INFLORESCENCE INITIATION IN LOLLUM TEMULENTUM L.

IX.* SOME CHEMICAL CHANGES IN THE SHOOT APEX AT INDUCTION

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Summary

Plants of L. temulentum were induced to flower by exposure to 1 long day. Shoot apices harvested from vegetative plants prior to the long-day exposure (day I) and others harvested on days IV and VI were analysed for length, fresh weight, total and residual (after extraction for solubles and lipids) dry weight, total and residual nitrogen content, residual phosphorus, and RNA and DNA phosphorus content. Cell number per apex was also established for two harvests.

Dry weight was 17–19% of the fresh weight of the apices, and soluble substances 27–31% of the dry weight. In the vegetative apices, protein comprised about 58% of the residual dry weight, nucleic acids, 14%, and cell wall materials 27%. The RNA/DNA ratio in the vegetative apex was low, about 1.0.

Between days I and IV the RNA content per apex increased by 46%, whereas residual nitrogen and DNA content per apex increased by only 11 and 16%, respectively. RNA content per apex increased by 140% between days IV and VI, during which time morphological differentiation of the spikelets occurred, while residual nitrogen increased by 160% and DNA content per apex by 95%.

The proportion of nitrogen in the soluble fraction increased markedly between days I and IV, but no comparable increases were found in soluble ninhydrin-positive or ultraviolet-absorbing substances. There was a pronounced diurnal fluctuation in the level of soluble, ninhydrin-positive substances in shoot apices.

I. INTRODUCTION

Many changes attend the transition of a shoot apical meristem from the vegetative to the reproductive state. Histochemical studies (e.g. Gifford and Tepper 1961, 1962; Bernier 1962, 1964; Nougarède et al. 1964; Knox and Evans 1966) and experiments using inhibitors of nucleic acid synthesis (e.g. Salisbury and Bonner 1960; Bonner and Zeevaart 1962; Zeevaart 1962; Evans 1964; Galun, Gressel, and Keyman 1964) on plants for which the timing of the transition is under experimental control have indicated that nucleic acid metabolism at the shoot apex is actively involved in flower induction.

Neither Ross (1962) nor Cherry and Huystee (1965) could detect a change in nucleic acid composition of apical buds following induction. On the other hand, a marked increase in the number of ribosomes in the central cells of the shoot apex following induction has been found by Lance-Nougarède and Bronchart (1965) in Perilla and by Healy and Jensen (1966) in Pharbitis.

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In contrast to root apices, no quantitative estimates of nucleic acid contents of shoot apices have been published. The shoot apex of Lolium temulentum L. lends itself to chemical analysis because its morphology allows a rather precise isolation of the apex by simple transection. Moreover, exposure of plants of L. temulentum to only 1 long day is sufficient to induce flowering, and earlier work has shown that RNA synthesis at the shoot apex on the morning following the long day is essential to induction (Evans 1964), and that an increase in RNA content can be detected histochemically the day after (Knox and Evans 1966). In contrast to the results of Lance-Nougarède and Bronchart (1965), this increase in RNA was localized in the peripheral rather than the central cells of the apex.

Some quantitative information is available on the nitrogenous constituents of shoot apices from the work of Steward et al. (1954) and of Sunderland, Heyes, and Brown (1956, 1957) with Lupinus. Steward et al. (1954) found about half of the apical nitrogen to be in the soluble fraction, mostly asparagine, while Sunderland, Heyes, and Brown (1956) found 13–60% of the total nitrogen to be soluble. With barley apices, Sen (1964) found a marked stimulation of protein synthesis after exposure to 5 long days.

The aims of the present study were to examine changes in DNA, RNA, total nitrogen, and soluble nitrogen contents in shoot apices of L. temulentum following induction of flowering. The changes were examined after two intervals, on the third day after the long day (day IV), before morphological differentiation was evident, and on day VI by which time spikelet primordia had differentiated.

II. Methods

(a) Plant Culture and Harvest

Lolium temulentum plants were grown for 6 weeks from sowing, in 9 cm pots of perlite in cabinets under daylight from 8.30 a.m. until 4.30 p.m. at a temperature of 25°C, the temperature during the dark period being 20°C. On day I of the experiment some plants received long-day treatment by illumination with an incandescent light source giving an intensity of 50 f.c. at plant level from 4.30 p.m. until 8.30 a.m. of day II. The plants, which had 6–7 leaves on the primary shoot, were randomized in blocks of 30 prior to the long-day treatment.

