EFFECT OF CERTAIN GROWTH REGULATORS
ON SEED STALK DEVELOPMENT
IN LETTUCE AND CELERY

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EFFECT OF CERTAIN GROWTH REGULATORS
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Introduction

For many years, farmers have been interested in the process of seed stalk formation because of its great economic importance to them. From their point of view, seed stalk formation, commonly called bolting, may be either desirable or undesirable. In the case of lettuce and celery, for instance, the development of seed stalks eventually destroys the value of the market crop. On the other hand, seed stalks are essential to the production of the seeds from which these crops are grown.

In commercial lettuce and celery production, seed stalk development is controlled either by using varieties resistant to bolting or by confining the plants to those environmental conditions which are not conducive to the initiation and elongation of seed stalks. Neither of these methods of control is entirely satisfactory. Slow bolting lettuce varieties may lack vigor and fail to produce seed readily, and "non bolting" celery varieties tend to be low in quality. It is impossible, of course, to control environmental factors in the field. Fluctuating weather conditions make it difficult to adjust planting dates in such a way as to avoid temperatures conducive to seed stalk formation. Obviously,
then, a better method of controlling seed stalk development would be desirable.

Interest in seed stalk formation, however, is not limited to farmers. Plant physiologists also have a keen interest in the process because, in many plants, it marks the morphological transformation from the vegetative to the reproductive phase of development. This transformation has been studied extensively in certain crops, and much has been learned concerning the environmental factors which affect it. Nevertheless, plant physiologists have as yet discovered little concerning the biochemical mechanism which causes it. The discoveries of Van Overbeek (27), (28), and others (5), (6), however, indicate that, in some plants at least, certain already identified chemicals having the properties of phytohormones play a role in the complex responsible for the initiation of reproduction. If these chemicals should be found capable of influencing the flowering of still other plants, then we shall have increased our knowledge not only of reproduction in plants but also of the mode of action of phytohormones. The use of these growth regulating substances might help to solve the practical problem of controlling seed stalk development in either of two ways: (1) by delaying seed stalk development in the crop produced for market or (2) by hastening the development of seed stalks of slow bolting
varieties in the crop grown for seed. It was with these considerations in mind that the present work was undertaken.
REVIEW OF LITERATURE

It is very difficult to consider intelligently the influence of one factor on flowering without also giving consideration to other interacting factors influencing reproduction in plants. This review, however, shall be limited to experiments concerning the effect of synthetic growth regulators on flowering. Such a limitation of the subject seems desirable in view of the fact that other workers have already published comprehensive reviews of experiments involving the effects of environmental factors on plant development. A recently published symposium by Murneek and Whyte et al. (19) and a book by Whyte (31) contain such reviews. Specific factors affecting seed stalk development in lettuce have been discussed by Thompson and Knott (25) and by Bremer (1). Those factors affecting seed stalk initiation in celery have been studied by Thompson (24) and by Starring (20).

Although papers concerning the effects of growth substances on flowering are relatively few, the results reported in them are very interesting, not only because of their practical significance, but also because of their implications concerning the biochemical mechanism involved in flower initiation.

One of the earliest observations of an effect on flowering produced by the application of synthetic growth
substances was that reported by Hitchcock and Zimmerman (13). They observed that the flowering of Turkish tobacco was hastened by applying indolebutyric, indolepropionic, phenylacetic, and phenylpropionic acids to the soil in which the plants were growing. They obtained most pronounced effects by applying these substances three to five weeks prior to the normal date of flowering. Murneek (16), on the other hand, applied indoleacetic, indolepropionic, and phenylacetic acids to soybean plants and obtained no effect on flowering. Hamner and Bonner (12) treated Xanthium plants with various substances, including indoleacetic acid, and likewise discovered no effect on flower initiation. Galston (10) applied 2,3,5 triiodobenzoic acid to soybeans and discovered that, although it did not induce flowering of plants exposed to long days, it increased the number of flowers on photo-induced plants.

Thimann and Lane (23) found that plants produced from oat and tomato seeds which had been soaked in indole-3-acetic acid flowered from three to seven days earlier than control plants. The results with tomato, however, were inconsistent. Nevertheless, Stier and Du Buy (21) found that the treatment of seeds and seedlings at the time of transplanting with indolebutyric and naphthylacetic acids caused significantly earlier flowering in Master Marglobe tomato
plants. Tang and Loo (22) also found that mustard, tomato,
and rice plants grown from seeds treated with indole-3-
acetic acid began to flower from three to seven days earlier
than plants grown from untreated seeds.

Some of the most pronounced effects of growth
substances on flowering have been observed in the pineapple
plant. Clark and Kerns (5) working with this plant were
able to hasten flowering by applying low concentrations of
naphthaleneacetic acid and to delay flowering with higher
concentrations of the same substance. Cooper (6) discovered
that, in Florida, naphthaleneacetic acid in solutions of
.01, .005, .001 per cent had no effect when applied to plants
in July, but when applied to plants in October, it caused
them to flower a month ahead of the normal flowering date.
By the application of 0.25 milligram of naphthaleneacetic
acid or 2,4-D per plant, Van Overbeek (27) and (28) was able
to induce practically 100 per cent flowering in plants of
the Cabezona variety of pineapple even a year before they
were due to flower naturally. What is more, he was able to
accomplish this regardless of the season of the year.

The vegetative-reproductive condition of plant shoots
has been reversed through the application of growth regulators
by Zimmerman and Hitchcock (33) and by Dostal and Hosek (9).
By applying triiodobenzoic acid at the rate of 25 to 500
milligrams per liter to tomato plants, Zimmerman and Hitchcock caused the normally vegetative axillary shoots to produce flowers instead of leaves. Dostal and Hosek prevented the flowering of "flower-ready" Cercaea stem tips by treating them with indoleacetic acid.

The suppression of flowering by growth substances has been reported by several workers. Johnson (16) treated annual stocks with aqueous solutions of alpha and beta naphthoxyacetic acids. At the end of her experiments, 6 per cent of the controls, 46 per cent of the beta treated plants, and 86 per cent of the alpha treated plants were without inflorescences. Thurlow and Bonner (26) by the application of sprays containing 500 parts per million indoleacetic acid or naphthaleneacetic acid prevented induction of flower primordia in Xanthium even when the plants were exposed to short days conducive to photoperiodic induction. Scions from treated plants also failed to cause flowering when they were grafted as donors on receptor plants. Green and Fuller (11) applied indole-3-acetic acid to a number of plants and found that it delayed the appearance of blossom buds and retarded their development after they had appeared. In celery, Wittwer, Coulter, and Carolus (32) and Coulter (7) obtained complete inhibition of seed stalk elongation in cold-induced Cornell 19 plants by applying 100 parts per
millions alpha ortho chlorophenoxyproprionic acid to the plants just previous to the induction treatment. Plants treated with 50 parts per million 2,4-D under the same conditions, on the other hand, showed an acceleration in the rate of seed stalk elongation.

Although the results are somewhat inconsistent, it is apparent from the literature cited that synthetic growth regulators are capable of influencing reproductive-vegetative responses in plants.
GENERAL METHODS AND MATERIALS

The experiments herein described were carried out both in the greenhouse and in the field. In the greenhouse, night temperatures ranged from 60-70°F except during the early fall and late spring when warmer weather prevailed. In the field, usual commercial methods were followed in every respect possible.

The growth substances used in these experiments were 2,4-dichlorophenoxyacetic acid, alpha naphthaleneacetic acid, alpha ortho chlorophenoxypropionic acid, para chlorophenoxyacetic acid, and 2,3,5 triiodobenzoic acid, hereafter designated as 2,4-D, NAA, ClPP, ClPA, and TIBA respectively. Unless otherwise specified, the chemicals were used in the form of the true acids. Stock solutions of the growth substances were prepared by dissolving one gram of crystals in 100 ml. of 95 per cent ethyl alcohol. These stock solutions were then diluted with tap water to produce the desired concentrations. The concentrations of the growth substances are expressed as parts per million (ppm).

