additional studies aimed toward obtaining non-sprouted vernalized seed are indicated. Perhaps pre-soaking for 12 hours and maintaining seed at 40°F in a high atmospheric humidity may permit satisfactory vernalization without the emergence of radicles.

Light Quality and Duration: The effects of light quality on lettuce seed germination have been studied for many years (5, 6, 17, 38). In the present study the delay with light from 100 watt mazda lamps at 40°F offers an interesting cool temperature/light interaction worthy of further study.

In 1951 Vogt (75) reported the destruction of 3-Indoleacetic Acid in the roots of cereal crops with ultra-violet light. Terpstra (65) obtained comparable results with oat coleoptile tips. The effects of ultra-violet light on seedlings before and during chilling were studied, therefore to associate indirectly the effects of auxins and vernalization on subsequent floral initiation. The plants from irradiated seedlings were transferred to the field August 16. Possibly because of cool night temperatures and decreasing daylengths during September and October, seedstalk development was prevented and only firm heads were formed.

The recognized influence of photoperiod on plants suggested that duration of light during germination might also affect flowering. When plants were subjected to different daylengths during vernalization, however, at 50°F only heads were formed. This is further evidence for the predominant effects of night temperature on flowering irrespective of treatment during vernalization.

Chemical Growth Regulators: Growth regulator appli-

cations during and subsequent to seed germination and vernalization have influenced flowering in lettuce (1, 12, 13, 14, 79). A series of experiments were performed to evaluate the effects of Maleic Hydrazide and 2,4-Dichlorophenoxyacetic Acid and of various other growth regulators on flowering in lettuce. Again, plants grown either at 50°F or in field plots when night temperatures were conducive to head formation, remained vegetative. The primary effectiveness of high night temperature in accelerating flowering of vernalized lettuce was displayed in a study of the effect of 60 and 70°F night temperature on plants from seedlings vernalized together with 20 or 40 ppm of MH. Only those plants grown at 70°F produced seedstalks without head formation irrespective of growth regulator treatment. Only at the higher temperature was MH effective in reducing leaf numbers preceding the developing inflorescence.

Andrew (1) concluded that differences in flowering response to the same growth regulators could be attributed to one or more factors: (a) concentration, (b) the "morphological age" of the treated plants, (c), the number of days from seeding. However, "the factor or factors affecting the degree and type of influence of growth regulators on the seedstalk development of lettuce is as yet uncertain". The results of the present investigation suggest that environment, subsequent to seed treatment or growth regulator application

to plants, is a primary factor which must also be considered.

In relation to the results of Franklin (18), Andrew (1), and Clark and Wittwer (13) it is interesting that Great Lakes and Cornell 456 plants in a field experiment with growth regulator application during and/or subsequent to vernalization formed firm vegetative heads when transplanted May 21, 1954. Apparently these factors were not effective in overcoming the influence of cool night temperatures preventing flowering of lettuce. In a recent communication Franklin (18) indicated that the stage of development for spraying was so critical that only during a delimited period of growth, measured in heat units, were 2,4-D sprays effective in accelerating seedstalk development in lettuce at Parma, Idaho.

It appears that the factors which normally stimulate flowering in lettuce are even more effective when operative in combination with vernalization. Seemingly, a combination of high night temperatures, high root temperatures, and long days contribute to acceleration of flowering and seedstalk development in head lettuce grown from vernalized seedlings.