In the main experiment plants were harvested on the mornings of days I, IV, and VI. The main shoots were kept in beakers with water until their dissection in the fresh state. The apex was laid bare, measured with an ocular micrometer, and isolated by a transverse cut directly above the first basal leaf primordium showing upwards growth. The interval between successive primordia reaching this stage is 6–4 days in summer (Evans 1960). Apices were isolated at the rate of about one per minute.

(b) Chemical Analysis

Some samples were used for estimation of fresh weight and total dry weight. Other samples were dropped into ethanol and immediately carried through an extraction procedure for soluble substances according to Williams and Rijven (1965).
At no stage of the analytical procedure was material subjected to powdering. The appearance of extracted apices can be judged from Figure 1. Extracted samples were used for the determination of residual dry weight, residual nitrogen, and phosphorus content, and for RNA and DNA contents.

(i) Weights

Weights were obtained using a Cahn electrobalance, accurate to about 1–2 μg. Samples for dry weight comprised 10 apices. Estimates of fresh weight were attempted using single apices, readings being made at precisely 50 and 120 sec after the cut isolating the apex from the shoot. The values of Table 1 designated “fresh weight” are those obtained at 50 sec, while those designated “corrected fresh weight” represent the sum of the fresh weights at 50 sec and the difference between fresh weights at 50 and 120 sec. Comparison of the two values gives a measure of the rate of desiccation and of the arbitrary correction applied.

(ii) Absorbancies

Absorbancies were measured using a Hitachi spectrophotometer, model EPU. In the analytical assays final volumes were often in the range of 0·3–2·0 ml, necessitating the use of micro-cells of 2 mm width and 1 cm light path length. The set of cells selected for this work permitted satisfactory results to be obtained.

(1) Nitrogen.—Nitrogen was estimated by direct Nesslerization of digests. The technique, derived from Levy (1936), involved digestion of 5–15 apices with 20 μl digestion mixture (1000 ml H₂SO₄, 100 g K₂SO₄, 1 g Se), addition of 2 ml 1·6% (w/v) NaOH, 2 ml water, and 50 μl Nessler reagent (Vanselow). The absorbancy at 450 mμ was read after 5 min.
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(2) Amino Nitrogen. When the soluble amino nitrogen content was estimated 10 apices per sample were dropped into 100 μl ethanol contained in small Pyrex tubes and later 50 μl water was added. The tubes were heated to 70°C for 10 min, 100 μl 70% ethanol was added, and after 1 hr the tubes were heated once more for 20 min. The volume was adjusted with 70% ethanol to 300 μl. Aliquots of 50 and 100 μl were transferred to empty tubes, which were then kept overnight in the oven at 60°C. The ninhydrin procedure of Moore and Stein, as described by Spies (1957),

![Ultraviolet absorption spectra](image)

Fig. 2.—Ultraviolet absorption spectra of two or three successive, hot, 0.5N perchloric acid extractions of shoot apices of *L. temulentum* harvested on days I, IV, and VI of the main experiment. Absorbancy values (at 260 mμ) per apex and standard errors and DNA phosphorus content per apex of replicate extracts (as measured by the diphenylamine test) for these harvests are given in the following tabulation:

<table>
<thead>
<tr>
<th>Absorbancy/ml</th>
<th>Day I</th>
<th>Day IV</th>
<th>Day VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard error</td>
<td>10.44 × 10^{-3}</td>
<td>13.82 × 10^{-3}</td>
<td>30.82 × 10^{-3}</td>
</tr>
<tr>
<td>DNA phosphorus (ng)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replicate 1</td>
<td>16.64</td>
<td>19.03</td>
<td>39.63</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>16.54</td>
<td>19.63</td>
<td>35.37</td>
</tr>
</tbody>
</table>

was followed using smaller volumes: 5 μl 70% ethanol and 50 μl reagent were added and, after the prescribed heating (which should not be vigorous), 250 μl diluent. Absorbancies were read at 570 mμ.

(3) Phosphorus.—Phosphorus was estimated directly on one occasion only. Digestion was in 80 μl 70% perchloric acid, 1 ml water was added, and thereafter 0.1 ml Deniges solution, 1 ml water, and 50 μl of the reductant chlorostannous acid (Ingle 1963).

