The Regulation of Cambial Division and Secondary Xylem Differentiation in Xanthium by Auxins and Gibberellin

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ABSTRACT

Cambial division continued in decapitated Xanthium plants without concomitant xylem fiber differentiation. The application of indoleacetic acid to these plants did not affect the production of cambial derivatives or induce xylem fiber differentiation. When naphthaleneacetic acid was applied either to the second internode or to the stump of a lateral shoot, xylem fiber differentiation was induced in the newly formed cambial derivatives on the xylem side of the cambium in the stem. When naphthaleneacetic acid was applied unilaterally, xylem fiber differentiation was restricted to that side of the stem in the first internode and hypocotyl. Naphthaleneacetic acid also enhanced the production of cambial derivatives. Gibberellin acid enhanced cambial derivative production but did not affect the differentiation of xylem fibers. Similar numbers of cambial derivatives were produced in some naphthaleneacetic acid-treated plants in which xylem fiber differentiation was induced and in gibberellin acid-treated plants which did not differentiate xylem. When naphthaleneacetic acid was applied 72 hours after decapitation, the oldest of the cambial derivatives on the xylem side failed to develop into fibers although younger cells did. These results suggest that auxin has its direct effect on the induction of xylem differentiation rather than the induction of divisions prerequisite to differentiation.

Little direct evidence has been presented to demonstrate the role of auxin in the induction of xylem differentiation from either the procambium or the cambium. Jacobs and Morrow (11) presented data correlating the differentiation of the primary xylem in Coleus with the auxin production in the leaf. Wangemann (30) demonstrated that exogenous auxin could replace the leaf in its xylemogenic effect in Coleus stems. However, in neither of these cases is it clear whether auxin is required for the induction of xylem differentiation, or for the maturation of xylem, or both. Young (33) found that exogenous IAA could not substitute for the developing leaf in the induction of primary xylem differentiation from the procambium in Lupinus albus.

Studies of secondary xylem differentiation in response to growth regulators are complicated by the involvement of the cambium. It has often been assumed that the induction of cambial division and xylem differentiation in response to a growth regulator demonstrated a requirement for the growth regulator in xylogenesis. Since the induction of cambial division is a prerequisite to secondary xylem differentiation, it cannot be assumed that a factor which induces the division is directly necessary for differentiation. Auxin induces cambial division in developing stems (23) and reactivates the cambium after a dormant period (9). The differentiation of the cambial derivatives into xylem in response to auxin may (5, 9, 18, 23) or may not (13, 19, 24, 25) occur. Auxin was fully effective in the induction of cambial division in those cases where it failed to induce normal xylem differentiation.

Auxin alone may not promote cambial division but will promote xylem differentiation if cambial division is invoked by gibberellic acid (31). There is apparently some difference in the response to gibberellic acid depending on the type of system used. Studies which deal with the application of GA alone to stem segments show that the effect of GA is to promote cambial division without accompanying xylem differentiation (31). Studies dealing with intact plants show that the effect of GA in that situation is to increase the production of xylem and the resulting cells differentiate (1–3, 14, 22), or there is no effect on cambial division but differentiation is enhanced (17), or there is only slight enhancement of division and no effect on differentiation (16).

The effects of auxins, gibberellins, and cytokinins on xylem differentiation have been noted in a great deal of work on wound xylem regeneration as well as various tissue and organ culture systems; for a recent review see Roberts (20).

The present report is concerned with the induction of xylem fiber differentiation in cambial derivatives in the stems of decapitated Xanthium seedlings. In this system cambial derivative production continues when the young leaves and buds are excised although xylem fiber differentiation ceases. Thus cambial derivative production can be experimentally separated from xylem differentiation. The basic system and the xylegenic effect of growing leaves were described previously (21).

MATERIALS AND METHODS

In this system the effect of applied growth regulators can be monitored easily. If a given growth regulator affects cell division only, then there will be an increase in the number of cambial derivatives as compared to the controls, and these cells will not be differentiated. If a growth regulator affects differentiation only, then there will be no increase over the controls in terms of cell production, but those cells produced will be differentiated. There could be an effect of the growth regulator on both cell division and xylem differentiation.

Burrs of Xanthium pennsylvanicum Wallr. (Chicago strain) were germinated and grown under the long day conditions described previously (21) to the stage where they had 9 ± 1...
leaves. The experiments were conducted in two ways which differed in the method and the timing of the application of the growth regulators.