BIOCHEMICAL STUDIES

Using paper partition chromatography for the separation of naturally occurring indole compounds in ether extracts of vernalized and non-vernalized lettuce seedlings, many spots which fluoresced in ultra-violet were detected. Several approximated those for compounds known to occur in plants. However, no single R_f value was found peculiar to chromatograms of either vernalized or non-vernalized lettuce seed extracts (Tables XI and XII). This may indicate that none or all of the compounds obtained are associated with the stimulative process. As suggested by the appearance of only a few colored spots with Salkowski's and Ehrlich's reagents on chromatograms of the extracts from lettuce seedlings, the concentration of indole compounds may have fallen below the range which is detectable by the chromatographic technique, thus preventing characterization of such compounds occurring in extremely minute quantities. Although many R_f values comparable to those of indole compounds occurring in plants were obtained by chromatographing seedling extracts on filter paper, it is possible that many resulted from lipids or were artifacts. This suggests the need for a bioassay and/or quantitative chemical analysis in conjunction with chromatographic separations to ascertain and relate specific biological activity to biochemical differences induced by vernalization

of lettuce seed. The significantly low percentage of L-tryptophan in vernalized, as compared to non-vernalized seed suggests direct utilization of L-tryptophan or conversion to another substance, conceivably 3-Indoleacetic Acid during vernalization, or may be indicative of other differences likely to occur in the auxin complex in the seedlings as a result of vernalization.

SUMMARY AND CONCLUSIONS

Seed vernalization, plant growing temperatures, daylength, and chemical growth substances, separately and interacting, markedly influenced flowering and seedstalk development in head lettuce as indicated by leaf numbers and days preceding the inflorescence and days to anthesis.

A minimum of 13 days at 40°F was required for vernalizing moist lettuce seed pre-germinated less than three days at 60 to 70°F. In comparison to seed vernalized in the usual manner, soaking with water for 6 or 12 hours, drying, then holding at 32 or 40°F was not effective. High temperature (60 or 90°F) interruptions of vernalization for two hours daily or at intervals of three days did not prevent subsequent acceleration of flowering.

Seed vernalization together with 20 or 40 ppm of Maleic Hydrazide resulted in plants which flowered after the appearance of fewer leaves (nodes) than those from seed vernalized with water alone.

Night temperatures above 65°F subsequent to seed vernalization accelerated flowering and resulted in seedstalks without preceding head formation. Below 65°F vernalized plants first produced a high percentage of firm, vegetative heads and then flowered. Non-vernalized plants flowered only at night temperatures above 65°F. Earlier flow-

ering resulted in plants exposed continuously to warm temperatures as compared to alternating cool (50°F) and warm (70°F) night temperatures. Apparently, the potential for flowering is greater following vernalization but a critical temperature for plant growth exists for reproductive expression.

Photoperiod, separately and interacting with night temperature, markedly affected flowering in vernalized head lettuce. The minimum night temperature following vernalization at which early flowering was favored was reduced from 65 to 60°F when plants were grown at a daylength of 16 hours. At 60°F with a 9-hour photoperiod, both vernalized and non-vernalized plants produced firm heads which flowered simultaneously, suggesting that this combination of environmental factors prevents expression of the seed vernalization stimulus.

Root (soil) temperatures influenced the rate of seedstalk development (measured as days to anthesis) rather than the expression of vernalization (leaf numbers preceding the appearance of the developing inflorescence). This supports the supposition that the apical meristem perceives the vernalization stimulus. At high soil temperatures (64 and 70°F) flowering in days was promoted, while at low temperatures (50 and 57°F) flowering was delayed.

Field studies with vernalized lettuce verified the controlling effects on flowering of photoperiod and tempera-

ture critically demonstrated with plants grown in the greenhouse. For seed production the practical utilization of long days, high night temperature, and high root temperature which accelerate seedstalk development in vernalized lettuce lies in the selection of appropriate planting dates or manipulation of the factors in the greenhouse for increasing breeding stock during the Winter. Conversely, head lettuce for market should be produced when night temperatures average below 60°F.

By early Spring transplanting or direct seeding, or late transplanting of vernalized Full Heart Batavian endive plants, flowering was accelerated by two months. This suggests that endive for market should be planted after May 1, but for seed production in Michigan, direct seeding in early April is desirable.

