Regulation of Endogenous Indoleacetic Acid and Keeping Quality of Poinsettia

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Abstract. A study was made of the changes with aging in the level of endogenous auxin and the activity of IAA-oxidase in the bracts of two poinsettia cultivars. The auxin level decreased with time in both cultivars. Diffusible auxin also decreased with time in both cultivars but faster in the poor keeper. The activity of the IAA-oxidase system and the level of hydrogen peroxide increased with aging. The auxin level could be related to the activity of IAA-oxidase, which in turn could be related to the appearance of new forms of peroxidase. A hypothesis is developed to explain differences in keeping quality among poinsettia cultivars based on changes in the level of endogenous auxin.

Keeping quality varies among poinsettia cultivars and is measured by the rate of bract abscission. Indoleacetic acid (IAA) at concentrations above $10^{-5}$M applied to the petiole stump has generally been found to delay abscission of explants (11). This is also true for poinsettia leaves and bracts (6). Intact plant studies have indicated a close relationship between the level of endogenous diffusible IAA and the propensity to abscise (20). A decrease in the level of auxin accelerates abscission. Several hypotheses have been advanced to explain this auxin effect in vivo; subscribing to a direct auxin effect, auxin interacting with aging and senescence, or auxin interacting with ethylene. The level of diffusible auxin may be regulated through synthesis, transport, or destruction. Control of abscission in cotton has been related to the activity of the IAA-oxidase system (19).

This study was conducted to determine the endogenous IAA auxin relationships in the poinsettia during normal aging and after experimental treatment. A study of the IAA-oxidase system was undertaken to ascertain its role in the regulation of the level of IAA.

Materials and Methods

The poinsettia cvs. Paul Mikkelsen, White Ecke, New Ecke Pink all $2n=28$ and Barbara Ecke Supreme $2n=56$ were used in these studies. 'Paul Mikkelsen', a good keeping cultivar, and 'Barbara Ecke Supreme', a poor keeping type, were used more intensively. Cultural methods, experimental methods, and preparation of plants were described previously (6).

Diffusible growth regulators were collected from either individual entire bracts of flowering stems cut directly above the primary bract point. They were embedded in 1% agar at 21° and 100% RH in the dark and left to diffuse for 24 hr. The agar was extracted 3 times with 50 ml aliquots of methanol which was reduced to near dryness in a flask evaporator and made up to 1 ml with ethanol. Duplicate 100 μl samples of each extract were spotted on Whatman No. 1 paper and developed in 1 ml of 10:1:1 (v/v/v) mixture of 5mM guaiacol and 1 mM H$_2$O$_2$ in 0.1 M phosphate-citrate buffer pH 6.1 and the reaction was stopped by flooding with 10% acetic acid.

Peroxidase activity was assayed by incubating the gels in a mixture of 5mM guaiacol and 1 mM H$_2$O$_2$ in 0.1 M phosphate-citrate buffer pH 6.1. Peroxidase activity was assayed by incubating the gels in a mixture of 5mM guaiacol and 1 mM H$_2$O$_2$ in 0.1 M phosphate-citrate buffer pH 6.1. Peroxidase or IAA-oxidase enzyme studies were carried out using fresh (petioles only) or freeze dried bract tissue. Freeze dried material was ground in a Wiley mill and extracted for 2 hr. at 0° with sufficient 0.1 M phosphate-citrate buffer pH 6.0 to give a final protein concentration of approximately 1 mg/ml (usually 25 ml/gm tissue). Fresh tissue was coarsely chopped then homogenized at 0° with 5 volumes of phosphate-citrate buffer. In either case the slurry was centrifuged at 100,000 x g, for 30 min. at all at 0°. The supernatant was used directly for enzyme activity or gel electrophoresis studies.