(4) Nucleic Acids.—Nucleic acid content was determined specifically by measuring on aliquots of successive 0.5N perchloric acid extracts (20 min, 70°C) their 260 mμ absorbancies, and by performing on other aliquots the diphenylamine test.
For the purpose in hand this procedure (for comparison with others see Williams and Rijven 1965) proved most satisfactory; the difficulties encountered on applying perchloric acid extraction to plant material, such as atypical ultraviolet-absorption spectra (Holdgate and Goodwin 1965), did not occur, perhaps because the apices were not powdered. Figure 2 shows the ultraviolet spectra of three successive perchloric acid extractions of apices harvested in the main experiment. 300 μl extractions were made on 20–50 apices in duplicate or triplicate. Ultraviolet absorbancy readings of the first extract were obtained by diluting a 50 μl aliquot sixfold. Undiluted 100 μl aliquots were used for the diphenylamine test following Burton (1956), but using a

![Graph showing seasonal effect of the long-day response in L. temulentum.](image)

Fig. 3.—Seasonal effect of the long-day response in *L. temulentum*, as displayed by length of apices dissected 3 weeks after the long-day treatment, in different experiments performed over a number of years, but treated similarly with respect to age, nutrition, and temperature conditions.

final volume of 300 μl. Two extractions sufficed for the complete hydrolysis of RNA and DNA. The computations made to arrive at RNA and DNA phosphorus content, nucleic acid, dry weight, and nitrogen content are detailed by Williams and Rijven (1965). The magnitude of errors attached to primary data on which nucleic acid estimates were based can be judged from the data given in the legend to Figure 2.

(c) Cell Number

Direct estimates of cell number were made through an enzymatic conversion of apices to a suspension of Feulgen-stained nuclei from which samples were precipitated on millipore filters for counting according to the procedure of Rijven and Wardlaw (1966). Estimates were also made by direct counting of nuclei in serial sections.
III. Results
(a) Seasonal Effects on Apex Size

Comparison of the results recorded in the following sections will reveal a variation in size and weight of apices used on different occasions. This is noteworthy because the plants were at a similar developmental stage of 6–7 visible leaves on the primary shoot throughout and were grown in similar environmental conditions, except for seasonal variations in daylight. In general, the apices were smaller in winter and in this season, although the plants were all committed to flowering after exposure to 1 long day, the growth rates of induced apices were much lower than those encountered in summer. Variation in the extension of lower internodes at the time of long-day treatment has also been noticed. Collation of long-day response data encountered over the last five years expresses this seasonal effect (Fig. 3).

Table 1
Some attributes of the shoot apex of L. Temulentum exposed to 1 long day on day I and harvested on days I, IV, and VI
Day I: February 2, 1965

<table>
<thead>
<tr>
<th>Shoot Apex Attribute</th>
<th>Day I</th>
<th>Day IV</th>
<th>Day VI</th>
<th>Day IV/ Day I</th>
<th>Day VI/ Day IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (mm)</td>
<td>0.55</td>
<td>0.61</td>
<td>1.30</td>
<td>1.10</td>
<td>2.13</td>
</tr>
<tr>
<td>Fresh weight (µg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Corrected&quot; fresh weight (µg)</td>
<td>22.3</td>
<td>60.6</td>
<td></td>
<td></td>
<td>2.72</td>
</tr>
<tr>
<td>Dry weight (µg)</td>
<td>3.44</td>
<td>4.29</td>
<td>10.55</td>
<td>1.25</td>
<td>2.46</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.303</td>
<td>0.206</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weight (%)</td>
<td>19.2</td>
<td>17.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual* dry weight (µg)</td>
<td>2.52</td>
<td>7.25</td>
<td>1.21</td>
<td></td>
<td>2.39</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.115</td>
<td>0.086</td>
<td>0.082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble substances (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrogen content (ng)</td>
<td>320</td>
<td>456</td>
<td>1003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual nitrogen content (ng)</td>
<td>293</td>
<td>326</td>
<td>850</td>
<td>1.11</td>
<td>2.60</td>
</tr>
<tr>
<td>Residual phosphorus content (ng)</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA phosphorus content (ng)</td>
<td>16.44</td>
<td>24.00</td>
<td>57.80</td>
<td>1.46</td>
<td>2.40</td>
</tr>
<tr>
<td>DNA phosphorus content (ng)</td>
<td>16.59</td>
<td>19.20</td>
<td>37.50</td>
<td>1.16</td>
<td>1.95</td>
</tr>
<tr>
<td>RNA phosphorus/DNA phosphorus</td>
<td>0.99</td>
<td>1.25</td>
<td>1.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cells (thousands)</td>
<td>14.7</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA phosphorus per cell (pg)</td>
<td>1.13</td>
<td>0.78†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (% residual dry wt.)</td>
<td>58</td>
<td>51</td>
<td>59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleic acids (% residual dry wt.)</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell wall (% residual dry wt.)</td>
<td>27</td>
<td>33</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* “Residual” means after extraction of solubles and lipids.
† Determined from serial sections.
‡ Determined on nuclear suspensions. For L. temulentum leaves 1.1 cm long, DNA phosphorus per cell = 1.06 pg.
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(b) Main Experiment