The prepared solutions were sprayed onto the plants with small household sprayers. Each plant was wet thoroughly and a special effort was made to reach the growing points with the solutions being applied. Contaminations were prevented by using a different sprayer for each material and
by placing a shield around the plants being sprayed to prevent the spray from drifting.

Whenever applicable, the data obtained in these investigations were analysed for statistical significance. Significance was determined either by the analysis of variance or by Student's "t" test, depending on which test was considered most appropriate for the data in question.
LETTUCE EXPERIMENTS

Object of Study

The lettuce experiments conducted in the course of these investigations were designed to determine the effects of growth substances on seed stalk development, and to determine the effects of various times and methods of application and of various concentrations of these substances on the response of lettuce plants to them.

Experiment 1

Methods

The first lettuce experiment was a preliminary one conducted for the purpose of determining, in a general way, whether certain growth substances might be used effectively to influence seed stalk development. It was designed also to ascertain the concentrations of growth substances which could be tolerated by young lettuce plants.

On October 26, 1946, Grand Rapids lettuce plants with six to eight leaves were transplanted from a greenhouse bed to six-inch clay pots. These potted plants were arranged into eleven groups of five plants each in such a way that the plants in each group were as nearly as possible the same size as the plants in each of the other groups. On October 30, each of the eleven groups of plants was given
one of the following spray treatments:

A. Control  G. 25 ppm ClPP
B. 10 ppm NAA  H. 0.5 ppm 2,4-D
C. 50 ppm NAA  I. 2.5 ppm 2,4-D
D. 100 ppm NAA  J. 10 ppm 2,4-D
E. 1 ppm ClPP  K. 25 ppm 2,4-D
F. 5 ppm ClPP

After treatment, notations were made of the morphological modifications produced by the chemicals applied. Measurements of seed stalks were also made several times during the period of elongation.

Results

No pronounced modification of foliage was apparent on the plants treated with ClPP and NAA. The foliage of all of the plants treated with solutions of 2,4-D, however, was noticeably modified. The modification was slight for plants treated with 0.5 ppm. Plants treated with 25 ppm, on the other hand, were greatly affected. New leaves of these plants had much dwarfed, cupped, leaf blades and elongated petioles. Several days after treatment, bulbous tumors began to form at the base of the stems of these plants. These tumors finally reached a diameter of an inch or more and produced shoots from their bases. These adventitious shoots elongated and eventually produced blossoms.
Plants treated with 10 ppm of 2,4-D did not produce tumors, but their leaves were markedly modified. Their leaf surfaces had less savoying than the leaf surfaces of the control plants, and their leaf margins were distinctively fringed. The leaves of plants treated with 2.5 ppm of 2,4-D were similar to those of plants treated with 10 ppm except that the modifications were not so pronounced.

Seed stalk elongation was not apparent in any of the treatments until January 15. It was apparent first in the plants treated with ClPP and 2,4-D. It appeared in the control plants several days later. It was evident from a comparison of the control and treated plants that the treatments had not profoundly altered the course of development of the plants in this experiment. Nevertheless, the data presented in Table I show that the rate of seed stalk elongation was increased by the treatments. The most rapid elongation was produced by the higher concentrations of ClPP and lower concentrations of 2,4-D. Although 25 ppm of 2,4-D tended to hasten the maturity of lettuce plants, its general dwarfing effect produced plants with shorter seed stalks than those of the other treatments.
Experiment 2

Methods

Experiment two was another preliminary experiment designed to determine the relative effect of single versus repeated applications of growth substances applied to plants of different ages. In this experiment, seeds of Grand Rapids
and Slobolt lettuce were planted in greenhouse flats on November 7, 1946. On December 13, plants from each variety were set in a ground bed of the greenhouse in 14 plots of five plants each. At the proper time, plants in two plots of each variety were treated with 10 ppm of 2,4-D according to the following plan:

A. Control
B. Single treatment when 6 weeks old
C. Single treatment when 8 weeks old
D. Single treatment when 10 weeks old
E. First treatment when 6 weeks old, treatments repeated when 8 and 10 weeks old
F. First treatment when 8 weeks old, treatments repeated when 10 and 12 weeks old
G. First treatment when 10 weeks old, treatments repeated when 12 and 14 weeks old

Results

The results of this experiment are best shown by figure 1. It can be seen from this figure that the repeated treatments had a greater effect on stem elongation than did single treatments. It is apparent, also, that treatments applied to six-week old plants had a pronounced dwarfing effect which retarded the development of these plants.

The difference in response between the Grand Rapids
Figure 1. Effect of spraying 10 ppm 2,4-D on lettuce. Upper row; Grand Rapids, single treatment; left - 6 weeks old; center - 8 weeks old; right - 10 weeks old. Middle row; Grand Rapids, three treatments; left - 6, 8, and 10 weeks old; center - 8, 10, and 12 weeks old; right - 10, 12, and 14 weeks old. Bottom row; left - Grand Rapids, control; right - Slobolt, three treatments, 10, 12, and 14 weeks old.
and Slobolt varieties is indicated by a comparison of the plants receiving repeated treatments at 10, 12, and 14 weeks of age. The plant of the Grand Rapids variety shows marked stem elongation, whereas only slight elongation is apparent in the stem of the Slobolt plant. This difference in response between the two varieties was characteristic for all of the treatments.

Experiment 3

Methods

After it had been discovered that growth regulating substances had an effect on seed stalk elongation in winter crops of greenhouse lettuce, and that this effect was greatest when applications were repeated, an experiment was set up to determine the effect of repeated applications of several growth regulators on a spring crop of greenhouse lettuce. This experiment was designed to determine the effect of growth substances not only upon the treated plants themselves, but also upon their progeny. In testing the effect of growth regulators on the progeny of treated plants, seeds were saved from the plants of the several treatments. These seeds were sowed and the resulting plants grown to maturity without further treatments. Thus, differences in seed stalk elongation of the second generation plants could result only from an effect of growth substances carried over from the
previous generation.

First generation observations in this experiment were made on both Grand Rapids and Slobolt varieties. Second generation observation were made only on the Grand Rapids variety.

The plants to be treated were produced from seeds planted in a greenhouse flat on February 3, 1947. On February 15, seedlings were pricked off to other flats, and on March 30, the plants were transplanted to a greenhouse ground bed. The plants of each variety were set into 12 plots of five plants each and on April 5, and each week thereafter for five weeks, two plots of plants from each variety received one of the following treatments:

A. Control D. 5 ppm ClPA
B. 10 ppm NAA E. 50 ppm TIBA
C. 25 ppm ClPF F. 5 ppm 2,4-D

The seeds produced by the treated Grand Rapids lettuce plants were harvested in July and were stored in the laboratory until September. On September 8, 1947, seeds from plants of each treatment were planted in a greenhouse flat. On September 22, the resulting plants were transplanted to a ground bed in the greenhouse. The plants were set in two blocks, each of which was composed of six plots of five plants each. Each plot contained second generation plants from one of the six treatments previously described.
The above experiment on the progeny of treated plants was repeated in the spring of 1948. The experimental design of this second trial was the same as that of the first except that there were three replications in the second trial, whereas there were only two in the first. The season of the year, of course, was also different for the two trials. In the second trial, the seeds were planted on March 3 and the resulting plants were transplanted on April 10.

