Physiology of Flowering in Peas

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The garden pea, *Pisum sativum* L., has been used rather extensively in studies of flowering (1, 2, 10, 11, 14, 17, 21, 22, 25). The early varieties commonly flower at the ninth or tenth node and are insensitive to vernalization and photoperiod (1). The late varieties, which commonly do not flower below the 15th node, usually behave as quantitative long-day plants and are vernalizable (1, 26).

Many workers who have studied the process of flowering have believed in the concept of a specific flowering hormone which controls floral initiation in a positive and promotive manner (e.g. 8, 12, 18, 19). However, there is no unanimity of opinion that floral initiation is mediated by a flower-promoting hormone in all angiosperms or in all varieties of *Pisum*. Highkin (10) reported on the preparation and flower-promoting activity of pea seed diffusates, and he suggested that the active principle might be a flowering hormone or hormone precursor. Barber and his associates have studied flowering in several varieties of peas (1, 2, 22, 25). Barber (1) developed in full his concept that flowering in late varieties of *Pisum* is mediated by the destruction of a flower-inhibiting hormonal substance or "colysanthin," which is destroyed preferentially by low temperatures and long days. Early varieties of *Pisum* were believed not to produce colysanthin.

Thus the current information regarding the hormonal regulation of flowering in peas is marked by conflicting interpretations. In view of this situation a series of experiments has been conducted to investigate the flowering of the late pea variety Dwarf Telephone. The techniques used most extensively were vernalization and devernalization, treatments with aqueous pea seed extracts and treatments with glutathione and 2-thiouracil.

Materials & Methods

The Dwarf Telephone variety of *Pisum sativum* L. was used as the experimental plant. All pea seeds were surface-disinfected with 5% sodium hypochlorite and then rinsed with distilled water. The disinfected seeds were soaked for 4 to 12 hours, depending upon the particular experiment, in distilled water, chemical solution, or pea extract prior to the beginning of cold treatment or to direct planting. Seedlings were vernalized in plastic trays of sterile vermiculite.

After seedlings had been subjected to cold treatment or other pre-plant treatment, they were transplanted into sterile soil in the greenhouse. The plants were grown under a 16-hr photoperiod; the photoperiod temperature ranged from 20 to 22 C, and the nyctotherm temperature from 14 to 16 C. The light intensity at plant level during the photoperiod varied between 1,000 and 2,000 ft-c. All the experimental plants were grown to anthesis, and they were harvested when the internode below the first flower had elongated.

Results

Vernalization Experiments. The vernalization procedures were modified from the methods of Highkin (11). Seeds were soaked in distilled water for 4 to 6 hours. The germinating seeds were then placed immediately in a dark cold chamber where lots received cold treatment at 4 to 7 C for periods of 7, 14, 21, 28, 35, or 56 days. A group of controls was planted in the greenhouse after imbibition of distilled water. The experiment was repeated at least once for each treatment period.

Cold treatment (7-28 days) induced three measurable effects: A: reduction in the number of nodes to the first flower (fig 1), B: decrease in height to the first flowering node (fig 2), and C: decrease in the number of days to anthesis (fig 3). Data on peas vernalized for 35 and 56 days are not reported since it was found that approximately 28 days of cold treatment induced maximum effects.

If average internode length at anthesis is plotted versus the number of days of cold treatment, as in figure 4, it can be seen that internode length is apparently not constant, as would be expected if the reduction in height to the first flowering node were a simple function of reduction in the number of nodes to the first flower.

Negative results were obtained when 14-day-old peas were cold treated for 28 days, as far as any effect on the number of nodes to flower is concerned. The ontogenetic limit to thermoinduction of early flowering clearly is attained before the plants are 2 weeks old.
In another experiment germinating pea seeds were vernalized for 14 and 28 days at 6 to 7°C and then subjected to 10 continuous days at high temperature. During the devernalization procedure the plants were given a 16-hr photoperiod, and the air temperature at plant level was maintained through the devernalization period at 30 to 32°C. Ten days of heat treatment immediately following vernalization partially annulled the vernalization effect on the flowering node in both the 14- and 28-day groups. The vegetative effect was not significantly reversed by the heat treatment.