Method 1: Immediate Growth Regulator Application. The first experiments tested the effects of IAA (0.1-10.0 mg/liter \(\pm\) GA (100 mg/liter), or naphthaleneacetic acid (1-15 mg/liter). The plants were decapitated, a tube was attached to the second internode, and the treatments were applied as shown in Figure 1. Five or 10 plants were used for each concentration of growth regulator tested. In the case of the IAA experiments 10 plants were used at each concentration and the entire experiment was replicated twice. GA (100 mg/liter) was tested only once. The NAA* experiment was run only once utilizing this method of growth regulator application. In all cases the test period was 14 days. At that time the plants were fixed and processed for sectioning as previously described (21).

Method 2: Delayed Growth Regulator Application. This method was employed after it was found that NAA had an effect on xylem differentiation. The procedure was to decapitate the plants in the second internode and treat as shown in Figure 1, c and d. Growth regulators were applied 72 hr after decapitation by one of two methods as shown in Figure 1, c and d. In both cases a thin slice of tissue was cut off the stump of the lateral shoot prior to attachment of the tubes. Two factorial experiments were conducted by method A and one factorial experiment conducted by method B. A fourth experiment tested the effect of NAA only. The test period with method 2 (delayed application) was 15 days with the growth regulators applied during the last 12 days. The 3-day lag was employed so that the xylem fibers which differentiated in response to applied growth regulators might be spatially separated from the xylem formed prior to growth regulator application.

In both test methods the tubes containing growth regulators were refilled as they emptied into the plants. In addition all tubes were emptied by hand and refilled with fresh solution in the middle of the test period.

IAA and NAA solutions were made by first dissolving the solute in 2 to 3 ml of 95% (v/v) ethanol and diluting with distilled water. Serial dilutions of the stock solution were made to arrive at the desired concentration series. GA solutions were made up in the same manner as the IAA and NAA solutions except in the one experiment where GA was made up in 95% (v/v) ethanol and applied to the plants as a 12-\(\mu\)l drop.

At the end of treatment the first internodes and hypocotyl of the plants were fixed and processed (21). A slit was made in the side of the stem to indicate the side treated with growth regulator if the growth regulator was applied unilaterally.

For the first internode the following measurements were taken from transverse sections of the first internode: (a) the

*Abbreviation: NAA: naphthaleneacetic acid.

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**Table I. Effect of IAA, GA, or Both on Production of Cambial Derivatives**

<table>
<thead>
<tr>
<th>GA Concn (mg/liter)</th>
<th>IAA Concn (mg/liter)</th>
<th>Cambial Derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>43±</td>
</tr>
<tr>
<td>0</td>
<td>0.1</td>
<td>41±</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>39±</td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>39±</td>
</tr>
<tr>
<td>0</td>
<td>3.0</td>
<td>35±</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
<td>42±</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>56±</td>
</tr>
<tr>
<td>100</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.0</td>
<td>54±</td>
</tr>
<tr>
<td>100</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10.0</td>
<td>58±</td>
</tr>
</tbody>
</table>
Figs. 3 and 4. Transverse section of a vascular bundle from an NAA-treated plant. The plants were treated 12 days with NAA (30 mg/liter) beginning 72 hr after decapitation. The section is from the first internode on the side of the stem directly below the insertion of the lateral branch bearing the NAA containing tube. Fig. 3: the whole vascular bundle. Note the parenchymatous layer and the zone of fibers differentiated as a result of NAA treatment. X 47. Fig. 4: higher magnification of the new xylem fibers of Figure 3. X 147. PL: Parenchymatous layer; C: cambium; F: fibers; V: vessels.

RESULTS

Method 1: Immediate Growth Regulator Application. IAA treatment for 14 days at concentrations of 0.1 to 10.0 mg/liter had essentially no effect (Table I).

GA treatment for 14 days did not induce the differentiation of xylem fibers, but GA enhanced the production of cambial derivatives. The simultaneous application of GA and IAA did not increase cambial derivative production above that induced by GA alone (Table I) and did not induce xylem fiber differentiation.

NAA applied to the second internode promoted the differentiation of xylem fibers. Only qualitative data were collected in this instance, but concentrations less than 5 mg/liter appeared ineffective. The new xylem fibers formed in this case were difficult to distinguish from the pre-existing xylem since NAA was applied at the time of decapitation.

