Effects of Photoperiod on Growth Rate and Endogenous Gibberellins in the Long-Day Rosette Plant Spinach

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ABSTRACT

The earliest visible responses of spinach plants (Spinacia oleracea L., cv. Savoy Hybrid 612) transferred from short to long days (8 hours of high intensity light supplemented with 16 hours of low intensity illumination from incandescent lamps) were upright leaf orientation and increased elongation of the petioles. The effect of long days on growth rate was direct; i.e., there was no after-effect if the plants were transferred to short days. Gibberellin A₁ applied to plants under short days had an effect similar to that of long days, whereas application of the growth retardant AMO-1618 [2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate] under long days caused a growth habit typical of short-day conditions. Gibberellin A₁ caused more stem growth in plants under long days in which the endogenous gibberellin content had been reduced by AMO-1618 than in plants under short days not treated with the growth retardant.

Three gibberellin-like substances, called I, II, and III in order of increasing Rₚ value, were present in acidic extracts of spinach under short days. After transfer to long days, II increased, whereas I and III decreased, the latter below the level of detection in the d₅ corn assay. Following application of AMO-1618 the gibberellin content of plants under long days fell off more rapidly than in those under short days, indicating that gibberellin turnover was markedly higher under long days. This increased rate of gibberellin metabolism was established after 2 long days. When plants were returned to short days, the turnover of gibberellins declined. It is suggested that a higher rate of gibberellin biosynthesis combined with increased sensitivity to gibberellin is responsible for the observed growth responses in spinach under long days.

In many rosette LDP₁ application of GA under SD conditions causes stem elongation and flower formation (4, 11, 26). These observations have led to the idea that rosette plants on SD are deficient in GAs, while LD would raise the level of endogenous GAs which in turn would stimulate stem growth e.g., Ref. 3). Work in this laboratory with the LDP Silene armeria (4) failed to substantiate this hypothesis. Upon transfer of plants from SD to LD the GA content of extracts did not increase over the SD control by more than a factor of 2. However, results of diffusion experiments suggested that just prior to the onset of stem elongation both the biosynthesis of GA and its metabolism had increased.

Working with spinach, Radley (16) reported a sharp although transient increase in GA content after 1 LD. She suggested that the decrease in GA level after more LD cycles was due to greatly increased turnover of GA. In view of this marked increase in the GA content after 1 LD, the levels of native GAs in spinach have been re-investigated under different photoperiods, as well as the decrease in GA content following the application of the growth retardant AMO-1618. The results in this communication provide experimental support for the notion that the turnover of GAs in spinach is much higher under LD than under SD conditions. Preliminary reports of this investigation have been presented earlier (26, 27).

MATERIALS AND METHODS

Plant Material. Seed of spinach (Spinacia oleracea L., cv. Savoy Hybrid 612) was purchased from Joseph Harris Co., Inc., Rochester N. Y., and germinated in vermiculite. The seedlings were transplanted into 340-ml plastic containers filled with a gravel-vermiculite (1:2) mixture when the cotyledons had fully expanded. Growing conditions in the greenhouse under SD and watering with half-strength Hoagland's nutrient solution were similar to those used for Silene (4), except that the temperature during the 16-hr dark period was 23°C. The plants were ready for experimentation approximately 2 months after sowing. Prior to the start of an experiment, the plants were transferred to growth chambers and continued on SD for at least 1 week. Temperature in the growth chambers was maintained at 23°C throughout. SD consisted of an 8-hr light period of light from fluorescent and incandescent lamps (3000 ft-c) followed by 16 hr of darkness. For LD treatment, the plants were exposed to 8 hr of high intensity light as in SD, followed by 16 hr of low intensity supplementary illumination (40 ft-c) from incandescent lamps.

For petiole measurements, two young leaves on each plant with petioles between 10 and 20 mm long were selected and measured daily at the beginning of the high intensity light period from the base to the leaf blade. Stem height was determined from the base of the stem to the tip of the stem, or the inflorescence. When an experiment was discontinued and an inflorescence was not visible macroscopically, the shoot apices were examined under a dissecting microscope.

