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COTYLEDON EXCISION AND FLOWERING IN PISUM SATIVUM*

By MARGARET J. JOHNSTON† and R. K. CROWDEN‡

Investigations of the flowering behaviour of peas led Paton and Barber (1955), Sprent and Barber (1957), and Barber (1959) to propose the presence of a mobile flower-delaying substance, “colysanthin”, in cotyledons of late-flowering varieties. Moore (1964, 1965) and Sprent (1966) have recently obtained results consistent with the colysanthin hypothesis. However, the basis for early flowering of other varieties is not clear. Moore (1964) observed significant latening of two early-flowering varieties, Massey and Alaska, following cotyledon excision at 4 days after germination, and Haupt (1952) has reported a slight delay in the early variety Kleine Rheinlanderin when embryos, separated from the cotyledons within 8 hr of soaking the seed, were grown in nutrient culture. In contrast Cruickshank (unpublished data) did not observe any change in flower node of Massey following culture of embryos removed from dry seeds. Paton and Barber (1955) in separate experiments obtained conflicting evidence that a flower-promotive substance was present in Massey cotyledons. In the one experiment in support, only five plants were scored due to the high death rate of plumules following early excision of the cotyledons.

In our present experiments we have re-examined the effect of cotyledon removal on flowering in both late-flowering (Greenfeast) and early-flowering (Massey) varieties. Cotyledons were excised at intervals, after imbibition for 8 hr and then at 1,2,4,6, and 9 days. In order to overcome the high plumule mortality experienced by Paton and Barber, embryos of treatments at 8 hr through to 4 days were grown for an initial period in nutrient culture (White’s medium) before transferring to a vermiculite–gravel mixture in the glasshouse. Transfer was usually effected when the leaves at node 4 were beginning to unfold. It was confirmed in separate experiments that plants left to flower in nutrient agar showed no variation in node to first flower to those transferred to glasshouse conditions. In the 6- and 9-day treatments, seeds were planted directly into vermiculite–gravel. Plants were grown under two photo-period regimes: (1) short days (8 hr); and (2) long days (normal spring day supplemented to 18 hr with artificial illumination). The results are summarized in Table 1.

The results obtained for Greenfeast confirm and extend the observations of Sprent (1966) and are interpreted to mean that early excision of cotyledons removes an inhibitor (i.e. colysanthin), thereby providing for earlier flowering. However, flowering becomes progressively delayed the longer the cotyledons remain attached to the developing plant before excision, due to the transfer of increasing amounts of inhibitor to the plumule. Movement of the inhibitor apparently starts at about day 4 of germination, and continues beyond day 9. Sprent and Barber (1957) have estimated from leaching experiments that removal of colysanthin is complete within 14 days from germination.

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### Table 1

**EFFECT OF COTYLEDON EXCISION ON FLOWERING IN MASSEY AND GREENFEAST PEAS**

<table>
<thead>
<tr>
<th>Days to Cotyledon Excision</th>
<th>Massey</th>
<th>Greenfeast: Long Day</th>
<th>Greenfeast: Short Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Plants Scored</td>
<td>Mean Flower Node ± S.E.</td>
<td>$t^+$</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>9.22 ± 0.147</td>
<td>0.6609</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>10.54 ± 0.207</td>
<td>5.876***</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10.75 ± 0.222</td>
<td>6.861***</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>11.00 ± 0.202</td>
<td>8.260***</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>9.70 ± 0.143</td>
<td>0.667</td>
</tr>
<tr>
<td>9</td>
<td>42</td>
<td>9.39 ± 0.102</td>
<td>0.188</td>
</tr>
<tr>
<td>Control</td>
<td>41</td>
<td>9.33 ± 0.096</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at 1% level.**  **Significant at 0.1% level.**  
$t^+$ Calculated for the difference in means between the control and the respective treatments.
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Our experiments further showed that cotyledon excision had no effect on the photoperiod response of Greenfeast, a quantitative long-day plant. Exposure to short days imposed a regular delay of 7–8 nodes on all treated plants, the effect being additive upon that of cotyledon removal. Barber (1959) had previously supposed that long photoperiods may destroy colysanthin or alternatively convert it to a florigen. These present results indicate that photoperiod and colysanthin have an independent effect on flowering.

The early-flowering variety Massey is insensitive to photoperiod and the respective photoperiod data obtained for Massey in these experiments have been combined in Table 1. The response of Massey to the removal of cotyledons shows two main features. One, there is progressive delay of flowering caused by early cotyledon excision, the effect becoming nearly maximal within 2 days (cf. results of Cruickshank and of Haut). Second, this effect is almost completely reversed within 6 days (cf. results of Moore and of Paton and Barber). A simple explanation of this behaviour might be that Massey cotyledons also contain a flower-inhibitory substance which is rapidly mobilized after germination commences, and which is subsequently deactivated or perhaps converted to a promotive substance as germination and development of the plumule progresses. Additional evidence supporting this explanation has been obtained through the use of extracts. Our preliminary experiments show that extracts prepared from both Massey and Greenfeast cotyledons will delay flower initiation of Massey. We are currently attempting to obtain definition of the inhibitory fractions from both varieties.

References

BARBER, H. N. (1959).—Physiological genetics of Pisum. II. The genetics of photoperiodism and vernalization. Heredity 13, 33–60.