Method 2: Delayed Growth Regulator Application. Figures 3 and 4 show the xylem fibers differentiated in the first internode in response to the unilateral application of NAA. Because of the 72-hr delay before applying NAA to the decapitated plants, the new xylem fibers differentiated in response to the NAA are separated from the xylem differentiated prior to treatment by a layer of parenchymatous cells. Vessel elements
Table II. Number of Cambial Derivatives Produced in Individual Experiments

Plants were decapitated and growth regulators were applied for a 12-day period after a 3-day lag. NAA and GA were applied together unilaterally in A and B; NAA unilaterally, GA in ethanol to surface of cut second internode in C. (Unilaterally means applied to cut surface of the lateral branch of the first node.) Data are the means of five replicates per treatment. Differences between means within experiments tested by Duncan's New Multiple Range Test after log₁₀ transformation of the data. Means are compared only within experiments. Numbers with the same letters are not significantly different.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>NAA Conc (mg/liter)</th>
<th>Cambial Derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>42 ± 2</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>32 ± 2</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>47 ± 2</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>44 ± 2</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>58 ± 2</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>63 ± 2</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>86 ± 2</td>
</tr>
</tbody>
</table>

are differentiated in this parenchymatous layer. The induction of fiber differentiation was greatest in terms of cell number on the side of the stem which received NAA. Cambial activity was also stimulated most on that side. The response declined very quickly in vascular bundles on either side of that bundle which showed the greatest effect. Fiber differentiation was not induced on the opposite side of the stem.

Cambial derivative production was greatly enhanced by the application of NAA (Table II). GA enhanced cambial derivative production in the presence of NAA, and GA also had a significant effect alone (Table II).

The number of cambial derivatives (cells per file), fibers, and radial rows of fibers formed in response to NAA are summarized in Figure 5. These data are presented as adjusted means ± the standard error. This was done because the various experiments did not always include the same concentrations of NAA and because the individual experiments varied in the absolute values of the measured responses. Because of the variation between experiments the over-all mean of the experiments does not represent the response of the individual experiments. The adjustment in all cases was toward the mean of the raw data for a given response at 15 mg/liter NAA. For each parameter in each experiment the following calculation was performed: F = (mean response of all experiments at 15 mg/liter NAA)/(mean response of individual experiment at 15 mg/liter NAA). The data obtained from each experiment were then multiplied by F to give the adjusted data. The adjusted data were then used for calculations of means and standard errors. The F value differs between experiments for one parameter but is similar for different parameters within an experiment. The same trends are obtained with or without adjustment of the data.

The number of cells per file of cambial derivatives and the radial width of the fiber zone show a linear response to NAA concentration between 7.5 and 30.0 mg/liter. The number of fibers formed per vascular bundle does not show a linear response to NAA concentration over the whole range of NAA concentrations employed. Fiber differentiation does not simply parallel cambial derivative production (Fig. 5).

The width of the parenchymatous layer did not change with NAA concentration.

The fibers and vessels formed and differentiated during NAA treatment of decapitated plants were clearly recognizable as either fibers or vessels in macerated material. The fibers differentiated under the influence of NAA had distorted tips and were significantly shorter than those formed in intact plants (Table III).

The average diameter of fibers, at the point of greatest diameter, was the same in both intact and NAA-treated plants (Table III). Vessels formed in NAA-treated plants were shorter than those formed in intact plants, but nearly the same as those formed in decapitated control plants. The diameter of vessels was reduced in both NAA- and water-treated plants as compared to intact plants (Table III).

**NAA Effects in Hypocotyl.** The application of NAA also promoted cambial derivative production and fiber differentiation in the hypocotyl. The response was the same in the hypocotyl relative to the water control as in the first internode at each NAA concentration. The promotion of fiber differentiation was generally evident in several vascular bundles of the hypocotyl.

![Fig. 5. A plot of the number of cambial cells per file, fibers per vascular bundle, and the width of the fiber zone against NAA concentration.](https://www.plantphysiol.org/)

**Table III. Fiber and Vessel Size in Plants Treated with NAA Compared to Intact and Decapitated Plants (Water-treated)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intact</th>
<th>Decapitated (+30 mg/liter NAA)</th>
<th>Decapitated (+ HoO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber L</td>
<td>450 ± 42</td>
<td>317 ± 40</td>
<td>⋯</td>
</tr>
<tr>
<td></td>
<td>24 ± 10</td>
<td>20 ± 10</td>
<td>⋯</td>
</tr>
<tr>
<td>Vessel L</td>
<td>146 ± 18</td>
<td>70 ± 10</td>
<td>63 ± 9</td>
</tr>
<tr>
<td></td>
<td>58 ± 9</td>
<td>30 ± 3</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>
DISCUSSION

The lack of response to applied IAA may be due to the presence of peroxidases and polyphenoloxidases at the cut surface of *Xanthium* stems (J. A. Lockhart, personal communication). Peroxidases have been shown to be capable of destroying IAA and other indole auxins (27).