GA, and AMO-1618 Applications. GA₃ was dissolved in water containing 0.05% Tween 20 at a concentration of 200 mg/liter; 0.05 ml of solution was administered to the shoot apex and surrounding leaves at each application. AMO-1618 was used in a concentration of 5 × 10⁻³ M, 10 ml of solution

1 Abbreviations: SD: short days; LD: long days; LDP: long-day plants; AMO-1618: 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate; TLC: thin layer chromatography.
being applied per plant via the soil on alternate days, or in some treatments daily.

Extraction and Purification Procedures. Unless specifically stated, plants were always harvested toward the end of the 8-hr high light intensity period. All aerial parts were collected. Following freezing and lyophilization, the same extraction procedure as described for Silene (4) was used with some minor changes. The dried plant material was homogenized with cold methanol in a Waring Blender. After filtration the tissue residue was extracted once more with methanol. The combined filtrates were evaporated to dryness under reduced pressure. The residue was taken up in a 0.5 M phosphate buffer of pH 8.2 and partitioned several times against petroleum ether (b.r. 30–60°C) until the organic phase was light green. The buffer was then extracted three times with equal volumes of ethyl acetate. This alkaline ethyl acetate phase was not discarded as before (4), but instead was concentrated to about 100 ml and extracted two times with 5 ml of 1 M NaHCO₃, followed by one washing with 5 ml of water. These extracts were combined with the phosphate buffer at pH 8.2. After lowering the pH to 2.5 with 6 N HCl, the acidic fraction was prepared by partitioning three times with equal volumes of ethyl acetate.

The acidic fraction was purified on a charcoal-Celite 535 (1:2, w/w) column (diameter 1.8 or 2.5 cm) (13). 1 g of Darco G-60 charcoal being used for every 10 g of dry plant material extracted. The acidic fraction was dissolved in water and pipetted on top of the column. Elution of all GA-like substances was achieved with 80% aqueous acetone. The acetone was evaporated and the acidic material in the water phase was partitioned into ethyl acetate at pH 2.5. In preliminary experiments this acidic fraction was used for chromatography, but in later experiments, particularly when this fraction was to be chromatographed in solvent system 2 (see below), an additional purification step on a silicic acid-Celite 535 (1:2, w/w) column was introduced (13). The column, usually containing 2 g of silicic acid (Mallinekrodt, 100 mesh) and 4 g of Celite 535, was packed in chloroform and had a dimension of 1.5 x 12 cm. The acidic fraction dissolved in ethyl acetate-chloroform (1:1; v/v) was applied to the column and eluted with the same solvent. A considerable amount of brown impurities was retained in the upper part of the column. The effluent was reduced to a small volume and chromatographed.

Thin Layer Chromatography. Preparative TLC was carried out on 20 x 20 cm glass plates coated with Silica Gel H layers, 0.3 to 0.4 mm thick. Two solvent systems were used. System 1 (18) consisted of chloroform-ethyl acetate-acetic acid (60:40:5, v/v). For solvent system 2 (7) carbon tetrachloride-acetic acid-water (8:3:5, v/v) were shaken in a separatory funnel and permitted to separate into two phases. The plates were equilibrated with the upper phase overnight and developed with ethyl acetate-lower phase (2:5, v/v). The increased ratio of ethyl acetate to lower phase as compared to the previously used ratio, 1:5 (4, 7), was essential to obtain adequate separation of GA₃ and GA₄ with this particular batch of Silica Gel H.