Results

An analysis of the seed stalk measurements of the treated plants of both the Grand Rapids and Slobolt varieties showed that, during the first three weeks of elongation, plants treated with 2,4-D and TIBA had seed stalks significantly longer than those of the control plants. During the last two weeks of seed stalk elongation, however, there were no significant differences in seed stalk length among the plants of the several treatments. The reason for the loss of significance as seed stalk development progressed is indicated by Table II.

As can be seen from Table II, the weekly increment in seed stalk elongation was greater for the treated plants than for the untreated plants during the early stages of elongation. During the final week of observation, on the other hand, growth increments were essentially the same.
TABLE II. The Effect of Growth Regulating Substances on the Weekly Increment in Length of Seed Stalk in a Spring Crop of Greenhouse Lettuce.

<table>
<thead>
<tr>
<th>Treatment (Grand Rapids)</th>
<th>Increment in Seed Stalk Length (inches)</th>
<th>May 10-May 17</th>
<th>May 17-May 24</th>
<th>May 24-May 31</th>
<th>May 31-June 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.9</td>
<td>6.1</td>
<td>6.3</td>
<td>14.0</td>
</tr>
<tr>
<td>NAA</td>
<td></td>
<td>1.9</td>
<td>5.8</td>
<td>6.3</td>
<td>15.0</td>
</tr>
<tr>
<td>ClPP</td>
<td></td>
<td>3.1</td>
<td>7.7</td>
<td>6.9</td>
<td>13.7</td>
</tr>
<tr>
<td>ClPA</td>
<td></td>
<td>2.8</td>
<td>7.8</td>
<td>8.7</td>
<td>14.9</td>
</tr>
<tr>
<td>TIBA</td>
<td></td>
<td>3.9</td>
<td>8.6</td>
<td>7.8</td>
<td>14.3</td>
</tr>
<tr>
<td>2,4-D</td>
<td></td>
<td>4.7</td>
<td>10.6</td>
<td>10.4</td>
<td>14.7</td>
</tr>
</tbody>
</table>

Least signif. differences, 5% level

<table>
<thead>
<tr>
<th>Treatment (Slobolt)</th>
<th>Increment in Seed Stalk Length (inches)</th>
<th>May 24-May 31</th>
<th>May 31-June 7</th>
<th>June 7-June 14</th>
<th>June 14-June 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>.9</td>
<td>2.1</td>
<td>8.7</td>
<td>10.3</td>
</tr>
<tr>
<td>NAA</td>
<td></td>
<td>1.7</td>
<td>1.6</td>
<td>8.4</td>
<td>12.1</td>
</tr>
<tr>
<td>ClPP</td>
<td></td>
<td>1.8</td>
<td>2.5</td>
<td>6.4</td>
<td>9.5</td>
</tr>
<tr>
<td>ClPA</td>
<td></td>
<td>1.7</td>
<td>2.4</td>
<td>6.4</td>
<td>10.0</td>
</tr>
<tr>
<td>TIBA</td>
<td></td>
<td>2.5</td>
<td>1.0</td>
<td>8.7</td>
<td>12.2</td>
</tr>
<tr>
<td>2,4-D</td>
<td></td>
<td>1.1</td>
<td>3.6</td>
<td>9.4</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Least signif. differences, 5% level

| Least signif. differences, 5% level | 1.36 | 3.18 | 4.16 | 5.17 |
for all plants regardless of treatment. It is apparent, then, that the effect of growth substances is most pronounced early in the process of seed stalk elongation and that it becomes negligible during the latter stages.

A comparison of the responses of Grand Rapids and Slobolt lettuce plants showed that the treatments did not overcome the inherent slow bolting characteristic of Slobolt lettuce. Elongation in Slobolt lettuce did not start until two weeks after it had occurred in Grand Rapids lettuce. The growth substances, however, did hasten the seed stalk elongation of the Slobolt plants after it had been initiated. The general pattern of response of Slobolt lettuce plants was the same as that of Grand Rapids plants, but the resulting differences were of a lower magnitude.

The progeny of the treated lettuce plants showed no distinguishable morphological differences during the vegetative phase of their development, regardless of the treatment of the parent plants. Physiological differences, however, became apparent during the process of seed stalk elongation. This is clearly indicated by the data for the two trials presented in Table III. It can be seen from this table that, except for the additional precision obtained by including three replications in the second trial, there were essentially no differences between the results of the two trials. It is evident from the data presented that seed
TABLE III. The Effect of Growth Regulating Substances on the Length of Seed Stalks in the Progeny of Treated Lettuce Plants.

<table>
<thead>
<tr>
<th>Treatment of Parent Plant</th>
<th>Length of Seed Stalk of Progeny (inches)</th>
<th>First Trial</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nov. 22</td>
<td>Nov. 29</td>
<td>Dec. 6</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>10.9</td>
<td>14.1</td>
<td>19.5</td>
</tr>
<tr>
<td>NAA</td>
<td></td>
<td>12.1</td>
<td>17.8</td>
<td>23.8</td>
</tr>
<tr>
<td>C1PP</td>
<td></td>
<td>12.9</td>
<td>18.3</td>
<td>26.5</td>
</tr>
<tr>
<td>C1PA</td>
<td></td>
<td>13.4</td>
<td>19.7</td>
<td>25.7</td>
</tr>
<tr>
<td>TIBA</td>
<td></td>
<td>11.5</td>
<td>15.9</td>
<td>22.2</td>
</tr>
<tr>
<td>2,4-D</td>
<td></td>
<td>11.8</td>
<td>16.2</td>
<td>25.0</td>
</tr>
<tr>
<td><strong>Least signif. differences, 5% level</strong></td>
<td></td>
<td>2.47</td>
<td>5.04</td>
<td>6.55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment of Parent Plant</th>
<th>Second Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May 26</td>
</tr>
<tr>
<td>Control</td>
<td>8.3</td>
</tr>
<tr>
<td>NAA</td>
<td>10.0</td>
</tr>
<tr>
<td>C1PP</td>
<td>10.9</td>
</tr>
<tr>
<td>C1PA</td>
<td>12.7</td>
</tr>
<tr>
<td>TIBA</td>
<td>10.3</td>
</tr>
<tr>
<td>2,4-D</td>
<td>10.8</td>
</tr>
<tr>
<td><strong>Least signif. differences, 5% level</strong></td>
<td></td>
</tr>
</tbody>
</table>
stalk elongation was more rapid in the progeny of plants that had been treated with growth substances than it was in the progeny of the plants that had not been treated. The weekly growth increments of these plants showed that in the second generation, as in the first, the effect of the growth substances took place early in the process of seed stalk elongation. During the last week of elongation, the seed stalks of all of the plants, regardless of the treatment, were elongating at essentially the same rate.

Figure 2 presents a comparison of the responses of treated plants and their progeny to growth regulating substances. It can be seen from this figure that NAA, C1PP, and C1PA had about the same effect on the progeny as on the treated plants themselves. The effects of TIBA and 2,4-D, on the other hand, were much diminished in the second generation. Evidently, the effects of these two latter substances are not so persistent as the effects of the other three.

Experiment 4
Methods

Lettuce experiment four was set up to determine the effect of growth substances on lettuce grown in the field under natural conditions. In this experiment, growth substances were applied to a slow-bolting head lettuce variety, Cornell 456, and to the Grand Rapids leaf lettuce variety
Figure 2. Relative responses of treated lettuce plants and their progeny to growth regulating substances.
previously used in greenhouse experiments.