This appears to be the first reported instance of a quantitative response to an auxin in terms of cambial derivative production. The number of cells per file of cambial derivatives produced at the highest NAA concentration is comparable to the number of cambial derivatives formed in intact plants of the same age (21).

Though GA also promoted cambial division, it had a slight effect in comparison to the effect of NAA. The appropriate analysis of variance tests were run on the data to test for interactions. An interaction of NAA and GA with respect to cambial division was suggested by the data, but a conclusive demonstration of this interaction requires that the growth regulators be supplied in saturating doses (15). Saturation was not achieved with either GA or NAA.

The induction of xylem fiber differentiation in response to exogenous auxin was achieved in a situation where exogenous auxin was not needed for cambial division. At the highest concentration tested the number of fibers per vascular bundle approached the number found in intact plants. The promotion of cambial division and tracheary element differentiation by auxin is an established phenomenon. This appears to be the first instance where xylem fiber differentiation from the cambium was induced as a specific response to auxin without the need for the induction of division. In this work it was shown that there is a quantitative response to NAA in both the production of cambial derivatives and the number of cells induced to differentiate. The possibility that xylem fiber differentiation was induced in NAA-treated plants simply because of the increased rate of cambial division seems to be ruled out by the following: Rates of cambial division were achieved in GA-treated plants which were as great or greater than the rates achieved in some NAA-treated plants in which xylem fiber differentiation occurred.

NAA specifically induced the differentiation of xylem fibers. In other work (4, 7, 10, 28, 30, 32), some of which utilized very different experimental systems, the effect of auxin was to induce the development of tracheary elements. Auxin was not apparently limiting vessel differentiation here, as shown by the fact that vessels develop relatively normally in decapitated plants not treated with auxin.

A common feature of other experimental systems is that the induction of tracheary element differentiation occurred in cells which were not derived from a cambium. These cells may have undergone division prior to differentiation and may have been derived from a cambial-like meristem. This difference in cell origin might be a reason for the difference between the tissue culture, xylem regeneration studies, and the differentiation of xylem fibers in *Xanthium* stems. If fibers develop from fiber initials which exist in the cambium, then it may be perfectly reasonable that they do not develop in the parenchymatous cells of tissue cultures or from parenchyma in the regeneration of xylem. Vessel initials need not exist within the cambium, but they may develop from a postcambial division and may therefore develop independently of a cambium.

The inability of GA to induce any aspect of xylem differentiation is in agreement with previous literature dealing with excised stem segments (31). The decapitated *Xanthium* system is similar to isolated stem segments in this respect.

Torrey (29) suggested that there might be a relationship between cell division and the induction of xylem differentiation. Stockdale and Topper (26) found that DNA synthesis, cell division, or both are required for initiation of differentiation in mammary gland epithelium. Using the xylem regeneration system in *Coleus*, Fosket (6) found that agents which block cell division or DNA synthesis also block wound-vessel membrane formation. Finally, Fosket and Torrey (8) concluded from their work with soybean callus cultures that cell division is a prerequisite to the auxin-cytokinin-induced differentiation of trachaeary elements.

Jeffs and Northcote (12) suggested that the role of auxin in their tissue culture system was to induce cell division and that sucrose actually determined whether or not differentiation of xylem occurred.

The inducibility of xylem fiber differentiation in the cambial derivatives in this system is apparently related to the age of the cells or to mitosis. In any secondary xylem differentiation system it will be found that cell division is required at least for the production of potential xylem elements. In this system cambial division occurs in the decapitated plants, but the cells do not differentiate normally. The effect of GA was to enhance cambial division but not to induce fiber differentiation. NAA was capable of inducing xylem fiber differentiation and enhancing cambial division. However, NAA did not induce fiber differentiation in all of the cells produced by the cambium. Specifically, NAA did not induce fiber differentiation in the older cambial derivatives, and this resulted in the formation of a parenchymatous layer of cells which remained as such within the normally differentiated xylem. This event may be interpreted in several ways. (a) The cells which became parenchyma had, by the time the auxin reached them, passed the stage during which they were receptive to induction; i.e., they had differentiated into a cell which was no longer a potential xylem fiber; (b) for xylegenesis to be induced the cells must divide in the presence of a sufficient level of auxin or auxin plus other factors; (c) the cells did not receive NAA because they were too far removed from the transport system by the time NAA reached that level in the stem. Either a or b would seem to be the most logical explanation. If a explains the response, then xylem fiber differentiation is induced just after cell division and cells retain this potential for only a short period of time. If b explains the situation, then xylem fiber differentiation would only be inducible at the time of cell division, and cells would not retain the potential to develop into xylem fibers unless they divided again.

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LITERATURE CITED