After developing the plates to 15 cm, each chromatogram was divided into 10 or 15 equal zones, and the silica gel was scraped into centrifuge tubes. GA-like substances were eluted two times with water-saturated ethyl acetate, followed by one elution with methanol. The combined eluates of each zone were evaporated to dryness and used for bioassay. Cochromatography of authentic GAs with spinach extracts was performed in the same manner as described for Silene (4). Purified extracts were applied as a narrow band approximately 1 cm long and 0.5 cm wide. Authentic GAs were spotted in the middle of this band. After development of the chromatogram, the middle portion of the plate was left intact to detect the reference GAs (4), while the rest of the silica gel was scraped off, eluted, and used for bioassay.

Bioassays. The dwarf corn d5-mutant was used for bioassay to estimate the GA content as in previous work (4). To avoid slight variations in response from one bioassay to the next, care was taken to test all samples of one experiment in the same run of an assay. Thus, comparisons on an absolute quantitative basis can be made between different treatments of an experiment, but not between separate experiments. The total amount of GA-like substances in each eluate was determined by interpolation from a GA₃ standard curve. The results on GA content are expressed as µg equivalents of GA₃ per 100 g dry weight (µg GA₃ eq/100 g dry wt).

Preliminary tests were also performed with the barley aleurone system (9), and with the lettuce hypocotyl assay (6).

RESULTS

Petiole Growth. Transfer of spinach plants from SD to LD resulted in marked differences in growth habit which were noticeable after 1 day. Under SD the petioles were short and the leaves were in a horizontal position. On plants shifted to LD the younger leaves became almost vertically oriented, and the ultimate petiole length increased considerably. The more rapid growth rate of petioles under LD was measurable after 1 day (Fig. 1). It is obvious that this increased growth took place during the 16-hr period of supplementary light from incandescent lamps, since both SD and LD treatments received identical high intensity light during the first 8 hr of the 24-hr period. In several separate experiments the increment in petiole length after 1 LD was two to four times that of petioles on SD. When plants were returned from LD to SD, petiole growth practically stopped during the next day, and then continued at a rate comparable to that of plants kept continuously under SD (Fig. 1).

To investigate the possible role of GAs in controlling petiole growth, plants were treated with AMO-1618 under both SD and LD conditions, and GA₄ was applied simultaneously as an antidote of the growth retardant. The results in Table I demonstrate once more the striking effect of LD on petiole growth. When GA₄ was applied under SD conditions, the plants exhibited a LD growth habit with petioles two times longer than those of the control. AMO-1618 had a slight inhibitory effect under SD, but reduced petiole length on LD by 50%. Applied GA₄ completely overcame the inhibitory effect of AMO-1618.

Stem Elongation. Whereas 1 LD cycle had a clear cut effect on petiole growth, stem elongation did not start until after 7 LD. When transferred to permanent LD, a high growth rate was established after 12 days (Fig. 2). If, on the other hand, plants were returned to SD after 8 LD, which is sufficient to cause 100% flowering (see below), little stem elongation took place, again indicating the absence of a photoperiodic after-effect on growth in spinach. When the same plants were transferred once more to LD after 12 SD, a rapid rate of growth was reached 4 days later (Fig. 2).

Table II shows the results of an experiment in which the effects of applied AMO-1618 and GA₄ on stem growth were studied. Plants were either kept continuously under SD or returned to SD after exposure to 10 LD. Application of AMO-1618 almost completely suppressed stem elongation induced by 10 LD. GA₄ treatment of plants on permanent SD caused very little stem growth as compared to the stem elongation it caused on plants exposed to 10 LD in which the endogenous GA level had been reduced by AMO-1618. It is apparent from these data that plants under LD are more responsive to applied GA with respect to stem growth than are plants under
the influence: florescences under inhibition. this were more than few plants usually but when has (Table elongation during than all times, and thus at AMO-1618, AMO-1618 was applied on alternate days, starting 2 days before transfer to LD. GA₄ was applied five times on alternate days, 10 μg per plant, for a total of 50 μg; applications were started on first LD. Two petioles of leaves that developed during treatments were measured on each plant after 10 days. Ten plants per treatment.