Seeds of the two varieties of lettuce were planted in flats in the greenhouse on April 1, 1947. The resulting plants were transplanted to the field on May 1-3. In the field, each variety was set in four blocks of 18 rows each. The rows were 10 feet long and 28 inches apart, and each row contained 10 lettuce plants. The two outer rows of each block served as guard rows, and each of the other 16 rows received one of the following treatments at the age indicated:

A. Water, single application, 9 weeks old
B. 10 ppm 2,4-D, single application, 9 weeks old
C. 200 ppm TIBA, single application, 9 weeks old
D. 50 ppm ClPP, single application, 9 weeks old
E. Water, single application, 11 weeks old
F. 10 ppm 2,4-D, single application, 11 weeks old
G. 200 ppm TIBA, single application, 11 weeks old
H. 50 ppm ClPP, single application, 11 weeks old
I. Water, three applications, 9, 11, and 13 weeks old
J. 10 ppm 2,4-D, three applications, 9, 11, and 13 weeks old
K. 200 ppm TIBA, three applications, 9, 11, and 13 weeks old
L. 50 ppm ClPP, three applications, 9, 11, and 13 weeks old
M. Water, three applications, 11, 13, and 15 weeks old
N. 10 ppm 2,4-D, three applications, 11, 13, and 15 weeks old
O. 200 ppm TIBA, three applications, 11, 13, and 15 weeks old
P. 50 ppm ClPP, three applications, 11, 13, and 15 weeks old
The treatments were applied in random order to the plots within each block. The effects of the treatments were determined by measuring the lengths of seed stalks at intervals during elongation and by observing the dates of appearance of blossom buds and of first open blossoms.

**Results**

The effects of the applied substances on the seed stalk development of plants of the Grand Rapids variety are shown in Table IV. It can be seen from this table that the 2,4-D treatments produced highly significant variations from the control (water) treatments, not only in length of seed stalk, but also in date of blossom bud appearance. Neither 2,4-D

<table>
<thead>
<tr>
<th>Growth Substance</th>
<th>Days from Transplanting</th>
<th>Length of Seed Stalk (inches)</th>
<th>Days from Appearance of Blossom Buds to Open Blossoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>79</td>
<td>8.9 15.0 24.5</td>
<td>24</td>
</tr>
<tr>
<td>2,4-D</td>
<td>75*</td>
<td>14.1* 21.0* 28.8*</td>
<td>24</td>
</tr>
<tr>
<td>TIBA</td>
<td>79</td>
<td>9.9 15.0 23.9</td>
<td>22</td>
</tr>
<tr>
<td>ClPP</td>
<td>79</td>
<td>10.5 15.9 25.0</td>
<td>22</td>
</tr>
</tbody>
</table>

* Significant variation from water treatments.

Student's "t" test.
nor any of the other treatments, however, had any significant
effect on the interval between blossom bud appearance and
the appearance of open blossoms. Neither TIBA nor CI1PP had
any significant effect on seed stalk elongation of these
field grown Grand Rapids lettuce plants.

Table V shows the effect of the time and method of
application on the response of field grown Grand Rapids
lettuce to 2,4-D. None of the differences in response were
great enough to be statistically significant. This is probably
due to the fact that there were only three degrees of freedom
available for measuring significance. There is some indication,
however, that the repeated treatments applied to plants when

TABLE V. Effect of Time and Method of Application on Response
of Field Grown Grand Rapids Lettuce to 10 ppm of 2,4-D

<table>
<thead>
<tr>
<th>Method of Application</th>
<th>Days from Transplanting</th>
<th>Length of Seed Stalk (inches)</th>
<th>Days from Appearance of Blossom Buds to Appearance of Open Blossoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 application, 9 weeks old</td>
<td>76</td>
<td>11.9 19.4 27.6 22</td>
<td></td>
</tr>
<tr>
<td>1 application, 11 weeks old</td>
<td>76</td>
<td>13.7 19.6 27.3 25</td>
<td></td>
</tr>
<tr>
<td>3 applications, 9, 11, and 13 weeks old</td>
<td>72</td>
<td>17.4 24.7 31.6 25</td>
<td></td>
</tr>
<tr>
<td>3 applications, 11, 13, and 15 weeks old</td>
<td>76</td>
<td>13.5 20.3 28.5 24</td>
<td></td>
</tr>
</tbody>
</table>
they were 9, 11, and 13 weeks old were more effective in hastening seed stalk elongation than other treatments.

Table VI shows the effect of growth regulators on field grown Cornell 456 lettuce. In this case, significant variations from the control were observed in only two instances. At the time of the first measurement, the seed stalks of plants treated with TIBA were significantly shorter than those of control plants. At the time of the last measurement, the seed stalks of plants treated with 2,4-D were significantly longer than those of control plants.

The behavior of treated Cornell 456 lettuce plants indicates again, as in the case of Slobolt lettuce, that varieties which are resistant to bolting are resistant as well to the effects of growth regulators on seed stalk elongation.

TABLE VI. Effect of Growth Substances on Seed Stalk Development in Field Grown Cornell 456 Lettuce

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>95</td>
<td>16.7</td>
<td>23.5</td>
<td>30.2</td>
</tr>
<tr>
<td>2,4-D</td>
<td>94</td>
<td>16.7</td>
<td>25.7</td>
<td>32.3*</td>
</tr>
<tr>
<td>TIBA</td>
<td>95</td>
<td>14.3*</td>
<td>21.9</td>
<td>28.6</td>
</tr>
<tr>
<td>ClPP</td>
<td>95</td>
<td>15.6</td>
<td>22.3</td>
<td>29.2</td>
</tr>
</tbody>
</table>

* Significant variation from water treatments. Student's "t" test.
Experiment 5

Methods

Although Coulter (7) found that ClPP retarded seed stalk development in celery, no instance of significant retardation by ClPP was observed in the lettuce experiments described above. In fact, ClPP tended to hasten seed stalk development in all of the experiments where it was used. Since Coulter’s results were obtained by treating young celery plants before induction had occurred, it was thought that seed stalk development in lettuce might be retarded if the plants were treated at a relatively young stage. It was thought, also, that concentrations of ClPP greater than those already used might have a retarding, rather than an accelerating, effect of seed stalk development.

To test these possibilities, plots of one-month-old Grand Rapids lettuce plants were treated on October 17, 1947 with eight different concentrations of ClPP. The concentrations used were 5, 25, 50, 75, 100, 200, 300, and 400 ppm. An additional plot was left untreated as a control. The plants used in this experiment were grown in the greenhouse in the manner already described. The treatment plots contained five plants each and were replicated three times in randomized blocks. Records were kept of the dates of the first appearance of blossom buds and of first open blossoms. After elongation had occurred, seed stalks were measured at weekly intervals.
Results

Table VII shows the effect of the C1PP treatments on the dates of the appearance of blossom buds and first open blossoms. It can be seen from this table that treatments of 75, 300, and 400 ppm of C1PP significantly delayed the appearance of blossom buds. None of the treatments, however, had a significant effect on the length of the interval between the appearance of blossom buds and the appearance of open blossoms.

Figure 3 shows the effect of the C1PP treatments on the elongation of seed stalks on the treated plants. The points on the graph indicate the average heights of the seed stalks of the plants of each treatment on the date specified, and the vertical distances between points indicate the increments in length of seed stalk.