In biochemical studies, with extracts of both vernalized and non-vernalized seed, a series of fluorescent spots (ultraviolet light) and occasionally colored spots (normal light were separated by paper partition chromatography. Such extracts repeatedly yielded fluorescent spots comparable to those of 3-Indoleacetic Acid, Tryptophan, Indoleacetonitrile, and Ethyl Ester of Indoleacetic Acid in both water and isopropanol-ammonia water (8:1:1 V/V) and water systems. Fluorescent spots also occurred on chromatograms of both vernalized and non-vernalized seed extracts at an R_f value of 0.55, where no biologically

active substances have been reported insofar as the author is aware. A lower percentage of L-tryptophan was found in vernalized, as compared to non-vernalized seedlings, suggesting that during seed vernalization L-tryptophan is either utilized directly or converted to another compound, conceivable 3-Indoleacetic Acid.

LITERATURE CITED

1. Andrew, W. T. 1953. Seedstalk Development of Lettuce as Affected by Growth Regulators, Vernalization, Temperature and Photoperiod. Doctoral Thesis. Michigan State College.

2. Arthur, J. M. and J. D. Guthrie, 1927. Effect of Light, Carbon Dioxide, and Temperature on Flower and Fruit Production. Mem. Hort. Soc. N. Y. 3:73-74.

3. Avery, G. S., J. Berger, and B. Shalucha, 1942. Auxin Content of Maize Kernels During Ontogeny from Plants of Varying Heterotic Vigor. Amer. Jour. Bot. 29: 765-772.

4. Bailey, L. H., 1891. Some Preliminary Studies of the Influence of the Electric Arc Light Upon Greenhouse Plants. Cornell Univ. Agr. Expt. Sta. Bul. 30:83-139.

5. Borthwick, H. A., S. B. Hendricks, E. H. Toole, and V. K. Toole, 1954. Action of Light on Lettuce Seed Germination. Bot. Gaz. 115:205-225.

6. Borthwick, H. A. and M. W. Parker, 1952. Light in Relation to Flowering and Vegetative Development. Rept. of the 13th International Hort. Congr.:801-810. The Royal Horticultural Society, London.

7. Bremer, A. H., 1929. Hovudsalat (Lactuca sativa capitata) i drivbenk og pa friland. Scientific reports from the Agricultural College of Norway. IX: 1-112.

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

9. Chakravarti, S. C., 1954. Inhibition of Vernalization in Linum usitatissimum Linn. by Certain Synthetic Hormones. Nature. 174:461-463.

10. Chakravarti, S. C., and V. N. Krishna Pillai, 1955. Auxin-vernalization Relationships. I. The Effects of Certain Synthetic Auxins and their Antagonists on the Vernalization of Brassica campestris L. Phyton. 5:1-17.

11. Cholodny, N. G., 1939. The Internal Factors of Flowering. Herb. Rev. 7:223-247.

12. Choudri, R. S., and V. B. Bhatnagar, 1955. Response of Lettuce to Spraying with Maleic Hydrazide. Phyton 5:19-30.

13. Clark, B. E., and S. H. Wittwer, 1949. Effect of Certain Growth Regulators on Seed Stalk Development in Lettuce and Celery. Plant Physiol. 24: 555-576.

14. Crafts, A. S., H. B. Currier, and B. E. Day, 1950. Response of Several Crop Plants and Weeds to Maleic Hydrazide. Hilgardia. 20:57-80.

15. Emsweller, S. L., and H. A. Borthwick, 1937. Effects of Short Periods of Low Temperature on Flower Production in Stocks (Matthiola). Proc. Amer. Soc. Hort. Sci. 35:755-757.

16. Evanari, M. and G. Neumann, 1953. The Germination of Lettuce Seed. III. The Effect of Light on Germination. Bull. Res. Counc. Israel. III:137-145.