Experiments With Aqueous Extracts From Pea Seeds. The methods employed in the preparation of aqueous extracts from pea seeds were modified from the method of Highkin (10). Two variations on a basic method of preparation are described below.

I. To each of seven 250-ml Erlenmeyer flasks were added 50 ml of glass-redistilled water. To one other flask were added 50 ml of 2% sucrose solution and a wad of spun glass. All flasks were plugged with cotton and autoclaved. Pea seeds (50) were added to each of six flasks containing water, and

![Fig. 1](image1.png)

**Fig. 1.** Effect of cold treatment on the number of nodes to the first flower.

![Fig. 2](image2.png)

**Fig. 2.** Effect of cold treatment on the height to the first flowering node.

![Fig. 3](image3.png)

**Fig. 3.** Effect of cold treatment on the number of days to anthesis following removal from the cold chamber.

![Fig. 4](image4.png)

**Fig. 4.** The relationship between average internode length at anthesis and the number of days of cold treatment.
these flasks were incubated in a dark cold chamber at 4 to 7°C for 7, 9, 14, 21, or 28 days. After 24 hours of incubation, 5 ml of water were removed from each flask so as to leave a partially submerged single layer of seeds on the bottom of each flask.

Cotyledons (100), which had been excised from seeds soaked at room temperature, were added to the remaining flask containing water only. The submerged cotyledons were incubated for 28 days at 4 to 7°C. To the flask containing 50 ml of 2% sucrose solution were added 24 excised embryos. The spun glass kept the embryos moist but prevented them from being submerged throughout the 28-day incubation period.

Upon removal from the cold chamber the embryos from all preparations gave a positive viability test in 0.1% 2,3,5-triphenyltetrazolium chloride solution. The extract from each vessel was used to soak groups of new pea seeds for 6 hours at room temperature.

In table I is summarized the data taken on plants which had been treated with the extracts described above. It is apparent that several of the diffusates were without significant effect on the flowering behavior of the treated plants. However, the 9-day diffusate and the 28-day-cotyledon diffusate significantly delayed flowering to a higher average node, and the plants treated with these two preparations were 4 to 8 cm taller than the controls. On the other hand, the 28-day-embryo diffusate caused the treated plants to flower at a lower average node than the controls.

In II. A second method of preparing diffusates was similar to a method used by Highkin (10). Six 600-ml beakers were used as incubation vessels. To each of four of the beakers were added 350 ml of distilled water; 150 ml were added to each of the other two beakers. Pea seeds (80) were added to each of the six beakers. The seeds were supported 5 cm above the bottoms of the vessels in the four vessels containing the large aliquots of water. When the seeds were fully turgid, enough water was removed from each vessel to leave the seeds partially submerged. All six preparations were incubated in darkness at 3 to 5°C for 25 days. Fresh pea seeds were then soaked for 12 hours in darkness at room temperature in each of the two types of diffusates and controls in distilled water.

In table II is summarized the data taken on the plants soaked in these dilute and concentrated diffusates before planting. The term "concentrated diffusate" deserves attention. This diffusate was very concentrated as compared to the dilute diffusate. But since much smaller aliquots of concentrated diffusate were used to soak the groups of fresh seeds, the actual seed treatment was such that seeds treated with both diffusates imbibed about equal amounts of active material. As is apparent in table II, both diffusates induced the plants to flower at a significantly lower node than the controls.