On December 20, none of the treatments varied significantly from the control in respect to length of seed stalk. On subsequent dates, plants treated with the concentrations given below had seed stalks significantly shorter than the control:

December 27 - 25, 75, 100, 300, and 400 ppm
January 3 - all concentrations except 5 ppm
January 10 - all concentrations
January 17 - all concentrations
January 24 - all concentrations except 5 and 200 ppm
TABLE VII. Effect of Various Concentrations of ClPP on the Time Required for the Appearance of Blossom Buds and Open Blossoms on Lettuce Plants

<table>
<thead>
<tr>
<th>Concentration ClPP, ppm</th>
<th>Days from Seeding to Treatment</th>
<th>Days from Treatment to Appearance of Blossom Buds</th>
<th>Days from Appearance of Blossom Buds to Open Blossoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>82</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>85</td>
<td>33</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
<td>86</td>
<td>32</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>85</td>
<td>31</td>
</tr>
<tr>
<td>75</td>
<td>30</td>
<td>87*</td>
<td>33</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>86</td>
<td>33</td>
</tr>
<tr>
<td>200</td>
<td>30</td>
<td>85</td>
<td>33</td>
</tr>
<tr>
<td>300</td>
<td>30</td>
<td>89*</td>
<td>32</td>
</tr>
<tr>
<td>400</td>
<td>30</td>
<td>88*</td>
<td>33</td>
</tr>
</tbody>
</table>

* Significant variation from control as determined by analysis of variance.
LEN T H O F S E E D S T A L K (I N C H E S )

CONCENTRATION CL PP (PPM)

Figure 3. Effect on subsequent seed stalk elongation of various concentrations of CLPP applied to month-old lettuce plants.
During the weeks indicated, plants receiving the following concentrations of ClPP showed a significantly smaller increment in seed stalk length than the control plants:

December 20-27; 25, 100, 300, and 400 ppm
December 27-January 3; 5, 25, 75, 100, 300, and 400 ppm
January 3-10; 25, 75, 100, 200, 300, and 400 ppm
January 10-17; 5, 25, 75, 300, and 400 ppm
January 17-24; none

Two interesting points are brought out by figure 5 and the discussion above. The first point is that there were no significant differences in seed stalk length on the first date of measurement, December 20. The second point is that, during the last week of the experiment, January 17-24, there were no significant differences in the increment in seed stalk length. It is apparent from these facts that all of the significant effects of the various concentrations of ClPP on seed stalk elongation took place between December 20 and January 17. That was the period during which the seed stalks elongated from approximately 7 inches to approximately 30 inches in length.
The lettuce experiments already described showed that, under certain conditions at least, growth regulating substances are capable of influencing the rate of seed stalk development. They did not indicate, however, whether growth substances influenced only the rate of seed stalk elongation, or whether they also affected the date of the differentiation of floral organs. Neither did these experiments give any indication of the role of the natural phytohormones in seed stalk development.

In order to gain information on the latter subjects, an experiment was set up to determine the effects of variety, induction treatment, and the application of growth regulators on the morphological differentiation, phytohormone content, and elongation of celery stems. Celery, rather than lettuce, was used in this experiment because it is easier to control seed stalk initiation in celery, and because celery is not as seriously injured in the field by aster yellows as is lettuce.

General Procedure

Two varieties of celery were selected for this study, Cornell 19, which bolts quite readily, and Tall Strain, Non-Bolting Golden Plume, a variety resistant to bolting.
Seeds of the two varieties of celery were planted in No. 2 vermiculite on February 9, 1948. The resulting seedlings were transplanted to flats of muck soil on March 12 to 17. They were set 70 seedlings to a flat with a spacing of 2 inches by 2 inches between seedlings. After the seedlings had been transplanted, they were placed in a 60-70°F. greenhouse.

On April 13, plants from each of the two varieties were separated into three groups for treatment with growth substances. Those of the first group were treated with 50 ppm 2,4-D, those of the second group with 100 ppm ClPP, and the plants of the third group received no treatment.

The growth substances were sprayed onto the plants with one quart "Sure-Shot" compressed air sprayers. Each plant was thoroughly wet with the solution.

Two days after the celery plants had been treated with growth substances, half of the plants of each treatment were moved to a cold frame where they remained until moved to the field on May 19. The other plants were left in the greenhouse until they were similarly transferred to the field on May 19.

The treatments thus applied to the celery plants
are summarized below:

A. Cornell 19  No growth substance  Greenhouse
B. Cornell 19  No growth substance  Cold frame
C. Cornell 19  50 ppm 2,4-D  Greenhouse
D. Cornell 19  50 ppm 2,4-D  Cold frame
E. Cornell 19  100 ppm ClPP  Greenhouse
F. Cornell 19  100 ppm ClPP  Cold frame
G. Golden Plume  No growth substance  Greenhouse
H. Golden Plume  No growth substance  Cold frame
I. Golden Plume  50 ppm 2,4-D  Greenhouse
J. Golden Plume  50 ppm 2,4-D  Cold frame
K. Golden Plume  100 ppm ClPP  Greenhouse
L. Golden Plume  100 ppm ClPP  Cold frame

On April 14, one-third ounce of 12-52-17 fertilizer and one-third ounce of ammonium nitrate were applied in solution to each flat.

On April 30, the tops of all of the plants were clipped in the manner normally practiced in commercial production in Michigan.

The plants were set in the field into plots of muck soil on the Muck Soils Experimental Farm of Michigan State College. The transplanting was done May 22-26. Plants of each treatment were set into two areas in the field. The first area contained five blocks, each of which was composed of 12 randomized plots corresponding to the 12 treatments
described above. Each plot in this area consisted of a row 33 feet long and containing 66 plants. The plants used for morphological observations and phytohormone determinations were taken from this area.

The second area of plants was arranged in the same way as the first with the exception that each plot consisted of a row 7 feet long and containing 10 plants. These plants were allowed to produce seed stalks which were measured at weekly intervals.

Morphological Observations

Methods

On April 27 and each week thereafter until July 27, five celery plants for morphological observations were taken from each of the treatments described above. Each of these plants was taken from a different flat while the plants were in the greenhouse and cold frame and from a different plot after the plants had been moved to the field. After the leaves and roots had been removed from the selected plants, excess tissue was trimmed from the area around the growing points. The pieces of tissue thus trimmed, were killed and fixed in F.A.A. killing and fixing solution. The tissues were then dehydrated by the tertiary butyl alcohol method described by Johansen (15). The dehydrated tissues were
embedded in paraffin and sections 14 microns thick were cut from them on a rotary microtome. The cut sections were stained with Delafield's Haematoxylin and mounted in balsam. After slides had been prepared from the celery growing points, they were observed under a microscope and a record was kept of the date of the first ascertainable seed stalk differentiation in plants from each treatment.

Results

Stages in the development of terminal growing points of celery stems are shown in figure 4. The first three stages in figure 4 show a widening of the growing point. This widening was not associated with seed stalk development. It occurred in all of the plants regardless of whether floral primordia and seed stalk elongation subsequently occurred. Stages 4, 5, and 6, on the other hand, were associated with seed stalk development. None of these stages occurred in any plants which did not subsequently produce seed stalks. Stage 4 shows the first indication of seed stalk elongation. In this stage, the internodes in the region of the growing point have elongated slightly to produce a cone-shaped tip on the celery stem. Stage 5 is characterized by a still greater elongation in the vicinity of the growing point and by the appearance of primordial axillary shoots. In stage 6, recognizable floral primordia
Figure 4. Stages in the development of terminal growing points of celery stems.
are present and the axillary shoots are considerably larger than in stage 5.

In this experiment, the growing points of the plants of all treatments remained in stage 1 from the time of the first collection of samples on April 27 through the collection on June 8. On June 15, all of the plants had terminal growing points which had reached stage 3. Only treatments B, D, and F (Cornell 19 plants which had received an induction treatment in the cold frame) produced plants whose terminal growing points advanced beyond stage 3. The growing points of all other plants remained in stage 3 until the end of the experiment on July 27.

Plants in the three treatments which produced seed stalks had terminal growing points in stage 4 of figure 4 approximately June 29. Their growing points had reached stage 5 about July 6, and stage 6 about July 13. Beyond stage 3, however, the development of growing points of different plants proceeded at different rates so that not all of the plants of a single treatment were in the same stage at a given date.