The method for vertical disc gel electrophoresis was based on that of Davis (3). Routinely 0.2 ml enzyme extract was run in 7% polyacrylamide gel at pH 8.3 with 2.5 m amps/gel. Peroxidase activity was assayed by incubating the gels in a mixture of 5mM guaiacol and 1 mM H$_2$O$_2$ in 0.1 M phosphate-citrate buffer pH 6.1 and the reaction was stopped by flooding with 10% acetic acid.

Hydrogen peroxide assay was based on the method of MacNevin and Uplone (14). Fresh petioles were homogenized in excess cold ethanol. Peroxidase was complexed by the addition of 0.5 ml of 12.5% Ti Cl$_4$ and precipitated with excess NH$_4$OH. After centrifugation for 5 min at 2,000 x g the supernatant was discarded and the precipitate solubilized in 10 ml of 3.0 N HCl. The yellow color was read at 405 mμ.

Results

Diffusible growth regulators in the course of aging. The growth regulators diffusing from the flowering head were measured at weekly intervals on 'Paul Mikkelsen' and 'Barbara Ecke Supreme' plants held in a simulated home environment.

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The zone corresponding to IAA (Rp 0.3 to 0.4) stimulated coleoptile growth (Fig. 1). During the first week there was a small increase then a steady decline. The pattern for both cultivars was the same except that 'Barbara Ecke Supreme' declined more rapidly. A second major zone of stimulation 0 to 0.1 also changed with time. In both cultivars it steadily declined at approximately the same rate. The zones 0.1 to 0.2 to 0.3 could be separate growth regulators or tails of the 2 major peaks. The zones 0.6 to 0.7 and 0.8 to 0.9 were the only ones consistently active as inhibitors of coleoptile growth. No gross difference was apparent between the cultivars nor was there any consistent change with time for the inhibitors.

Evidence to support the hypothesis that stimulation in the zone Rp 0.3 to 0.4 was due to endogenous IAA was obtained by colorimetric measurement of diffusate after reaction with Salper reagent (8). Twenty bracts were sampled at 6 day intervals from plants of 'Paul Mikkelsen' and 'Barbara Ecke Supreme' held in a home environment from the time of anthesis. Half the plants of each cultivar had been given a single spray with 10⁻⁵ M IAA at anthesis. The results expressed in IAA equivalents are presented in Table 1. Both the untreated and treated samples showed a relative decline in diffusible IAA with time. The treated samples showed higher levels of IAA throughout but the treatment differential decreased with time in both cultivars. The IAA level in 'Barbara Ecke Supreme' declined more rapidly.

IAA-oxidase activity. Preliminary experiments using freeze dried whole bracts of 'Paul Mikkelsen', 'White Ecke', 'New Ecke Pink' and 'Barbara Ecke Supreme', indicated different activities of the enzyme among cultivars and at different sampling times (Table 2). There was also a marked differential in response to added peroxide in the assay procedure. Both at anthesis and senescence 'Paul Mikkelsen' and 'Barbara Ecke Supreme' held in a home environment from the time of anthesis. Half the plants of each cultivar had been given a single spray with 10⁻⁵ M IAA at anthesis. The results expressed in IAA equivalents are presented in Table 1. Both the untreated and treated samples showed a relative decline in diffusible IAA with time. The treated samples showed higher levels of IAA throughout but the treatment differential decreased with time in both cultivars. The IAA level in 'Barbara Ecke Supreme' declined more rapidly.

### Table 1. Diffusible IAA from bracts of two poinsettia cultivars at different sampling times after anthesis in μg IAA/10 bracts. Treated plants were sprayed with 1 mM IAA at anthesis.

<table>
<thead>
<tr>
<th>Cultivar and treatment</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul Mikkelsen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>6.1</td>
<td>6.7</td>
<td>5.3</td>
<td>4.9</td>
</tr>
<tr>
<td>Treated</td>
<td>9.2</td>
<td>8.0</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Barbara Ecke Supreme</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>5.9</td>
<td>5.8</td>
<td>4.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Treated</td>
<td>7.6</td>
<td>6.0</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. IAA-oxidase activity in bracts of four poinsettia cultivars sampled at anthesis and senescence with and without H₂O₂ in the incubation solution. Activity measured in VOD at 562 nm after reaction with p-dimethylaminocinnamaldehyde.