This experiment was designed to establish the trend of the major constituents of the apices after long-day treatment, based on the harvest of 300 apices during day I, 200 apices on the morning of day IV, and 100 apices on the morning of day VI. Results are shown in Table 1. The main aim was to establish the trends in RNA and DNA content, and precautions were taken that the supply of apices for those estimations was adequate; the primary data for the nucleic acid values recorded can be traced from Figure 2. However, the total nitrogen value for apices of day I rests on a single observation.

Comparison of the last two columns of Table 1 demonstrates a slow rate of increase for all constituents during the 48 hr following the arrival of the flowering stimulus at the apex on the morning of day II (Evans and Wardlaw 1966), and a marked acceleration during the following 48-hr period.

Table 2
NUCLEIC ACID CONTENT OF SHOOT APICES OF L. TEMULENTUM EXPOSED TO 1 LONG DAY ON DAY I AND HARVESTED ON DAY I AND DAY IV, AND OTHERS NOT EXPOSED TO 1 LONG DAY BUT ALSO HARVESTED ON DAY IV
Day I: March 9, 1965

<table>
<thead>
<tr>
<th></th>
<th>Harvested Day 1</th>
<th>Not Exposed to Long Day, Harvested Day IV</th>
<th>Exposed to Long Day, Harvested Day IV</th>
<th>Ratio B/A</th>
<th>Ratio C/A</th>
<th>Ratio C/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (mm)</td>
<td>0.57</td>
<td>0.60</td>
<td>0.77</td>
<td>1.16</td>
<td>1.50</td>
<td>1.29</td>
</tr>
<tr>
<td>Residual dry weight (µg)</td>
<td>2.38</td>
<td>2.75</td>
<td>3.56</td>
<td>1.11</td>
<td>1.50</td>
<td>1.35</td>
</tr>
<tr>
<td>RNA phosphorus content (µg)</td>
<td>18.10</td>
<td>20.05</td>
<td>27.11</td>
<td>1.11</td>
<td>1.25</td>
<td>1.13</td>
</tr>
<tr>
<td>DNA phosphorus content (µg)</td>
<td>15.65</td>
<td>17.32</td>
<td>19.53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA phosphorus</td>
<td>1.16</td>
<td>1.16</td>
<td>1.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA phosphorus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The dry weight data in Table 1 can be used to estimate relative growth rates of the induced apices over the two intervals, while those in Table 2 yield relative growth rates for both induced and vegetative apices over a 3-day interval. For vegetative apices the relative growth rate was extremely low, being 0.05 g/g/day. For induced apices over the interval days I–IV, it was 0.07 and 0.13 g/g/day in the two experiments, and 0.45 g/g/day for the interval days IV–VI. This latter value compares with that of 0.4 g/g/day found by Williams (1966) for young wheat inflorescences.

During the first interval after induction RNA was the apical component to increase most rapidly, consistent with the results of the histochemical study of the L. temulentum apex and with the key role attributed to RNA in developmental changes. Also noteworthy are the RNA/DNA ratios, which were very low on day I and increased thereafter. Comparison of the total with the residual nitrogen values suggests that day IV was characterized by a high soluble nitrogen content. This
point is examined further below, but it may be observed here that the residual nitrogen value of day IV accords the apex a somewhat lower value for percentage protein content. It is also believed that the DNA phosphorus content per cell for day VI is somewhat too low, partly because one of the diphenylamine test replicates may have been a low variant (see legend to Fig. 2).