The effect of growth substances on the date on which terminal growing points of plants of treatments B, D, and F had reached stage 6 of figure 4 is shown in Table VIII. It can be seen from this table that none of the growing points of the treated plants contained recognizable floral
primordia on June 29, but that all of the growing points of the five plants of each treatment collected on July 27 contained floral primordia. Between these two dates, the rate of differentiation of floral primordia appeared to be more rapid in control plants and plants treated with ClPP than in plants treated with 2,4-D. These results, however, could hardly be considered conclusive.

TABLE VIII. The Effect of Growth Substances on the Differentiation of Floral Primordia in the Terminal Growing Points of Celery Stems

<table>
<thead>
<tr>
<th>Treatment</th>
<th>June 29</th>
<th>July 6</th>
<th>July 13</th>
<th>July 20</th>
<th>July 27</th>
</tr>
</thead>
<tbody>
<tr>
<td>B Control</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>D 2,4-D</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>F ClPP</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Phytohormone Determinations

Methods

On April 27, and every week thereafter until July 27, 25 plants were taken from each of the treatments listed above for phytohormone extractions. The plants were taken in proportional numbers from each flat while the plants were
in the greenhouse and cold frame, and from each plot after the plants had been moved to the field.

Upon removal from the soil, these plants were severed at the juncture of their stems and roots. After this, the leaves were pulled from the stem plate and discarded. These operations produced pieces of stem tissue free from roots and leaves. In the case of the young plants, these pieces of tissue were sliced and prepared for extraction without further treatment. From older plants, a cylinder of tissue about 5 mm. in diameter and 4 mm. high was cut from the stem tissue surrounding the apical meristem. This was then sliced and prepared for extraction. In both the young and old plants, the sliced stem tissue was frozen immediately with dry ice. The procedure outlined provided three to five grams of frozen tissue from each of the 12 treatments. This frozen tissue was taken to the laboratory where three-gram portions were weighed out from each lot of tissues and placed in Erlenmeyer flasks. The tissue in each flask was then covered with 20 ml. of freshly distilled, peroxide-free ether. At the end of six hours, this ether was decanted and was replaced with another 20 ml. portion. At the end of another 15 hours, the second portion of ether was decanted and replaced with a third. The third portion was decanted at the end of another 22 hours. The first two portions of ether were refrigerated until the third portion was decanted.
Then all three portions were placed together in an evap-
oration dish and to them was added another 5-ml. portion of
ether used in rinsing the tissue in the Erlenmeyer flask.

The ether in the evaporating dish was evaporated
before a fan until only an aqueous residue remained. This
residue was further evaporated until less than 2 ml. re-
mained in each evaporating dish. Each residue was then
brought to a volume of 2 ml. to produce the solution to be
assayed for phytohormone content.

The phytohormone* determinations were made by means
of a modification of the cucumber injection method described
by Kribben (17). This method was used primarily because
facilities for the standard Avena test were not available.
There was, however, another reason for using this test.
Experiments with selective herbicides have shown that some
growth substances produce an effect on broad leaf plants
different from their effect on the grasses. Therefore, it
might be expected that a dicotyledonous plant like the
cucumber would give a better indication of the phytohormone
content of celery growing points than would the Avena
coleoptile.

* The term phytohormone as used here refers to substances
capable of causing curvature of cucumber hypocotyls. It is
not known whether these are the same substances as those causing
curvature of the Avena coleoptile and commonly called auxins.
The cucumber test as described by Kribben was carried out on a revolving table in the greenhouse. It was found in tests preliminary to this experiment, however, that the accuracy of the test could be improved by placing the plants in a high humidity incubator previous to testing. If the plants were not placed in a saturated atmosphere previous to injection, curvature was produced even by injections of pure water. This apparently resulted from unilateral hydration of the hypocotyl and may account for some of the erratic results obtained in the early use of this test.

To supply seedlings for the cucumber tests, seeds of the National Pickling variety were planted in No. 2 vermiculite. As soon as the seedlings had emerged and their cotyledons had separated, they were transplanted to trays of soil in the greenhouse. The soil trays were three inches wide, three inches deep, and 17 inches long. Ten seedlings were transplanted into a single row in each tray in such a way that their cotyledons were arranged crosswise of the long axis of the tray as indicated in figures 5 and 6. About four days after transplanting, as soon as the first true leaf was visible, the seedlings were moved to an incubator in a dark room. The incubator was kept at 30°C and its air was kept saturated with moisture by placing open containers of water on its shelves. The trays of cucumbers were also well watered before being placed in the incubator.
Figure 5. Method of injecting phytohormone extracts into cucumber cotyledons.

Figure 6. Response of cucumbers to hypodermic injections. 
Left - 1 ppm indole acetic acid. Right - water.
After the cucumber seedlings had been in the incubator for 24 hours, the trays were removed one by one, and the extracts to be tested were injected into the seedling cotyledons. All of these operations were carried out with the aid of a red "safe" light in a dark room so that there would be no phototropic curvature of the hypocotyls.

The method followed in injecting solutions into cucumber cotyledons is illustrated in figure 5. At about the center of each cotyledon there are two veins which branch from the midrib. The hypodermic needle was inserted between the upper and lower epidermises of the cotyledon in the area in the axil of one of these veins. After the needle had been inserted, .05 ml. of the extract solution was injected into the cotyledon. A small droplet of solution was usually forced out around the needle, but this droplet was later reabsorbed. The injections were made with a tuberculin syringe graduated in hundredths of a milliliter and equipped with a 27 gauge needle one-half inch long.

In making the phytohormone tests, extract solutions were injected into one cotyledon of each cucumber. The extract from each treatment was injected into one tray of ten seedlings. Thus, 12 trays of seedlings received injections of extracts each week. In addition to the extract injections, one tray of seedlings was injected with water and another with a 1 ppm solution of Indole acetic acid. These two
treatments served as controls. Figure 6 shows trays of seedlings having received these two treatments.

After treatment, the cucumber seedlings were returned to the incubator for a period of two hours. At the end of the two-hour period, they were removed from the incubator and the curvature of the hypocotyls was measured. In order to measure the curvature of the hypocotyls, the hypocotyl was severed just below the cotyledons and again at the surface of the soil. The lower end of the hypocotyl was placed along a vertical line on a piece of paper. A line was then traced along the side of the upper end of the hypocotyl where it departed from the vertical line. Later, this line was extended and the angle of divergence from the vertical line was measured with a protractor. This angle of divergence represented the curvature of the hypocotyl produced by the injected extract, plus curvature occurring naturally in the growth of the seedlings. The curvature produced by a given extract, therefore, was obtained by subtracting the degrees of curvature of the control (water injected) seedlings from the degrees of curvature of the seedlings injected with the extract in question.

Results

The phytohormone determinations made in this experiment indicated that, in respect to phytohormone activity,
there are three critical periods in the development of the terminal growing points of celery stems. In this experiment, the first critical period began about three weeks after the plants had been exposed to induction temperatures in the cold frame and extended over a period of four weeks (May 4-25). The second period (June 15-22) occurred at the time that heart development was taking place. This was approximately two weeks previous to the elongation of seed stalks and differentiation of flowers. The period of initial seed stalk elongation and differentiation of flower primordia (July 6-13) constituted the third critical period. On dates other than those just indicated, the phytohormone activity of the celery stem extracts was so low that it could not be measured accurately by the methods employed in this experiment.

The results of phytohormone determinations made during the critical periods described above are presented in Table IX. The negative values in Table IX represent curvature of the cucumber hypocotyl toward the cotyledon receiving the injection. Positive values represent curvature away from the injected cotyledon. Curvature toward the injected cotyledon (positive curvature) was the result of inhibition of growth by the injected extract. Curvature away from the injected cotyledon (negative curvature) was the result of stimulation of growth by the injected extract.
Extracts causing inhibition were encountered only on June 15 and even these extracts, upon dilution, were found to stimulate growth. It is apparent, therefrom, that the growth inhibition produced by these extracts was merely an expression of their high concentration of phytohormones. On all dates other than June 15, the curvature of cucumber hypocotyls produced by celery stem extracts was less than that produced by the standard solution of 1 ppm Indole-acetic acid.