<table>
<thead>
<tr>
<th></th>
<th>Anthesis</th>
<th>Senescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>H₂O₂</td>
<td>Control</td>
</tr>
<tr>
<td>Paul Mikkelsen</td>
<td>0.21</td>
<td>0.38</td>
</tr>
<tr>
<td>White Ecke</td>
<td>0.18</td>
<td>0.35</td>
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<tr>
<td>New Ecke Pink</td>
<td>0.32</td>
<td>0.46</td>
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<tr>
<td>Barbara Ecke Supreme</td>
<td>0.28</td>
<td>0.45</td>
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</table>

Measurements of IAA-oxidase activity were made at 6 day intervals with and without 1 mM H₂O₂ in the incubation mix. The results are shown in Fig. 2. The peroxide level showed a general increase with time. Plants treated with IAA had a slower rate of increase. Although the 2 cultivars had the same level at anthesis, 'Barbara Ecke Supreme' increased more rapidly and had a higher level at the termination of sampling.

For IAA-oxidase without added peroxide the general trend was a small increase in activity with increasing age. The addition of peroxide during incubation resulted in increased activity in all samples and the trend with time was a general increase disproportionately larger than without peroxide. Initial treatment with IAA delayed the surge of IAA-oxidase activity with peroxide.

The addition of 5 mM IAA to the peroxidase incubation mix did not reveal any new bands nor noticeably alter the relative intensity of staining but did appear to result in sharper, more clearly defined bands. The zymogram is shown in Fig. 3. The approximate intensity of the staining of a band is indicated by the width of the line in the zymogram.

In all cases there were multiple forms of the enzyme and the greatest number visible in one gel was 11. As a general rule, the number of forms and relative intensity of staining increased with age for all samples. Three heavy staining bands at Rp 0.55, 0.60, 0.65 appeared in every instance as did, in general, the light staining bands at Rp 0.85 to 0.90. Other bands, notably at Rp 0.10 to 0.15, 0.40 to 0.45 and 0.70 varied with cultivar, treatment and age. The single band Rp 0.70 was confined to ‘Barbara Ecke Supreme’ except for IAA treated plants where it was most intense and appeared in both cultivars. The 1 or 2 bands at Rp 0.40 to 0.45 appeared after anthesis and developed more intense staining with time in ‘Barbara Ecke Supreme’. The second band appeared in intact plants of ‘Barbara Ecke Supreme’ only. The 2 bands at Rp 0.10 to 0.15 were absent at anthesis but developed into intensively stained bands with time. ‘Barbara Ecke Supreme’ showed more bands and deeper staining than did ‘Paul Mikkelsen’. Treatment with IAA appeared to stimulate the formation of new bands.

Discussion

Our results provide the basis for the development of a hypothesis to explain control of abscission in poinsettia bracts and differences in keeping quality among cultivars. The hypothesis states that the primary control of abscission lies in the level of endogenous auxin. Above a certain threshold level, auxin maintains the bracts in situ; below this level, senescence and abscission result. Further, differential bract abscission rates among cultivars may be related to a variable rate of decline in the auxin level. The increment of decline is the result of the level of activity of the IAA-oxidase system.

The results of this and previous studies on poinsettia bract abscission (6) support this hypothesis. The only exogenously applied growth regulator that had any consistent effect was IAA which could substitute for the blade in inhibiting abscission of debladed petioles (6). The diffusible growth regulator studies showed a general decline in auxin after anthesis with the rate of decline the major difference between cultivars having different keeping qualities. Jacobs (11) has also demonstrated a negative correlation between auxin level and propensity to abscise. A sketch illustrating the hypothesis that senescence and abscission is governed by several factors determining the level of endogenous auxin is presented in Fig. 4. The 4 independent variables; respiration (unpublished data), growth, IAA-oxidase, and peroxide have been equally weighted and are assumed to run from zero to unity. The interaction of the independent variables is presented in the form of a cumulative vector analysis which determines the shape of the dependent variable, the level of endogenous auxin. The derived auxin curve fits closely with that observed for diffusible auxin. Other known parameters of senescence and abscission such as membrane permeability, polysome formation, nucleic acid synthesis and protease activity would probably contribute to the level of endogenous auxin, but the exact mode of actions in poinsettia has yet to be studied.