(c) Subsidiary Experiments

Other experiments were performed to investigate whether some observations of the main experiment could be proved reproducible.

(i) Nucleic Acids.—Table 2 presents the data of an experiment in which apices were collected on day I, and on day IV from two groups of plants, one having and one not having received a long-day treatment. Although differences may be noticed between this and the main experiment, it may be seen that it confirms the more rapid increase of the RNA fraction compared with DNA. This effect of the long-day treatment is expressed most clearly in the comparison between the induced and non-induced apices of day IV; also the RNA/DNA values increased as a result of the long-day treatment. For the vegetative apices the RNA/DNA values were somewhat higher than in the main experiment, which could be due to the plants being harvested during a period of more active apical growth.

Table 3

| Nitrogen data of shoot apices of L. temulentum exposed to 1 long day on day I and harvested on day IV, and others not exposed to 1 long day but also harvested on day IV |
|---|---|---|
| Day I: April 3, 1965 |
| | Not Exposed to Long Day, Harvested Day IV (A) | Exposed to Long Day, Harvested Day IV (B) | Ratio B/A |
| Length (mm) | 0.59 | 0.72 | 1.22 |
| Dry weight (µg) | 3.16 | 4.13 | 1.31 |
| Residual dry weight (µg) | 2.41 | 3.14 | 1.30 |
| Total nitrogen content (ng) | 320 | 436 | 1.36 |
| Residual nitrogen content (ng) | 303 | 338 | 1.12 |
| Soluble nitrogen content (%) (± S.E.) | 5 ± 4* | 22 ± 6* |

* Difference significant at P < 0.05.

(ii) Soluble Nitrogen.—Table 3 summarizes the results of an experiment in which the total and residual nitrogen content were determined for induced and non-induced day IV apices. In order to arrive at a more reliable evaluation of soluble nitrogen content by difference, five samples of 15 apices each were analysed for each entry. The results are in close agreement with those of the main experiment and confirm a higher relative soluble nitrogen content for long-day apices of day IV. The increase was established with a probability of 5% having been due to chance.
In a third experiment no increase was found in the proportion of soluble nitrogen in day IV apices; however, in this experiment the proportion of soluble nitrogen in no case exceeded 3% of the total. The total and residual nitrogen contents were estimated in separate runs and this may account for the anomaly.

In view of this variation, and of the difference in the proportion of nitrogen in soluble compounds between the *L. temulentum* apices and those of *Lupinus* (Steward et al. 1954; Sunderland, Heyes, and Brown 1956), attempts were made to determine the soluble nitrogen directly rather than by difference. Since the nitrogen pool in the apices presumably represents the balance of an import–export plus synthesis–degradation complex, it may fluctuate considerably during the course of the day.

Figure 4 presents the results of determinations of soluble amino nitrogen content of day VI and day VII apices harvested at different hours of the day. It can be seen from the extracted dry weight data, that these were slowly responding apices, in keeping with the winter season plant culture (cf. Fig. 3). The results indicated a marked diurnal fluctuation, with a minimum in the night period and a fairly constant and high amino level during the light period. The soluble amino nitrogen content of apices of plants which were held in a dark room during the "day" period and harvested at the end of it, was of the same magnitude as that of the daylight controls. This suggests that the fluctuation is not under direct control of light. Similarly, the daily temperature fluctuation can be excluded because the "dark" plants of day VI were held at 20°C, the temperature of the preceding night, until harvest.

The soluble amino nitrogen content of induced and non-induced day IV apices was compared in four experiments. No increase due to induction was found in any experiment. For example, about 14 ng soluble amino nitrogen per apex was found in both the long-day treated and the short-day control plants in one experiment; since the extracted dry weight of the induced apices was slightly higher (3.4 μg,
The ultraviolet absorbancy at 260 m\(\mu\) of 70\% ethanol extracts of induced and non-induced day IV apices was also compared, in three experiments, but in no case was any marked increase due to induction evident.