SD. When stem growth was inhibited by AMO-1618, GA₄ caused more elongation when applied during the LD treatment than during subsequent SD. These differences in stem height persisted when the experiments were continued for longer times, and thus they cannot be ascribed to the different times at which GA₄ applications had started.

Flower Formation. Savoy Hybrid 612 is a fast bolting spinach cultivar, and, as reported earlier (26), 7 to 8 LD suffice under the conditions used in this study to induce the subsequent formation of macroscopically visible inflorescences in all plants. However, there was considerable variation in the time until inflorescences became visible, male inflorescences usually appearing earlier than female ones.

On permanent SD, plants of this variety grew strictly as rosettes, but when more than 3 months old, floral primordia could usually be detected under the dissecting microscope in some plants (Table II). Thus, the strain Savoy Hybrid 612 has no absolute LD requirement for floral initiation, although LD were required for the further development of the inflorescence. GA₄ applied to plants on SD caused a slight stem elongation (Table II); if the GA treatment was continued for more than 1 month, male inflorescences became visible in a few plants but failed to develop further.

Application of AMO-1618 delayed the appearance of inflorescences under LD (Table II), and applied GA₄ reversed this inhibition.

GA Extractions. Results of a representative experiment on the influence of photoperiod on the GA content in spinach are presented in Table III. The GA level gradually increased with longer exposure to LD, but since dry weight also increased, the increase in GA content as expressed on a dry weight basis amounted to only 20%. GA activity was detected in two zones of the chromatogram at Rₜ 0.1 to 0.3, and at Rₜ 0.4 to 0.6 (Fig. 3). In extracts of plants on SD the GA activity in the latter zone was lower than in the slower moving zone. After 7 LD the GA activity in the faster moving zone was considerably higher than in the comparable zone of the SD chromatogram (Fig. 3).

In the experiment of Table III no increase in GA activity after 1 LD was detected. Since one of the objectives of this study was to reproduce the reported increase in GA content after 1 LD cycle (16), several additional extraction experiments were carried out in which plants were harvested at the beginning or toward the end of the high intensity light period after 0, 1, or 2 LD. In no case was a large increase in GA content ob-

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**Table I. Influence of AMO-1618 and GA₄ on Petiole Length in Spinach under Short- and Long-Day Conditions**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Petiole Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td>Control</td>
<td>42 ± 1.8</td>
</tr>
<tr>
<td>+ GA₄</td>
<td>85 ± 3.5</td>
</tr>
<tr>
<td>+ AMO-1618</td>
<td>30 ± 1.0</td>
</tr>
<tr>
<td>+ AMO-1618, + GA₄</td>
<td>...</td>
</tr>
</tbody>
</table>

SD: When stem growth was inhibited by AMO-1618, GA₄ caused more elongation when applied during the LD treatment than during subsequent SD. These differences in stem height persisted when the experiments were continued for longer times, and thus they cannot be ascribed to the different times at which GA₄ applications had started.

**Figure 1.** Petiole growth of spinach plants as affected by photoperiod. Arrow indicates when one group of plants was returned to SD after exposure to 3 LD.

**Figure 2.** Stem growth of spinach plants transferred permanently to LD, and of plants exposed to 8 LD, followed by 12 SD, and then returned to LD. ↓: Transfer to SD; ↑: transfer to LD.

**Table II. Influence of AMO-1618 and GA₄ on Stem Elongation and Floral Initiation in Spinach on Short and Long Days**

Long-day treatment was started 70 days after sowing date. AMO-1618 was applied on alternate days, starting 3 days before transfer to LD, and terminated on 9th LD. GA₄ was applied on alternate days, 10 μg per plant, for a total of 50 μg of GA₄ per plant.

