Influence of (2-Chloroethyl) Trimethylammonium Chloride (Cycocel) and Daylength on Gibberellin-like Activity in the Root Exudate of the Poinsettia Euphorbia pulcherrima, Willd. cv. ‘Paul Mikkelsen’

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Abstract. Three substances with gibberellin-like activity in the dock and barley endosperm bioassay systems were detected in the root exudate of poinsettia cultivar ‘Paul Mikkelsen’. In the isopropyl ether:acetic acid (95:5, v/v) solvent system on 250 µ layers of silica gel G, these substances had Rf values of 0-0.1, 0.4-0.6, and 0.7-0.8. The extracted exudate material at Rf 0-0.1 generally showed greater activity in plants grown under long day conditions while the relative proportion of the other 2 fractions to the first increased under short day conditions. Following a drench with (2-chloroethyl) trimethylammonium chloride (Cycocel), the activity of all 3 fractions was reduced in comparison to the control. The Rf 0-0.1 material evidenced lower activity in relation to the other 2 fractions in exudate from Cycocel spray-treated plants.

The occurrence of gibberellin-like substances in the bleeding sap has been demonstrated for a number of different plant species (3, 8, 10, 13). The level of gibberellin-like activity ranged from 0.007 to 30 µg gibberellic acid (GA3) equivalents per plant per day, depending upon the plant species. Such regular endogenous doses of gibberellin-like substances were considered to exert a significant effect upon the growth of the plant. The sites of synthesis of these materials may include both the aerial organs and roots.

Qualitative and quantitative changes in the levels of gibberellin-like substances in plant exudates have been reported. Reid and Carr (11) proposed that Cycocel effected such changes through altered GA synthesis in the roots. Kende and Sitton (8) reported a quantitative relationship between gibberellin-like substances occurring in poinsettia root exudate. Curtis and Kende (7) have shown a promoting effect of Cycocel upon flower initiation in plants growing under long (16 hr) and marginal (13½ hr) photoperiods. The previously cited reports suggested that this could be related to an effect of Cycocel upon the gibberellins involved in flower initiation.

Studies were undertaken to demonstrate that gibberellin-like substances occur in poinsettia root exudate. Additionally, an attempt was made to show that either quantitative or qualitative changes in gibberellin-like activity may be associated with transition to reproductive status as induced by short photoperiods or treatment with Cycocel.

Materials and Methods

Vegetative plants of 'Paul Mikkelsen' poinsettia were grown in a 22°C greenhouse with a 4-hr incandescent light break (10 ft-c minimum) from 10 pm to 2 am. Eight hour photoperiods were achieved by covering the plants with black cloth from 4 pm to 8 am. Cycocel was applied as a soil drench at the rate of 1 g per 6 inch pot or as a 0.25% foliar spray.

Root exudate was collected from 3-month-old plants.

Dock leaf senescence bioassay. The procedures of Whyte and Luckwill (16) were followed using leaves collected from clonally propagated broadleaved dock, Rumex obtusifolius, L., plants grown in the greenhouse. In this assay gibberellins prevent the degradation of chlorophyll in leaf discs kept in the dark. The amount of chlorophyll remaining is proportional to the gibberellin concentration. Chlorophyll remaining in the leaf discs after 5 days was extracted with methanol and the absorbency (optical density) read at 665 mp on a Bausch and Lomb Spectronic 20. The sensitivity of the assay was such that the top of the plant was cut off to leave a stump about 3 inches tall. A short piece of latex rubber tubing was affixed to the stump. An L-shaped glass spout was wedged in a short collar of tygon tubing which was forced into the rubber tube on the stump. The exudate was either collected in separate containers attached to the spout or drained into a central vessel through fine plastic tubing. The accumulated exudate was bulked twice a day and stored at —18°C until used.

Frozen exudates were thawed and filtered through Whatman No. 1 filter paper to remove debris, latex, and precipitated substances. The aqueous exudate was measured and lyophilized to a powder.

Dried residues were handled according to a procedure adapted from Aung and de Hertogh (1). The dry exudate was taken up in equal parts (v/v) of 0.33 M KH2PO4 buffer at pH 8.0 and petroleum ether. Following shaking and separation from the petroleum ether, the aqueous phase was extracted twice with ethyl acetate. The ethyl acetate was discarded, and the aqueous phase acidified to pH 2.5 with HCl and partitioned 3 times against ethyl acetate. The aqueous phase was discarded and the excess water present in the ethyl acetate was frozen out. The acidic ethyl acetate fraction was evaporated to a yellow gummy residue. This residue was taken up in a small portion of acetone or redistilled ethyl acetate for streaking on a 250 µ layer of silica gel G. The thin layer plate was developed at room temperature for 15 cm by ascending chromatography with isopropyl ether:acetic acid (95:5, v/v).