It can be seen from Table IX that, on May 11, 18, and 25, the stem tissue of plants in the cold frame had a significantly higher phytohormone content than that of plants in the greenhouse. Also, on May 4 and 11, stem tissue of the Cornell 19 plants was higher in phytohormone content than that of the Golden Plume plants. It is apparent from the data presented that differences resulting from the induction treatment occurred later than those due to variety. The reason for this is that the Cornell 19 plants responded to the cold treatment more rapidly than the Golden Plume plants. Therefore, there were significant differences between the two varieties relatively early in the induction period and then, after both varieties had responded to the cold treatment, the difference between varieties became insignificant, but the differences between cold and warm temperature treatments became significant.

On June 15 and 22, the situation was the reverse of that occurring during the induction period. At this time,
TABLE IX. Effect of Experimental Factors on the Phytohormone Content of the Meristem Region of Celery Stems

<table>
<thead>
<tr>
<th>Experimental Factor</th>
<th>Degrees Curvature of Cucumber Hypocotyls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May 4</td>
</tr>
<tr>
<td>Induction Treat.</td>
<td></td>
</tr>
<tr>
<td>Cold frame</td>
<td>13.7</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>16.2</td>
</tr>
<tr>
<td>Differences</td>
<td>2.5</td>
</tr>
<tr>
<td>Variety</td>
<td></td>
</tr>
<tr>
<td>Cornell 19</td>
<td>22.3</td>
</tr>
<tr>
<td>Golden Plume</td>
<td>7.6</td>
</tr>
<tr>
<td>Differences</td>
<td>14.7*</td>
</tr>
<tr>
<td>Growth Substance</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.5</td>
</tr>
<tr>
<td>2,4-D</td>
<td>13.7</td>
</tr>
<tr>
<td>ClPP</td>
<td>7.7</td>
</tr>
<tr>
<td>Differences</td>
<td>5.2</td>
</tr>
<tr>
<td>Control-2,4-D</td>
<td>0.8</td>
</tr>
<tr>
<td>Control-ClPP</td>
<td></td>
</tr>
</tbody>
</table>

* Stage of Development Induction Predifferentiation Differentiation

* Significant difference. Student's "t" test.
the phytohormone content of the stem tissue of Cornell 19 plants was lower than that of the Golden Plume plants and the stem tissue of plants which had received a cold exposure was lower in phytohormone content than that of plants which had received no cold exposure. It is impossible, however, to determine the significance of differences observed on June 15 because the negative readings recorded were not true mathematical representations of the phytohormone activity of the extracts involved.

On July 6-13, the stem tissue of Cornell 19 plants was significantly higher in phytohormone content than that of Golden Plume plants. The stem tissue of the cold-induced plants also averaged somewhat higher than that of plants which had received no induction treatment. The application of growth substances did not at any time produce any significant effect on the phytohormone content of the stem tissue of the celery plants.

Bolting Observations

Methods

Plants in the field area set aside for bolting observations were watched closely for seed stalk elongation, and after seed stalks became apparent on July 23, their heights were measured at weekly intervals until August 27. The measurements were made from the surface of the soil to the highest part of the seed stalk.
Results

Treatments B, D, and F were the only three treatments of the twelve in this experiment which resulted in any seed stalk elongation. These three treatments were the treatments containing Cornell 19 plants which had been exposed to an induction period in the cold frame. The plants of treatment F (C1PP treatment) were not significantly different in seed stalk height from those of treatment B (Control treatment) at any time during the period in which measurements were taken. The plants of treatment D (2,4-D treatment), on the other hand, had seed stalks which were significantly shorter than those of the control plants on the first two dates of measurement (July 23 and 30) but which were not significantly different from the controls on succeeding dates. The reason for the significant differences in the early stages of elongation and lack of significance in the later stages are indicated by the data in Table X. This table shows the weekly increments in the length of seed stalks of the celery plants in this experiment. It can be seen from this table that during the first two weeks of elongation the growth increments for the 2,4-D treated plants were smaller than those for the control plants. During the last two weeks, however, the growth increments were essentially the same for both lots of plants. The growth increments for the C1PP treated plants were essentially the same as those of the control plants throughout the period of elongation.
TABLE X. The Effect of Growth Regulating Substances on the Weekly Growth Increment of Seed Stalks in Celery

<table>
<thead>
<tr>
<th>Period of Growth</th>
<th>Control</th>
<th>C1PP</th>
<th>2,4-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 23-30</td>
<td>5.3</td>
<td>5.5</td>
<td>3.5</td>
</tr>
<tr>
<td>July 30-Aug. 6</td>
<td>4.9</td>
<td>5.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Aug. 6-13</td>
<td>6.4</td>
<td>6.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Aug. 13-20</td>
<td>5.3</td>
<td>5.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Aug. 20-27</td>
<td>7.3</td>
<td>8.0</td>
<td>7.5</td>
</tr>
</tbody>
</table>

It appears from the results just presented that the effects of growth substances on seed stalk development in celery, as in lettuce, occur during the early stages of elongation.
DISCUSSION

Variability in the Response of Plants
to Synthetic Growth Substances

One of the outstanding features observed in the application of synthetic growth substances to lettuce and celery plants was the variability in the results obtained. In several experiments, for instance, the rate of seed stalk elongation in lettuce tended to be accelerated by application of ClPP to two-month-old plants. In one experiment where ClPP was applied to one-month-old plants, on the other hand, the rate of seed stalk elongation was retarded. Since the same retarding effect was produced by concentrations ranging from 5 to 400 ppm, it does not appear that the observed response was a function of concentration as it was in pineapple experiments reported by Clark and Kerns (5).

In celery, Wittwer, Coulter, and Carolus (32) and Coulter (7) observed that seed stalk elongation was completely inhibited during the 1947 season by the application of 100 ppm ClPP and that it was accelerated by 50 ppm 2,4-D. The results presented above, on the other hand, show that, during the 1948 season, 100 ppm ClPP had no effect on seed stalk elongation and 50 ppm 2,4-D caused a slight retardation of elongation. These results were obtained in spite of the fact that the procedure followed in 1948 was as nearly as possible identical to that of 1947.
It is apparent that conditions not under experimental control influenced the results of these experiments. What these conditions were was not determined. In lettuce experiments, the age of the plant appeared to be the factor determining the response to growth regulators applied. In celery, however, this did not appear to be the case.

Variations in the flowering responses of plants to growth regulators were also reported by Thimann and Lane (23) for tomato plants, and by Cooper (6) for pineapple plants.

Transmission of the Effects of Growth Substances to Second Generation Plants

Lettuce experiment 3, in which plants were grown from seeds produced by Grand Rapids plants which had been treated with growth substances, demonstrated clearly that the effect of growth substances on seed stalk elongation can be transmitted from one generation to the next. Just how this transmission was accomplished, however, is not clear. It is hardly possible that the applied growth substances could have had a direct effect upon the developing embryo because the last application of growth substances was made fully a month before fertilization occurred. Possibly, though, the growth substance, or some derivative of it, was stored in the plant tissue and was later trans-
located to the developing seed and thereby transmitted to the succeeding generation. Another possibility is that the growth regulators, at the time of application, produced a more or less permanent physiological alteration of the protoplasm and that this alteration was transmitted through the embryo and remained to exhibit itself at the time of seed stalk elongation in the second generation.