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**Table III.**

<table>
<thead>
<tr>
<th>Photoperiodic Treatment</th>
<th>AMO-1618</th>
<th>GA₄</th>
<th>Stem Height after 20 days</th>
<th>Days until Inflorescence Visible</th>
<th>Flowering Response after 32 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>&gt;32</td>
<td>6/8</td>
</tr>
<tr>
<td>SD</td>
<td>—</td>
<td>+</td>
<td>1.3 ± 0.5</td>
<td>&gt;32</td>
<td>8/8</td>
</tr>
<tr>
<td>10 LD → SD</td>
<td>—</td>
<td>+</td>
<td>17.6 ± 4.2</td>
<td>12</td>
<td>8/8</td>
</tr>
<tr>
<td>10 LD → SD</td>
<td>+</td>
<td>—</td>
<td>0.9 ± 0.5</td>
<td>23</td>
<td>8/8</td>
</tr>
<tr>
<td>10 LD + GA₄ → SD</td>
<td>+</td>
<td>—</td>
<td>25.8 ± 3.8</td>
<td>13</td>
<td>8/8</td>
</tr>
<tr>
<td>10 LD → SD + GA₄</td>
<td>+</td>
<td>+</td>
<td>12.0 ± 3.2</td>
<td>17</td>
<td>8/8</td>
</tr>
</tbody>
</table>

1 No. of plants with inflorescence/no. of plants per treatment.
Table III. Influence of Photoperiod on Gibberellin Content

Nine plants were harvested per treatment. Acidic fractions were chromatographed in solvent system 1.

<table>
<thead>
<tr>
<th>No. of Long Days</th>
<th>Dry Weight</th>
<th>GA Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>µg GAs eq</td>
</tr>
<tr>
<td>0</td>
<td>21.9</td>
<td>0.14</td>
</tr>
<tr>
<td>1</td>
<td>22.1</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>21.9</td>
<td>0.15</td>
</tr>
<tr>
<td>7</td>
<td>27.5</td>
<td>0.21</td>
</tr>
<tr>
<td>10</td>
<td>27.8</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Fig. 3. Comparison of the gibberellin activity as measured in the d5 corn assay of acidic extracts prepared from spinach plants on SD, or after exposure to 7 LD. Thin layer chromatography was in solvent system 1.

erved. The same was true when the plants were grown in daylight instead of under fluorescent illumination. Similar results were also obtained with the spinach variety Winter Prickley used by Radley (16).

In order to confirm that AMO-1618 was inhibiting GA biosynthesis as expected, the level of endogenous GA was determined in plants treated with the growth retardant. The results in Table IV show that applications of AMO-1618 for a period of 19 days decreased the GA content to 10% of that in control plants while stem growth was almost completely suppressed. These findings combined with those presented in Table II clearly indicate that stem elongation in spinach is under the control of endogenous GAs.

Comparison of Spinach GAs with Authentic GAs. To compare authentic GAs with the GA-like substances obtained from spinach, purified acidic extracts were chromatographed in solvent system 2 along with GAs.b, GAs., GAs.a, and GAs. The histograms of Figure 4 show that in extracts of plants on SD GA activity was detected in three zones of the chromatogram. Spinach I (Rf 0.0–0.2) cochromatographed with GAs.b, spinach II (Rf 0.4–0.6) with GAs., and spinach III (Rf 0.73–0.93) with GAs.a. Extracts from plants exposed to 8 LD exhibited the same qualitative pattern of GA activity. Quantitatively, however, spinach II had increased as compared to the SD level, whereas both I and III had decreased, the latter to such a low level that it no longer caused a significant growth response in d5 corn.

Changes in Levels of Three Spinach GAs after Various Numbers of LD. Figure 5 gives a detailed picture of changes in the levels of the 3 GA-like substances present in spinach after various numbers of LD. Spinach III was low in SD and had disappeared after 2 LD. Fraction I decreased gradually while II increased with increasing number of LD. In other experiences, not included here, the level of II surpassed that of I after 10 to 12 LD. As a result of the decreasing levels of I and the concomitant increase of II, the total GA content, as estimated in the d5 corn assay, remained fairly constant after transferring plants from SD to LD.