Fractions of the chromatograms representing the origin and 10 zones 1.5 cm wide were scraped from the plate and eluted with water-saturated ethyl acetate. The ethyl acetate was evaporated and the residue taken up in distilled water for bioassay.
as to give an almost linear reading (Fig. 1) in proportion to the negative logarithm of the gibberellic acid concentration between $10^{-4}$ and $5 \times 10^{-3} \mu g GA_3/ml$. Standard $GA_3$ concentrations were run with each test.

The raw optical density readings from the replicated bioassay samples were used to find the standard deviation of the mean difference ($s_a$) which was used to find the Least Significant Difference value from the equation

\[ L.S.D. = s_a \times t_{0.05} \]

The $t$ value was selected for the one-tail test as the only differences for which this assay would be expected to be significant were those greater than the control. Since chlorophyll content was expressed as a percentage of the control, the LSD value was transformed into a control. The planned comparison for the expected to be significant were those greater than the LSD value was for the given treatment peaks with the control.

Barley endosperm bioassay. The technique used was as described by Jones and Varner (7). In this assay the release of $\alpha$-amylase by embryoless half seeds of Hordeum vulgare 'Himalaya' is reported as being proportional to the logarithm of the gibberellic acid concentration between 0.0005 and 0.05 $\mu g/ml$. Standard $GA_3$ concentrations were run with each test.

The data are given as optical density units according to the formula,

\[ O.D. \text{ units} = \frac{\Delta \text{ O.D.} \times T_v}{t \times v} \]

\[ T_v = \text{volume of supernatant} \]
\[ \Delta \text{ O.D.} = \text{O.D. of zero time control minus O.D. of sample} \]
\[ t = \text{time of incubation with starch} \]
\[ v = \text{volume of supernatant taken for incubation} \]

When 2 O.D. values were obtained for each sample which differed by no more than 4 on a scale of 1 to 100, their mean was taken and entered into the above formula. There was no need for statistical treatment because of the close internal agreement of the readings.

Presumably, the collected sap was translocated in the xylem, as injury to the phloem when the rubber tube was fitted on the stump did not appear to affect exudate flow. In the woody basal area of the stem, the exuding of latex from the laticifers in the bark was not a problem. Significant gibberellin-like activity was detected in the 0–0.1 Rf zone on the chromatogram of the first day's collection, but not in the second. Only the first day's collection was used unless otherwise noted. Fig. 1–4 are representative of the several trials which were analyzed.

The rate of flow from plants maintained on long days was somewhat greater than for plants subjected to short days or treated with Cycocel as a drench of spray (Table 1). The effect of a Cycocel treatment, either as a drench or spray, was also to decrease the volume of exudate collected. There was, however, no apparent injury to the root system of the Cycocel-treated plants which might account for the decrease in volume.

Three zones of gibberellin-like activity appeared in chromatograms of extracts of poinsettia exudate. The peak which appeared at Rf 0–0.1 (sometimes into 0.1–0.2) was designated Fraction A (a peak which occasionally appeared in the range 0.2–0.4 was designated A'); that in mid-range which appeared from 0.4–0.6, Fraction B; and that at Rf 0.7–0.9, Fraction C.

The data in Fig. 2 show relatively higher gibberellin-like activity in plants receiving 7 or 14 short days (SD) in comparison to their long day controls. In the barley assay under long days, however, the presence of Fraction A is not evident to the same extent as in the dock assay. The B and C components appear greater under short days than long. The gibberellin equivalent values when corrected for assay method and number of plants (Table 2) bear out this observation.

The application of 1.0 g Cycocel per 6 inch pot as a soil drench reduced the level of gibberellin-like substances in root exudate as evidenced by the dock assay (Fig. 3).