Effects of Pea Seed Diffusates on Flowering of Peas

<table>
<thead>
<tr>
<th>Seed treatment</th>
<th>No. plants</th>
<th>Avg. No. nodes to 1st flower*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Day diffusate</td>
<td>12</td>
<td>18.1 ± 0.24</td>
</tr>
<tr>
<td>9-Day diffusate</td>
<td>24</td>
<td>18.5 ± 0.12**</td>
</tr>
<tr>
<td>14-Day diffusate</td>
<td>20</td>
<td>18.0 ± 0.15</td>
</tr>
<tr>
<td>21-Day diffusate</td>
<td>16</td>
<td>18.1 ± 0.23</td>
</tr>
<tr>
<td>28-Day diffusates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole seeds</td>
<td>8</td>
<td>18.2 ± 0.41</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>16</td>
<td>18.3 ± 0.24***</td>
</tr>
<tr>
<td>Embryos</td>
<td>11</td>
<td>17.3 ± 0.20***</td>
</tr>
<tr>
<td>Water</td>
<td>27</td>
<td>17.8 ± 0.16</td>
</tr>
</tbody>
</table>

* Values are the means ± the standard errors of the means.
** Difference from controls significant at 1% level.
*** Difference from controls significant at 10% level.

In table III is summarized the data taken on peas treated with GSH and 2-thiouracil. Both chemicals were effective in inhibiting flowering, particularly when applied as a soak treatment before planting. The equimolar combination of GSH and 2-thiouracil was more effective than either chemical alone, and the same result was obtained by both methods of

<table>
<thead>
<tr>
<th>Table II Effects of Pea Seed Diffusates on Flowering of Peas II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed treatment</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Dilute diffusate</td>
</tr>
<tr>
<td>Conc diffusate</td>
</tr>
<tr>
<td>Water</td>
</tr>
</tbody>
</table>

* Values are the means ± the standard errors of the means.
** Difference from controls significant at 1% level.
Table III

<table>
<thead>
<tr>
<th>Chemical</th>
<th>No. plants</th>
<th>Avg. No. nodes to 1st flower*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soaked</td>
<td>24</td>
<td>18.9 ± 0.26**</td>
</tr>
<tr>
<td>Sprayed</td>
<td>25</td>
<td>18.5 ± 0.25</td>
</tr>
<tr>
<td>2-Thiouracil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soaked</td>
<td>24</td>
<td>19.0 ± 0.27**</td>
</tr>
<tr>
<td>Sprayed</td>
<td>27</td>
<td>18.6 ± 0.25</td>
</tr>
<tr>
<td>GSH + 2-Thiouracil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soaked</td>
<td>25</td>
<td>19.1 ± 0.25**</td>
</tr>
<tr>
<td>Sprayed</td>
<td>20</td>
<td>19.1 ± 0.38**</td>
</tr>
<tr>
<td>Controls</td>
<td>41</td>
<td>18.2 ± 0.18</td>
</tr>
</tbody>
</table>

* Values are the means ± the standard errors of the means.  
** Difference from controls significant at 5% level.

treatment. The height to the first flowering node was slightly greater in most of the treated plants than in the controls, this being most significant in the two groups treated with the combined chemicals. No superficially apparent abnormalities developed in the plants treated with either chemical.

Discussion

Vernalization Experiments. The results of these experiments are in good agreement with certain of those reported by other authors. The Dwarf Telephone pea responded to vernalization in a manner very similar to the (Tall) Telephone variety studied by Paton and Barber (22) and the variety Unica investigated by Highkin (11).

Highkin (11) reported a reduction in height to the first flowering node in vernalized Unica and Zelka varieties. Highkin suggested that the inductive effect of cold treatment on vegetative development appears to be distinct and separate from the effect on the number of nodes to the first flower. This observation has been confirmed in the present study. The experimental separation of these two inductive effects on vegetative and reproductive development is suggestive of a retardation in the auxin function of growth promotion and a concomitant promotion of hypothetical florigen activity. This interpretation would lend support to Galston's (7) concept of a functional association between growth and flowering hormones.