GA Turnover under SD and LD. To test the hypothesis that exposure to LD enhances GA turnover in spinach (16), AMO-1618 was applied to plants on both SD and LD for different periods of time. If the growth retardant blocks GA biosynthesis, then the decline of the GA content with time should be a measure of the rate of GA metabolism. In Figure 6, the results are expressed as the GA levels in percentages of the control plants. Under SD the GA content decreased gradually, the 50% level being reached after approximately 7 days. In plants on LD the GA level dropped much more rapidly, with more than 80% of the initial GA content disappearing within 2 days after AMO-1618 applications had started. It is of interest

Table IV. Influence of AMO-1618 on Gibberellin Content and Stem Growth of Spinach

AMO-1618 treatments were on alternate days, starting 19 days before harvest. Plants used for extraction were harvested after 10 LD.

<table>
<thead>
<tr>
<th>AMO-1618</th>
<th>GA Content</th>
<th>Stem Length1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg GAs eq/100 g dry wt</td>
<td>cm</td>
</tr>
<tr>
<td>–</td>
<td>0.65</td>
<td>11.3 ± 1.2</td>
</tr>
<tr>
<td>+</td>
<td>0.06</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

1 Plants received same treatment as those used for extraction but after 10 LD were returned to SD. Measurements were made 19 days after beginning of LD treatment.

Fig. 4. Comparison of gibberellin-like substances as measured in the d5 corn assay of extracts from spinach plants on SD, or after exposure to 8 LD. Authentic GAs cochromatographed in solvent system 2. Dry weight of each sample was 24 g.
GIBBERELLINS AND GROWTH IN SPINACH

3.2 Number of LD

FIG. 5. Changes in the levels of three GA-like substances in spinach as affected by different durations of LD treatment. Thin layer chromatography in solvent system 2.

In the next experiment plants in SD and in LD were again treated with AMO-1618 for different periods of time, and the decline of the level of each GA-like substance was determined separately following TLC in solvent system 2. The results in Figure 7 indicate that 2 days after application of the growth retardant to plants on SD, spinach III had disappeared. On the other hand, spinach I and II decreased more or less in parallel when expressed on a percentage basis. Under LD conditions a considerable decrease in the level of spinach I and II was noticeable 8 hr after the first AMO-1618 application, but at no time had the one GA-like substance entirely disappeared, while the other could still be detected. Thus, these data appear to rule out the possibility that spinach I and II have a precursor-product relationship.

In the experiments of Figures 6 and 7, differences in GA turnover were observed when comparing GA contents of plants continuously under SD and after exposure to 7 or 8 LD. A further experiment was designed to find out how soon after the transfer from SD to LD the turnover of GAs had increased. Conversely, plants were also returned from LD to SD to see if
to note that a small fraction of GA persisted in treated plants on LD for at least 10 days.

In the next experiment plants in SD and in LD were again treated with AMO-1618 for different periods of time, and the decline of the level of each GA-like substance was determined separately following TLC in solvent system 2. The results in Figure 7 indicate that 2 days after application of the growth retardant to plants on SD, spinach III had disappeared. On the other hand, spinach I and II decreased more or less in parallel when expressed on a percentage basis. Under LD conditions a considerable decrease in the level of spinach I and II was noticeable 8 hr after the first AMO-1618 application, but at no time had the one GA-like substance entirely disappeared, while the other could still be detected. Thus, these data appear to rule out the possibility that spinach I and II have a precursor-product relationship.

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FIG. 6. Decline of GA content in spinach plants on SD (upper part), or after 7 or 8 LD (lower part) following AMO-1618 application. Different symbols represent separate experiments. Thin layer chromatography was in solvent system 1.