![Fig. 1. Example of standard curve for gibberellic acid (GA3) in root exudate assay. Each point represents the mean of 10 bioassay standards for a given concentration. Two separate series of 10 bioassays each are shown as one curve as agreement was very close.](image_url)

Table 1. Mean volume and dry weight of collected poinsettia  
exudates expressed on a per plant per day basis.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>First day collections</th>
<th>Second day collections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume (ml)</td>
<td>Dry wt (mg)</td>
</tr>
<tr>
<td>Long days...</td>
<td>8.64 ± 4.65</td>
<td>30.5 ± 16.1</td>
</tr>
<tr>
<td>Short days..</td>
<td>6.03 ± 3.93</td>
<td>41.9 ± 31.3</td>
</tr>
<tr>
<td>Long days + Cycocel spray...</td>
<td>6.97 ± 1.22</td>
<td>16.3 ± 0.2</td>
</tr>
<tr>
<td>Long days + Cycocel drench...</td>
<td>4.86 ± 3.43</td>
<td>3.40 ± 3.40</td>
</tr>
</tbody>
</table>

Table 2. Gibberellin $A_3$ equivalents per plant for root exudate of long and short day plants as assayed in the dock and barley endosperm assays. Values derived from Fig. 2 following adjustments for number of plants and conditions of assay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction (GA3 equivalents in $\mu g \times 10^{-5}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Long days</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>2.7</td>
</tr>
<tr>
<td>Dock</td>
<td>5</td>
</tr>
<tr>
<td>Short days</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>6.7</td>
</tr>
<tr>
<td>Dock</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Fig. 2. Acidic ethyl acetate extracts of exudates from long day and short day poinsettia plants. A and B assayed in the dock leaf system; C and D assayed in the barley endosperm system. A, 665 ml (2.52 g dry wt) exudate from 60 long day (LD) plants. B, 730 ml (2.30 g dry wt) from 84 plants which received 7 short days (SD). C, 875 ml (2.68 g dry wt) exudate from 59 LD plants. D, 570 ml (1.25 g dry wt) exudate from 60 plants which received 14 SD. All extracts chromatographed on TLC. Solvent, isopropyl ether: acetic acid (95:5, v/v). In A and B, the LSD value indicates a significant difference from the control at the 5% level of risk. In C and D each peak represents the average of two optical density units values derived from separate replicates, but no test of significance was attempted. Control blanks contained barley half-seeds and distilled water instead of TLC chlort.

The active zones in the Cycocel assay, while still appearing at the expected Rf's were below the reliable extrapolation of the gibberellin standards. The GA3 equivalent activities of the long day peaks A, B, and C were 2 × 10−4, 1.6 × 10−4, and 4 × 10−4 µg/plant/day, respectively.

The barley endosperm assay was used to measure gibberellin-like activity in exudate of plants receiving a series of 0.25% Cycocel sprays at weekly intervals for 5 weeks (Fig. 4). The activity of the A fraction was greater in the long day plants which were not treated with Cycocel, but the B and C fractions were greater in the treated plants (Table 3). The effect of a spray on gibberellin-like activity in root exudate was less marked than the effect of a soil drench. The levels of gibberellin-like activity indicated in the Cycocel spray treatments were greater than the highest gibberellin standard of 1 × 10−2 µg GA3/ml. The fraction represented by the peak designated C appeared at a higher Rf than was usually found to be the case and may have been an artifact of the extraction solvent.

**Discussion**

Bioassays in repeated samplings of root exudate have revealed the presence of three fractions active as gibberellins. The first, designated Fraction A, was commonly observed in the Rf 0–0.1 zone but also in the origin and in the 0.1–0.2 Rf region of the isopropyl ether:acetic acid system. Gibberellins A1, A2, A3, A6, and A10 have been reported to occur in the origin and in the 0.1–0.2 Rf region with several others, A4, A5, A14, A15, and A13 occurring in the 0.1–0.2 region in this solvent system (4). All of the latter have been placed at higher Rf's with A5 at 0.2–0.3 and A6, A14, and A15 in the region of 0.3–0.4 (9). The B fraction of the exudate appeared variously at Rf 0.4–0.6 when it was evident at all. The known gibberellins for these regions (4) may include A9, A11, and A15, although A9 was also reported to have an Rf of 0.75 (9) and of 0.84 (Dr. B. O. Phinney, personal communication). The C fraction of gibberellin-like activity was regularly placed at 0.7–0.8 with occasional appearance in the 0.8–0.9 region and could correspond to A9.