The hypothesis is one of development among the quantitative relationships rather than qualitative differences. A change in the level of any factor controlling the level of auxin would influence the approach to senescence and abscission and a change in more than one factor would have a cumulative effect. What was observed were relatively small increments between the good and poor keeping cultivars. In every case, however, the situation favored lower auxin levels in the poor keeper.

The results indicated a differential capacity between cultivars for enzymatic oxidation of IAA. The activity of IAA-oxidase must be considered together with the hydrogen peroxide effect and the endogenous concentration of this compound. The activity of IAA-oxidase determined in the absence of H₂O₂ is a good approximation of the in vivo system whereas the activity in the presence of H₂O₂ would be an indication of the total amount of enzyme present. Hence, the former is the probable
A primary effect of auxin is to delay aging (2), and one effect of MAO may control the formation of new forms of IAA-oxidase. An increase in endogenous peroxide was associated with the previous investigations (10,18). It may also nonenzymatically increment of peroxide, yet an increased enzyme activity and fewer new isozymes. Prior treatment with IAA also gave an associated with a relatively smaller increase in enzyme activity for IAA-oxidase. Reason, Ockerse, Siegel and Galston (17), and Lavee, and Galston (12) have shown that the addition of IAA to extracts and the plant as a whole is able, either repressive-de-repression. On the basis of our studies, it would appear that high levels of IAA or peroxide are, either directly or indirectly, to induce the same new forms of peroxidase which would then function in decarboxylating the former and reducing the latter.

Endo (4) reported that IAA-oxidase and peroxidase may be both qualitatively and quantitatively different on a zymogram and that an analysis based solely on peroxidase may be misleading. Assay for IAA-oxidase using the method of Endo (4) or based on the method of Meuld and Gaines (15) did not prove consistent enough for routine analysis but when IAA-oxidase activity was observed it appeared in the 3 heavy staining bands Rp 0.55 to 0.65 and, in older tissue, also in the bands Rp 0.10 to 0.15. If this does indicate the actual case for IAA-oxidase in poinsettia, then it would fortify the hypothesis since the zymogram bands Rp 0.10 to 0.15 showed a close parallel to changes in the peroxide level and IAA-oxidase activity.

The IAA-oxidase system in poinsettia appears to differ from that in many other plants in its requirement for a phenolic co-factor. In oat coleoptiles (9), raw pea extract (7), cotton (19), and purified extract from horseradish (10) the IAA-oxidase system requires a monophenol such as 2,4-dichlorophenol as co-factor but not hydrogen peroxide. Raw cotton leaf extracts (13) have an inhibitor which stops the reaction from proceeding, while purified extract requires both a monophenol and hydrogen peroxide for activity. Raw poinsettia bract petiole extracts did not respond to added 2,4-dichlorophenol; however, after purification of the extract by passing it through Sephadex G-25 it then required 2,4-dichlorophenol for activity. Both raw and purified extracts responded to peroxide. The conclusion drawn is that raw extracts and the in vivo system contain sufficient phenolic co-factor that it is not limiting.

**Literature Cited**

Thinning Peaches with Bloom and Postbloom Applications of 2-Chloroethylphosphonic Acid

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Abstract. Bloom or postbloom sprays of 2-chloroethylphosphonic acid (Ethrel) thinned peaches. Applications at full bloom did not produce consistent thinning in 'Cardinal'. Postbloom sprays applied approximately at endosperm cytokinesis produced consistent thinning of 3 cultivars.