FIG. 7. Decline in the levels of three GA-like substances in spinach on SD, or after 7 LD following AMO-1618 applications. Thin layer chromatography was in solvent system 2.
this treatment would result in a lower GA turnover. The plants were exposed to the various photoperiodic treatments, and AMO-1618 was applied during the last 2 days before harvest. The retardant reduced the GA content of plants on permanent SD by only 16% (Table V). However, after 2 LD the GA content of plants treated with AMO-1618 was 21% that of control plants, indicating that a high rate of GA turnover had been established during the first 2 days under continuous illumination. After 8 LD followed by 4 SD the GA content was reduced somewhat more than in SD, but much less than after 2 LD. The histogram for GA activity in extracts of the treatment 8 LD → 4 SD was similar to that of extracts from plants in continuous SD (compare Fig. 4). As illustrated in Figure 2, stem growth initiated by 8 LD continued at a very low rate under subsequent SD. Thus, there appears to be a correlation between growth rate of petioles and stems, on the one hand, and the rate of GA metabolism, on the other.

**DISCUSSION**

The results described here clearly demonstrate that the growth rate of petioles increased immediately following the transfer of spinach from SD to LD. Since increased petiole growth is measurable after 1 LD, whereas at least 7 LD are necessary to induce flower formation, it follows that the elongating effect of LD on petioles is independent of stem growth and flower formation. A similar conclusion was reached by Stolwijk (20) from experiments in which spinach plants were given supplementary irradiation with different spectral regions. Longest petioles were observed under far red irradiation (700–800 nm) whereas green, yellow, and red irradiation resulted in short petioles. The situation was reversed with respect to flower formation, i.e., green, yellow, and red light were most conducive to flowering while the plants remained vegetative under far red. Day length extension in our experiments was obtained from incandescent bulbs which emit approximately equal energies in the red and far red regions of the spectrum. In spinach this combination favors rapid petiole growth as well as early flower formation and stem growth.

The cultivar Savoy Hybrid 612 used in this work ultimately produces flower primordia under SD conditions (Table II) and is therefore less suitable to assess the role of GA in flowering than a species with a qualitative long day response such as Silene armeria (4). However, as the results in Table II show, GA responses under SD did not significantly promote flower formation nor did AMO-1618 suppress it under LD. The tentative conclusion is therefore that flower formation in spinach is not under GA control. Previous workers (8, 22, 25) have observed that GA applications to spinach on SD cause stem elongation, but only Wittwer and Bukovac (25) also obtained flowering. However, in none of these studies were the shoot apices examined microscopically.

Extensive attempts to reproduce Radley's finding (16) of a large increase in GA activity in spinach after 1 LD cycle were unsuccessful (Table III). There is no obvious explanation for this discrepancy, but it should be noted that Radley states in a later report (17) that in some cases no peak of GA-like activity was found after I LD.

Following the temporary increase after 1 LD, Radley (16) observed that the GA content, as measured in the dwarf pea bioassay, decreased with increasing number of LD. A similar trend was observed for fraction I in the present study (Fig. 5). Fractions II and III, while active in the d5 corn assay, probably escaped detection in the pea bioassay (16) since GA4 is the most active GA in this test (2, 5). In subsequent experiments, Radley (17) estimated the GA content of spinach in the barley endosperm test. In our experiments only fraction I caused a response in this assay (unpublished results).

Using the lettuce hypocotyl test, Wareing et al. (24) detected two zones with GA-like activity in extracts of spinach. One peak cochromatographed with GA3 and was probably identical with our spinach I. The other zone cochromatographed with GA2 and GA4. No GA4 marker was used, but in the TLC system employed this GA runs at the same Rf as GA2 and GA4. Thus, both spinach II and III were presumably present in the second peak of Wareing et al. (24). Preliminary results (unpublished) with the lettuce hypocotyl assay of acidic extracts following TLC in solvent system 2 indicated activity for both I and II. The region of the chromatogram with III also contained ABA which inhibited hypocotyl growth.