Whyte and Luckwill (16) reported the dock bioassay to be most sensitive to GA3 while GA1 was only a tenth as effective with other known gibberellins still less effective (GA2 one-hundredth; GA6 one-thousandth). However, as their data have received no separate confirmation and as the substances extracted in these studies have not been positively identified as gibberellins, the assay data should be interpreted cautiously. Similarly, Jones and Varner (7) reported that GA5 induced less response in a 24-hr period in the barley assay than GA1, GA3, GA4, or GA7 while Stoddart and Lang (15) reported GA9 to be only 0.1% as active as GA3 in this system. Thus, suspected GA2-like and GA6-like components may be underestimated by this assay, and interpretations as to their presence or absence should be made cautiously.

Assuming the flow rates of Table 1 and the GA equivalent activity of Table 3, a calculation was made estimating the production at 0.0012 µg GA3 equivalents/plant/day or approximately 0.14 µg/1. Such a figure is comparable to other reports (10, 13).

The presence of gibberellin-like activity in the sap does not necessarily confirm its origin as a product of the root. Jones and Phillips (6) recognized this and provided evidence for considering the root as one organ involved in gibberellin synthesis. Their use of the agar block diffusion technique with roots, coupled with extraction of root tissue, confirmed that the apical 3–4 mm of the root actively synthesized gibberellin. Butcher (2) had previously isolated gibberellin-like substances in excised tomato roots in vitro, and Sitton et al. (12) demonstrated that isolated root apices incorporated C14-mevalonate into gibberellin intermediates. A preliminary experiment was conducted using the agar block diffusion technique to check the possibility that the root apex may be involved in the synthesis of gibberellin-like substances in poinsettia. The level of biological activity evidenced in the barley endosperm assay (Fig. 5) broadens the basis for considering the root apex as a source of gibberellin-like substances.

**Table 3.** GA3 equivalents per plant for root exudates from long day plants and long day plants which received five weekly sprays of 0.25% Cycocel. Values derived from Fig. 4 following adjustment for number of plants and condition of assay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction (GA3 equivalents in µg × 10−3)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long day</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td>0.4</td>
</tr>
<tr>
<td>Long day + Cycocel</td>
<td>0.16</td>
<td>1.6+</td>
</tr>
</tbody>
</table>

Fig. 3. Acidic ethyl acetate extracts of two-day exudate collections from LD and LD + Cycocel drench poinsettia plants. A. 465 ml (1.5 g dry wt) exudate from 30 LD plants. B. 465 ml (1.61 g dry wt) exudate from 34 LD plants which received 1 g Cycocel per 16 inch pot one week previously. Chromatographed on TLC. Solvent, isopropyl ether:acetic acid (95:5, v/v). Bioassay with dock leaf. LSD values indicate significant difference from the control at the 5% level of risk.

Fig. 4. Acidic ethyl acetate extracts of exudate from LD and LD + Cycocel sprayed poinsettia plants. A. 335 ml (1.22 g dry weight) from 50 LD plants. B. 290 ml (0.93 g dry weight) from 50 LD plants which received 5 weekly sprays of 0.25% Cycocel. Chromatographed on TLC. Solvent, isopropyl ether:acetic acid (95:5, v/v). Bioassay with barley endosperm. Peaks represent mean of two replicates.
Gibberellins. In Fig 4, for example, the A fraction (Rf 0-) in the poinsettia is of interest because of its expected if the shoot apex were the sole source of gibberellins. The production of the root. The sprays applied to the top of the root tip. These may interact or function independently.

Repeated applications of Cycocel reduce the gibberellin conversions, is less active in the exudate of treated plants. The effect of Cycocel in reducing the level of gibberellin or gibberellin precursor which is inhibitory to flower bud initiation in the poinsettia. Such a phenomenon may be restricted to conditions in which natural production is just barely sufficient to effect the inhibition, i.e., under marginal photoperiods.

Similarly, under short daylengths, the A fraction was lower in relation to the other fractions than in long day exudates. Stoddart (14) and Stoddart and Lang (15) advanced the concept of daylength-mediated steps in gibberellin metabolism. The alteration in gibberellin balance in root exudate during the transition from the vegetative to the floral state also has been documented (8). The possibility of conversion between gibberellins arising in the root and in the shoot complicates this concept but injects the thought that Fractions B and C may have their origin in the shoot apex and Fraction A in the root. Under short days flower initiation in the poinsettia may result as a response to a decreased ratio of Fraction A to other gibberellin-like substances or as a response to a higher level of the other gibberellin-like substances, no matter what the level of Fraction A.

**Literature Cited**