Degree of thinning was related to time of Ethrel application. Most thinning was obtained when Ethrel was applied near the end of Stage I or during Stage II. Ethrel sprays within a month after full bloom caused significant fruit size reduction that persisted 2 months or more after treatment. Ethrel sprays within 50 days of harvest accelerated maturity of several cultivars.

Renewed interest in chemical thinning of peaches has resulted from the successful thinning of several cultivars with 3-chlorophenoxy-a-propionamide (3-CPA) (1, 15). Unfortunately, many cultivars are not thinned satisfactorily with 3-CPA, and the search for a peach thinner with wider adaptability continues.

The abscission-promoting property of Ethrel has drawn attention to it as a possible thinning agent for peaches (3, 7). This paper reports experiments conducted during 1968 and 1969 to evaluate Ethrel as a thinner for several peach cultivars.

Materials and Methods

Location, experimental design. Experiments with 'Redskin' were located at Pendleton, S. C.; all others were conducted at the Clemson University Sandhill Experiment Station, near Columbia, S. C. All treatments consisted of a single Ethrel spray applied to runoff at 300 lb/in² pressure. Each experiment was a randomized complete block with single-tree plots and 4 replications.

Spray treatments: timing and concentration. Ethrel sprays were applied at 25 to 250 ppm to 11-year-old 'Cardinal' trees at full bloom in 1968, and on other trees at 100 and 200 ppm at full bloom in 1969. Postbloom applications were made to 'Cardinal', 4-year-old 'Redhaven', and 8-year-old 'Redskin' trees in 1968 and repeated on other trees in 1969. These sprays were applied in concentrations shown in Table 2 when fruit development, as indicated by ovule length, coincided approximately with endosperm cytokinesis (14). Other postbloom peach thinners are effective at this stage of fruit development (10, 11, 15).

To study the timing of treatment, a 100 ppm Ethrel spray was applied to 'Redskin' at 22 days after full bloom (AFB) in 1969 and at weekly intervals until the end of Stage I, the initial period of rapid fruit growth. In an additional timing study, 100 ppm of Ethrel was applied to 10-year-old 'Ranger' trees in 1969 at the beginning of pit hardening (Stage II), 2 weeks later, and 5 weeks later, during the final swell (Stage III).

The last spray was applied 2 weeks before anticipated harvest, but unusually slow development delayed harvest of control fruit until 3 weeks later.

Measurement. Blossom or fruit abscission was determined by calculating the number of peach per tree at harvest, using average fruit weight and yield data. We found previously (15) that this index of thinning is closely correlated with conventional fruit set counts and is an adequate substitute. Average fruit weight was determined at harvest in all experiments by weighing 30-fruit samples taken from each tree at each picking date. Fruit growth in the timing experiment with 'Redskin' was determined by weighing 15-fruit samples from each plot at each of several preharvest sampling dates. The number of peaches removed from each tree at each picking date in 1969 was also calculated. These data were used to compute a weighted-average harvest date for each plot. Harvest date is expressed for each treatment as days before or after the hand-thinned control.

Results and Discussion

Bloom sprays. Ethrel sprays at full bloom reduced the crop considerably (Table 1). None of these sprays had an adverse effect on the tree or foliage. These data indicate a relatively narrow range of concentration for satisfactory bloom-thinning, since all treatments either severely overthinned or failed to thin adequately.

The 100 ppm Ethrel concentration was much more effective in 1969 than in 1968 (Table 1), showing an inconsistency that characterizes many bloom-thinning agents. The 'Cardinal' trees used in our experiments had a very light crop in 1967 and a very heavy crop in 1968. This suggests that the ease of thinning with a given concentration of Ethrel may be related to blossom vigor as influenced by crop load the previous year.

Ethrel concentrations of 200 - 250 ppm, which overthinned 'Cardinal' in both years, are substantially lower than those reported to satisfactorily bloom thin the 'Maygold' peach in Florida (3).