The rapid decrease of spinach III after transfer to LD (Fig. 5), and also on SD following AMO-1618 application (Fig. 7), suggests that this GA-like material may be a precursor of the more polar GA-like substances I and II. If all three GA-like substances were on a single biosynthetic pathway, present knowledge of GA biogenesis (12, 19) suggests the sequence III → II → I. However, although the conversion of spinach II to I cannot definitely be ruled out, the data in Figures 5 and 7 offer no support for this idea.

The results on petiole and stem growth (Figs. 1 and 2; Tables I and II) indicate that both these processes are dependent on the presence of GA. AMO-1618 reduced petiole growth and blocked stem growth, while applied GA4 overcame the effects of the growth retardant and also caused stem growth in plants continuously under SD. Extraction experiments (Table IV, Figs. 6 and 7) confirmed the expectation that the GA content was much reduced after AMO-1618 application. On the other hand, rosette growth of plants in SD and stem elongation in plants under LD conditions were not simply correlated with the absence and presence, respectively, of endogenous GA. Upon transfer to LD, both spinach I and III decreased, while spinach II gradually increased (Fig. 5). Thus, if one wishes to consider a causal relationship between GA content and stem growth, only spinach II would qualify. A corollary of this consideration would be that substance II is more active in inducing stem growth than the other two substances. Bound GAs which can be released by acid hydrolysis (1) cannot be detected in extracts of spinach grown under SD or LD.

Spinach plants on SD had approximately the same GA content as plants on LD but failed to produce stems. It has been suggested (23, 26) that flower formation and stem growth under SD are prevented by the high levels of endogenous inhibitors which are reduced on transfer to LD. If this situation exists in spinach, the inhibitor is not identical with ABA, since the level of ABA increases rather than decreases when spinach is transferred from SD to LD (28).
The results on stem growth caused by applied GA_{\text{4}} in plants treated with AMO-1618 on LD, on the one hand, and in plants on SD, on the other, demonstrate that the sensitivity of spinach plants to GA is increased under LD, and are thus reminiscent of a similar situation in the rosette LDP Silene armeria (4).

Spinach plants on SD and LD exhibited a marked difference in GA metabolism. Following AMO-1618 application the GA level fell off much more rapidly under LD than under SD. Since the levels of extractable GAs under the two photoperiods were approximately equal, these results imply that the rate of GA biosynthesis was much more rapid in plants on LD than in those on SD. This drastic change in GA metabolism was established very shortly after the plants had been transferred to LD; the rapid turnover disappeared again when they were returned to SD (Table V). Likewise, the growth rate of petioles and of stems was determined by the prevailing photoperiod (Figs. 1 and 2). In Silene the amount of diffusible GAs markedly increased just prior to the onset of stem elongation. Since the GA content of Silene plants on LD as measured by extraction did not increase over that of plants in SD by more than a factor of 2, it was concluded that increased GA biosynthesis in Silene on LD was accompanied by increased GA utilization (4). Furthermore, ^{3}H-GA_{\text{4}} was more rapidly metabolized in Silene plants on LD than under SD (21). Thus, increased GA metabolism is closely associated with the beginning of stem growth in both the rosette plants Silene and spinach.

The question remains whether the more rapid GA turnover in plants under LD is significant for growth, or merely a result of the higher growth rate of the petioles and the increased mitotic activity in subapical tissues, as suggested by Radley (16). It should be noted that an increased growth rate is not necessarily associated with increased GA metabolism. For example, dwarf peas show marked differences in growth when kept in light or darkness, while metabolism of ^{3}H-GA_{\text{4}} was the same (10), and that of ^{3}H-GA_{\text{4}} only slightly different (15) under the two conditions. If GAs are biologically active in the chemically unchanged form as shown for GA_{\text{4}} and GA_{\text{5}} in peas (10, 15), metabolism of GA per se will not contribute to growth. However, more GA will be available to spinach plants under LD because of increased GA biosynthesis, and this, combined with the increased sensitivity to GA, could be responsible for increased petiole growth and stem elongation under LD.

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LITERATURE CITED