Reconsideration of the extraction procedure followed in obtaining the data given in Tables 1-3 suggested that soluble RNA might have been removed in the two steps using perchloric acid, first at 0·1N and then at 0·2N, each being for 30 min at 4°C, and that this condition might have been responsible for the differences found in the soluble nitrogen fraction. Accordingly, an experiment was done in which the day IV apices were first extracted with 70\% ethanol as for amino nitrogen determination, and then with 0·2N perchloric acid at 4°C for 30 min. The ultraviolet absorbancy of the perchloric acid extracts was very low, and of the same magnitude whether the apices were induced or not.

IV. DISCUSSION

The vegetative \textit{L. temulentum} apex is characterized by a low relative growth rate and a low RNA/DNA ratio. In Jensen’s (1958) study on \textit{Allium} and \textit{Vicia} root apices the RNA/DNA ratio in sections 100 \(\mu\) thick was lowest (about 2) for the one containing the “quiescent centre” and increased rapidly with distance from that section. The low values (about 1) encountered in the \textit{L. temulentum} apices were foreshadowed in a study of wheat leaf development (Williams and Rijven 1965), in which the RNA/DNA ratio increased with leaf size from a minimum value of 1·5 for the smallest primordia (0·6 mm) analysed. The vegetative apices also have an extremely low relative growth rate, about 0·05 g/g/day, which may be compared with the values found by Williams (1966) for wheat leaves of 0·4 g/g/day when small, and rising to 1·3 g/g/day. Contrary to the general impression, the shoot apex therefore appears to be a relatively inert tissue.

The nucleic acid and protein fractions comprised the greater part of both total and residual dry weights. Comparison with the data on protein content of shoot apices of lupin is possible. Sunderland, Heyes, and Brown (1957) expressed protein content on a volume and per cell basis and arrived at values of 20–34 \(\mu\)g protein nitrogen/mm\(^3\), and about 10 \(\mu\)g protein nitrogen/cell, in the lupin apex. The corresponding values for the \textit{L. temulentum} apex, derived from Table 1 assuming a density of 1, are 15–17 \(\mu\)g protein nitrogen/mm\(^3\) and about 20 \(\mu\)g protein nitrogen/cell. While these protein values are in good agreement, there is a marked difference between them and the data presented by Steward \textit{et al.} (1954). They found only about 20 \(\mu\)g protein nitrogen/mg dry weight in the lupin apex, whereas the values in Tables 1 and 3 give about 100 \(\mu\)g protein nitrogen/mg dry weight for the \textit{L. temulentum} apices. The difference may well be due to losses during the procedure followed by Steward \textit{et al.} (1954), in which protein content was derived from estimation of the individual amino acids after acid hydrolysis of the insoluble fraction.

A further difference between the data for \textit{L. temulentum} apices and those for lupin lies in the proportion of nitrogen in soluble compounds. This proportion was approximately 50\% in the data of Steward \textit{et al.} (1954), and between 13 and 60\%
in that of Sunderland, Heyes, and Brown (1956). In the vegetative apices of *L. temulentum* this proportion was 5–8%. The data given in Tables 1 and 3 suggest a marked increase in this proportion following induction, to 22–28% on day IV. A characteristic action of many hormones, such as insulin, is increased permeability to, and uptake of, compounds such as sugars and amino acids by the target tissues (cf. Wool 1965) and a higher proportion of soluble nitrogen could reflect a similar action by the floral hormone. However, we have been unable to demonstrate any marked increase in soluble amino nitrogen or ultraviolet-absorbing compounds in day IV apices following induction. Thus, if the increase in soluble nitrogen is real, it must reside in compounds extracted in the later steps of our extraction procedure.

While this study has not resolved the problem of a possible increase in soluble nitrogen in apices following induction, it has revealed the existence of a marked diurnal fluctuation in soluble amino nitrogen which was independent of diurnal temperature and light cycles.

The most pronounced change evident in day IV apices of the main experiment following induction is an increase in the RNA content relative to all other components. In the following 2 days, the sharp rise in the relative growth rate of the shoot apices was reflected in all components, and particularly in the protein content, but the first 2 days after induction were characterized by a relative increase in RNA synthesis. This confirms the histochemical observations (Knox and Evans 1966), in which the RNA increase was found to be localized in the peripheral cells between adjacent leaf primordia, at the sites of origin of the spikelets. The increase in RNA was evident, by histochemical means, in day III apices, and in Part X of this series (Rijven and Evans 1967) it will be seen to be detectable early on day II, at about the time of arrival of the floral stimulus at the shoot apex.

V. Acknowledgments

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VI. References