17. Flint, H. A., and E. D. McAlister, 1935. Wave Lengths of Radiation in the Visible Spectrum Inhibiting the Germination of Light Sensitive Lettuce Seed. Smith. Misc. Coll. 94:1-11.

18. Franklin, D. F., 1948. Using Hormone Sprays to Facilitate Bolting and Seed Production of Hard-headed Lettuce Varieties. Proc. Amer. Soc. Hort. Sci. 51:453-456.

19. Franklin, D. F., 1955. Personal Communication. Parma Branch Expt. Sta., Parma, Idaho.

20. Galston, A. W., 1943. The Physiology of Flowering with Special Reference to Floral Initiation in Soybeans. Doctoral Thesis. Univ. of Illinois.

21. Garner, W, W., and H. A. Allard, 1920. Effect of the Relative Length of Day and Night and Other Factors of the Environment on Growth and Reproduction in Plants. Jour. Agr. Res. 18:553-605.

22. Gassner, G., 1918. Beitrage zur physiologischen Characteristik Sommer und Winterannueller Gewachse, Insbesondere der Getreidepflanzen. Zeitschr. Bot. 10:417-30.

23. Gott, M. B., F. G. Gregory, and O. N. Purvis, 1955. Studies in Vernalization of Cereals. XIII. Photoperiodic Control of Stages in Flowering Between Initiation and Ear Formation in Vernalized and Unvernalized Petkus Rye. Ann. of Bot. XXI:87-126.

24. Gray, S. G., 1942. Increased Earliness of Flowering in Lettuce through Vernalization. Austr. Jour. Counc. for Sci. and Ind. 15:211-212.

25. Gregory, F. G., and O. N. Purvis, 1936. Vernalization. Nature. 138:249.

26. Gregory, F. G., and O. N. Purvis, 1938. Studies in Vernalization of Cereals. II. The Vernalization of Excised Mature Embryos, and of Developing Ears. Ann. of Bot. 2:237-251.

27. Hatcher, E. S. J., 1945. Studies in the Vernalization of Cerals. IX. Auxin Production during Development and Ripening of the Anther and Carpel of Spring and Winter Rye. Ann. of Bot. 9:235-266.

28. Hawthorne, L. R., and L. H. Pollard, 1951. Selection of Great Lakes Lettuce Strains for Higher Seed Yields. Proc. Amer. Soc. Hort. Sci. 57:323-328.

29. Highkin, H. R., 1955. Flower Promoting Activity of Plant Seed Diffusates. Plant Physiol. 30:390.

30. Hinsvark, O. N., W. H. Houff, S. H. Wittwar, and H. M. Sell, 1954. The Extraction and Colorimetric Estimation of Indole-3-acetic Acid and Its Esters in Developing Corn Kernels. Plant Physiol. 29:107-108.

31. Klein, W. H., and A. C. Leopold, 1953. The Effect of Maleic Hydrazide on Flower Initiation. Plant Physiol. 28:293-298.

32. Klippart, J. H., 1857. An Essay on the Growth, Origin, Diseases, etc. of the Wheat Plant. Ohio State Board of Agr. Ann. Rept. 12:562-816.

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

34. Lang, A., 1952. Physiology of Flowering. Ann. Rev. Plant Physiol. 3:265-306

35. Leopold, A. C., and F. S. Guernsey, 1953. Interaction of Auxin and Temperatures in Floral Initiation. Sci. 118:215-217.

36. Leopold, A. C., and F. S. Guernsey, 1954. Flower Initiation in the Alaska Pea. II. Chemical Vernalization. Amer. Jour. Bot. 41:181-185.

37. Lewis, M. T., 1951. Lettuce Head Removal Results in More Seed of Choice Lines. Penn. Agr. Expt. Sta. 64th. Ann. Rept. p.56.

38. Liverman, J. L., and James Bonner, 1953. The Interaction of Auxin and Light in the Growth Responses of Plants. Proc. Nat. Acad. Sci. 39:905-916.