The effect of synthetic growth substances on the progeny of treated plants was reported previously by Hitchcock and Zimmerman (14). They observed that dandelion plants which had been treated with 2,4,6 trichlorophenoxyacetic acid produced progeny which were markedly delayed in their flowering. Some treatments applied by Hitchcock and Zimmerman, however, resulted in the production of seeds of reduced viability. Seeds produced by the treated lettuce plants described above showed no loss of vitality and the seedlings produced by them displayed no visible formative effects.

Significance of Phytohormone Determinations

Before the significance of the results of phytohormone determinations herein reported can be evaluated properly, it will be necessary to discuss briefly some of the concepts that have been presented concerning the nature of the flower-inducing substance or substances.
Cailahjan (2) and (3) suggested that flowering in plants is caused by a hormonal substance which is produced in the leaves and transported to the growing point. He proposed the name "florigen" for this substance and stated it was distinct from the previously described auxins.

Cholodny (4), on the other hand, suggested that florigen might not be a specific flower producing substance at all but that it might be merely an ordinary auxin or auxin-like substance such as the natural phytohormones capable of causing geotropic and phototropic curvature in plants. He pointed out that the method used by Cailahjan to test for auxin in his plants may not have been sensitive enough to detect the small differences in auxin level which may be capable of causing flowering. It should be noted, too, that Cailahjan assumed that any ordinary auxin-like material present in his experimental plants could be detected by the Avena test. Recent experiences in the use of synthetic growth regulators as selective herbicides indicate that this may not be true. Results obtained with these substances suggest that there may exist natural phytohormones capable of producing pronounced effects on some plants, such as the bean, but having little or no effect on others, such as the grasses.

In an effort to identify the flower producing substance in Xanthium, Hamner and Bonner (12) treated shoots with a number of substances, including indoleacetic acid and
yeast extract, to determine whether these substances were capable of causing flowering. The fact that none of them stimulated flowering was presumed to be evidence that none of them was identical to the natural flower initiating substance. Such a conclusion seems to be based on the concept that flowering is caused by the mere presence within a plant of a certain flower producing substance. The theory of Cailahjan (2) and (3), however, suggests that such a substance must reach the growing point in sufficient quantities and must remain there for a sufficient period of time before flower induction can occur. The materials applied to Xanthium shoots by Hamner and Bonner may not have been applied in the proper concentration to produce flowering, or they may not have been translocated to the growing points, or they may not have remained at the growing points for a sufficient period to cause flower initiation.

That the distribution of phytohormones within a plant may be more important than the mere presence of a special substance in causing flowering, is suggested by an experiment performed by Curtis and Chang (8). By a special technique, these investigators grew celery plants under conditions in which the leaves and the crowns were exposed to different temperatures. Their experiments showed that the temperature of the crown and not that of the leaves determined whether a plant would produce seed stalks. If it
is assumed, as is commonly believed, that the flower producing substance is produced in the leaves, then it is apparent that, in the celery plants studied by Curtis and Chang, the temperatures of the crown must have controlled the translocation of that substance to the crown or it must have controlled the response of the crown to that substance.

It will be remembered that in the phytohormone determinations of the terminal regions of celery stems reported above, it was found that those conditions which resulted in flower differentiation and seed stalk elongation (cold frame treatment and Cornell 19 variety) were associated during the induction period with a significantly higher phytohormone content at the stem apices than those conditions which did not favor flowering. It would be presumptuous, of course, to claim that a direct cause and effect relationship has been established in this instance. Nevertheless, such a possibility does present itself.

The possibility that flowering in plants may be the result of the accumulation of ordinary phytohormones in the growing point is suggested also by an experiment performed by Van Overbeek and Cruzado (29). These investigators placed pineapple plants in a horizontal position and observed that these horizontally placed plants produced inflorescences whereas vertical control plants did not. Presumably, the horizontal position resulted in an accumu-
lation of auxin in the lower half of the terminal growing point and this accumulation of auxin caused the production of flower primordia in that portion.

Seemingly, the results obtained by Curtis and Chang, those obtained by Van Overbeek and Cruzado, and those obtained in our experiment do not conform very well to Cailahjan's florigen concept of flower initiation. These results are explained better by Cholodny's theory and still better by a more recent theory of Van Overbeek, deVazquez, and Gordon (30). These latter investigators analyzed the leaf bases and stem apices of pineapple plants for free and bound auxin. They found that the stem apices contained relatively large amounts of free auxin but little or no bound auxin. The leaf bases, on the other hand, contained comparatively large amounts of bound auxin but little free auxin. From this and other evidence (27) (28) (29) they concluded that flowering is caused by the transformation of the bound auxin of the leaf bases to free auxin and by the accumulation of this free auxin in the apical meristem of the plant.

This recent theory of Van Overbeek et al is in accord with present experimental evidence concerning flowering. It provides an explanation of the role of leaves in the initiation of flowering and it provides a possible explanation of the results obtained by Curtis and
Chang (8). (The temperature of the crowns of celery plants in their experiment may have regulated the conversion of bound auxin to free auxin in that part of the plant.) This theory is supported by the results obtained in the experiment of Van Overbeek and Cruzado (29) in which flowering was caused by geotropic stimulation and by the results of phytohormone determinations herein reported. It also has an advantage over the florigen theory in that it is based on biochemical assays of materials known to exist in plants and therefore does not require the invention of a hypothetical flower forming substance.
SUMMARY AND CONCLUSIONS

A series of experiments was conducted to determine the effect on seed stalk development of spraying aqueous solutions of several synthetic growth substances on lettuce and celery plants at various stages of development.

With lettuce, the synthetic growth substances used were 2,4-dichlorophenoxyacetic acid (2,4-D), alpha ortho chlorophenoxypropionic acid (C1PP), 2,3,5 triiodobenzoic acid (TIBA), para chlorophenoxyacetic acid (ClPA), and naphthaleneacetic acid (NAA). The effect of the chemicals was determined by periodic measurements of seed stalk heights and by observation of the dates of appearance of blossom buds and of first open blossoms.

In general, when any of these substances was applied to 8-12 week old plants, seed stalk elongation was hastened, the effect being greater for repeated than for single applications. In contrast, however, in the one case in which C1PP was applied to month-old plants, there was a significant retardation in the rate of seed stalk development.

The effects of growth substances, whether retarding or stimulating, occurred during the early stages of seed stalk elongation and were associated with a corresponding effect on the date of flowering.

Differences up to seven days in the date of the first appearance of blossom buds were produced by the
application of growth regulators.

The slow bolting varieties, Slobolt and Cornell 456, were less affected by growth regulators than Grand Rapids which bolted readily.

With the latter variety, alterations in seed stalk development induced by the application of growth substances were, in part, transmitted to the progeny.

In celery, an experiment was conducted to determine the effect of variety, induction treatment, and growth regulators (2,4-D and ClPP) on the morphological development and phytohormone content of stem apices and on subsequent seed stalk development.

Only cold induced Cornell 19 plants produced seed stalks and among these, elongation was slightly retarded by 50 ppm 2,4-D and was not affected by 100 ppm ClPP.

On a comparative basis, those factors which caused seed stalk formation (cold frame treatment and Cornell 19 variety) resulted in a high concentration of phytohormones in stem apices during the induction period, a low concentration during the period previous to flower differentiation, and a high concentration again at the time of the morphological differentiation of flower primordia.

The significance of the results of phytohormone determinations and alterations in seed stalk development
induced by synthetic growth regulators is discussed in relation to theories of the biochemical mechanism of flower initiation.

Because of the variability in the responses of plants to the application of synthetic growth regulators, these substances cannot as yet be recommended for the practical control of seed stalk development in lettuce and celery. Possibly, though, at some future date when our knowledge of the action of growth substances is more complete, it will be practical to use them for this purpose.
LITERATURE CITED


27. VAN OVERBEEK, J. Flower formation in the pineapple plant as controlled by 2,4-D and naphthalene acetic acid. Science 102:621. 1945.