Experiments With Aqueous Extracts From Pea Seeds. Both flower-promoting and flower-inhibiting extracts have been prepared from pea seeds. Whether the activity of such extracts is due to the relative concentration of a single substance or to the relative proportions of promotive and inhibitory substances is not known. Auxin would be expected to be present in the diffusates (13) and might be suspected as accounting for the inhibitory action of certain of the diffusates. Auxin was not detected even in the concentrated diffusates by paper chromatographic analysis in the present study. Highkin (10) was unable to detect auxin in his flower-promoting diffusates by sensitive bioassay. Highkin suggested that the active principle in his diffusates might be a flower-promoting hormone or hormone precursor.

Failure to detect auxin is not conclusive proof against its presence in the diffusates. And the possibility must be considered that the activity of the diffusates is determined by the concentration of a single substance which promotes flowering at low concentrations and inhibits the process at relatively high concentrations. Auxin has been reported to promote flowering in long-day plants under light conditions which were inadequate for photoperiodic induction (20). In the work of Leopold and Guernsey (15) auxin soak treatments of seeds of various species followed by growth under optimum conditions either inhibited flowering or were without effect. Thus the identity of the substance or substances responsible for the activity of pea seed diffusates in flowering must await further study.

Effects of GSH & 2-Thiouracil. Glutathione, in the relatively high concentration used, inhibited flowering. This effect is interesting, particularly in view of the report by Biddulph (3) and the statement of Bricas and Fromageot (4) that the concentration of GSH is particularly high in rapidly growing tissues. On the basis of the results of the present study it seems unlikely that the inhibition of flowering by GSH was due to a retardation in growth. It appears that GSH may inhibit flowering by enhancing an auxin-induced growth and antagonism of flowering.

Leopold and Guernsey (16) proposed a theory of auxin action based upon their evidence that auxin may form a thiol ester with coenzyme A. Bricas and Fromageot (4) stated that it is probable that GSH plays a protective role in maintaining the activity of CoA. Pilet (23) found that GSH inhibits indoleacetic acid oxidase in young carrot tissues. The data of Fries (6) show that GSH promotes the growth of decorticated peas in vitro.

2-Thiouracil also inhibits flowering in peas. No evidence was derived from the present study to account for the mechanism of this action. 5-Fluorouracil has been reported to inhibit photoperiodic induction in a short-day plant (24), and 2-thiouracil inhibits photoperiodic induction in another short-day plant (9). In both of these systems the inhibitory effects of the chemicals were attributed to interference with nucleic acid metabolism.

General Discussion. Certain of the data presented in this report do not seem to be explainable on the basis of a colysanthin. Particularly, the flower-promoting activity of certain of the aqueous extracts is in contradiction to such an interpretation. The data presented here seem to fit the concept of a flower-promoting hormone in Dwarf Telephone peas. In agreement with several authors (5, 7, 8, & others) the writers feel that auxin plays a decisive role in the flowering process.
Summary

The Dwarf Telephone variety of *Pisum sativum* L. is vernalizable. Fully imbibed seeds subjected to seven or more days of cold treatment at 4 to 7°C were induced to: A: flower at a lower node, B: attain less height to the first flowering node, and C: flower in fewer days, as compared to control plants. Maximum effects on these three responses were obtained with approximately 28 days of cold treatment.

Aqueous extracts have been prepared from Dwarf Telephone pea seeds which can either promote or inhibit flowering, depending upon the concentration of the extract.

Glutathione at a concentration of \(10^{-8} \text{M}\), applied as repeated spray treatments and as pre-plant seed treatment, inhibited flowering by delaying the position of the first flower to a higher node. This inhibition is interpreted as an enhancement of auxin antagonism of flowering. The pyrimidine 2-thiouracil also inhibited flowering in the pea.

The internal chemical control of flowering in the Dwarf Telephone pea is tentatively interpreted as being mediated by a flower-promoting hormone, the formation of which is influenced quantitatively by temperature and photoperiod, and the action of which is antagonized by high levels of endogenous auxin.

Literature Cited