39. Lysenko, T. D., 1946. Heredity and Its Variability. Kings Crown Press. New York.

40. McKinney, H. H., and W. J. Sando, 1933. Earliness and Seasonal Growth Habit in Wheat as Influenced by Temperature and Photoperiod. Jour. Hered. 24:169-179.

41. Melchers, G., 1939. Die. Bluhhorme. Deut. Bot. Ges. Berlin. 57:29-48.

42. Milthorpe, F. L., and B. Horowitz. 1943. The Effect of Length of Day and Temperature on the Flowering, Seed Production, and Growth of Vegetables. Agr. Gaz. New South Wales. 54:53-57.

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

44. Nitsch, J. P., 1955. Free Auxin and Free Tryptophan in the Strawberry. Plant Physiol. 30:33-39.

45. Philp, S., 1945. Personal Communication. National Veg. Res. Sta., Wellesbourne, Warwick, United Kingdom.

46. Pollard, L. H., and E. A. Davis, 1946. Heads and Seed from Overwintering Lettuce. Farm and Home. Sci. (Utah Agr. Exp. Sta.) 7(4):6-7,17.

47. Purvis, O. N., 1934. Analysis of the Influence of Temperature During Germination on the Subsequent Development of Certain Winter Cereals and Its Relation to the Effect of Day Length. Ann. of Bot. 48:919-55.

48. Purvis, O. N., 1940. Vernalization of Fragments of Embryo Tissue. Nature. 145:462

49. Purvis, O. N., and F. G. Gregory, 1937. Studies in Vernalization of Cereals. I. A Comparative Study of Vernalization of Winter Rye by Low Temperatures and by Short Days. Ann. of Bot. 1:569-91.

50. Purvis, O. N., and F. G. Gregory, 1953. Accelerating Effect of an Extract of Vernalized Embryos of Winter Rye on Flower Initiation in Unvernalized Embryos. Nature. 171:687-689.

51. Rappaport, Lawrence, and S. H. Wittwer, 1956. Stimulation of Flowering by Vernalization of Endive Seedlings. Proc. Amer. Soc. Hort. Sci. (In press).

52. Rappaport, Lawrence, and S. H. Wittwer, 1955. Temperature and Photoperiod Effects on the Development of Leaf Lettuce. Abstr. Amer. Soc. Hort. Sci. Meeting, Sept. 5-8. East Lansing, Michigan.

53. Roberts, A. N., 1953. Growth and Composition of the Strawberry Plant in Relation to Root Temperature. Doctoral Thesis. Michigan State College.

54. Rudorf, W., and G. Stelzner. Untersuchungen uber Lichtperiodische und Temperaturnachwirkung bei Sorten von Salat (Latuca sativa var Capitatal) und die Moglichkeit ihrer Ausnutzung im Gemausbau. Gartenbauwis. 9:143-153.

55. Sachs, J., 1865. Wirkung des Lichts auf die Bluthenbildung unter Vermittlung der Laubblatter. Bot. Ztg. 23:117-121, 125-131, 133-139.

56. Sachs, J., 1882. "Lectures on the Physiology of Plants." Clarenden Press, Oxford.

57. Sen, B., and S. C. Chakravarti, 1942. Studies in Vernalization in Mustard. Ind. Jour. Agr. Sci. 2:1.

58. Sen, B., and S. C. Chakravarti, 1946. Effect of High Temperature on Vernalized Mustard Seed. Nature. 157:266.

59. Sen, S. P., and A. C. Leopold, 1954. Paper Chromatography of Plant Growth Regulators and Allied Compounds. Physiol. Plantarum. 7:98-108.

60. Shuck, A. L., 1935. Light as a Factor Influencing the Dormancy of Lettuce Seeds. Plant Physiol. 10:193-196.

61. Simpson, A. C. 1943. Vernalization of Lettuce. Nature. 151:279-280.

62. Sprague, G., 1934. Experiments on "Iarovising" corn. Jour. Agr. Res. 48:1113-1120.

63. Stowe, B. B., and K. V. Thimann, 1953. Indolepyruvic Acid in Maize. Nature. 172:764.

64. Stowe, B. B., and K. V. Thimann, 1954. The Paper Chromatography of Indole-containing Auxins of Plant Tissues. Arch. Bio. Chem. and Biophys. 51:499-516.

65. Terpstra, W., 1953. Extraction and Identification of Growth Substances with Special Reference to the Avena Coleoptile Tip. Doctoral Thesis. Univ. of Utrecht, Netherlands.

66. Teubner, F. G., 1953. Identification of the Auxin Present in Apple Endosperm. Science 118:418.

67. Teubner, F. G., and S. H. Wittwer. Effect of N-m-Tolylphthalamic Acid on Tomato Flower Formation. Science 122:74-75.

68. Thompson, H. C., 1949. Vernalization of Growing Plants. From "Growth and Differentiation in Plants". Pp. 179-196. Iowa State Coll. Press. Ames.

69. Thompson, H. C., and J. E. Knott, 1933. The Effect of Temperature and Photoperiod on the Growth of Lettuce. Proc. Amer. Soc. Hort. Sci. 30:507-509.

70. Thompson, R. C., and W. Kosar, 1948. Vernalization and Seed Stem Development in Lettuce. Proc. Amer. Soc. Hort. Sci. 52:441-442.

71. Tincker, M. A. H., 1933. The Influence of the Length of Day on Lettuce. Gard. Chron. 93:404-405.

72. Van den Muijzenberg, E. W. B., 1952. The Influence of the Air Conditioned Glasshouse on the Growth of Plants. Rept. of the 13th. International Hort. Congr.: 895-903. The Royal Horticultural Society, London.

73. Verkerk, K., 1954. The Influence of Low Temperatures on Flower Initiation and Stem Elongation in Brussel Sprouts. Koninkl. Nederl. Akadamie Van Wetenschappen Amsterdam. 57:339-346.

74. Vlitos, A. J., and W. Meudt, 1955. Interactions between Vernalization and Photoperiod in Spinach. Contr. Boyce. Thompson Inst. 18:159-166.

75. Vogt, I., 1951. Uber den Hemmstoff und Wuchstoffgehalt in Getreidewurzeln. Planta. 40:145-169.

76. Warne, L. G. G., 1947. Vernalization of lettuce. Nature. 159:31.

77. Weller, L. E., S. H. Wittwer, and H. M. Sell, 1954. The Detection of 3-Indoleacetic acid in Cauliflower Heads. Chromatographic Behavior of Some Indole-Compounds. Jour. Amer. Chem. Soc. 76:629-630.

78. Went, F. W., 1944. Plant Growth Under Controlled Conditions. III. Correlation Between Various Physiological Processes and Growth in the Tomato Plant. Amer. Jour. Bot. 31:597-618.

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

80. Wittwer, S. H., 1950. Control of Seed-stalk Development and Fruit-setting. From Symposium; Physiological Aspects of Growth-regulators, with Special Reference to Reproduction. Abstr. Amer. Soc. Plant Physiol. Meeting Sept. 11-13. Columbus, Ohio.

81. Wittwer, S. H., H. Jackson, and D. P. Watson, 1954. Control of Seedstalk Development in Celery by Maleic Hydrazide. Amer. Jour. Bot. 5:435-439.

82. Wittwer, S. H., and F. G. Teubner, 1956. Cold Exposure of Tomato Seedlings and Flower Formation. Proc. Amer. Soc. Hort. Sci. (In press).

83. Zimmerman, P. W., and A. E. Hitchcock, 1942. Flowering Habit and Correlation of Organs Modified by Triiodobenzoic Acid. Contr. Boyce. Thompson Inst. 12:491-496.