

The control of vessel size and density along the plant axis

A new hypothesis

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Abstract. An hypothesis is presented which ascribes the general increase in vessel size and decrease in vessel density from leaves to roots to a gradient of decreasing auxin concentration from leaves to roots. Application of auxin in a lanolin paste to decapitated bean stems produced a very sharp basipetal gradient of decreasing vessel number and increasing vessel size. The high auxin concentration at the point of application resulted in numerous vessels that remain small because of their rapid differentiation. The low auxin concentration further down resulted in slow differentiation and therefore fewer and larger vessels.

Introduction

The xylem is the water-conducting tissue of the plant. In angiosperms, the water and soil nutrients move from roots to leaves in vessels composed of specialized vessel elements arranged end-to-end in defined files along the axis of the plant. There is generally a gradual increase in vessel diameter and at the same time a decrease in vessel density from leaves to roots. An increase in vessel diameter was recently found along leaves [10, 23]. A continuous increase in vessel diameter and vessel length was demonstrated from twigs to branches, down along the stem and into the roots of *Acer rubrum* trees [36]. Vessel diameter was found to continue to increase in the long conducting roots with increasing distance from the stem [12, 21]. This basic pattern was found in dicotyledons as well as in monocotyledons [8, 29, 35]. The widest vessels found anywhere in the plant kingdom are those of roots [19]. The basipetal increase in vessel diameter is associated with a basipetal decrease in vessel density (number of vessels per transverse-sectional area). Vessel density is generally greater in branches where vessels are smaller than in roots where they are larger [8, 9, 13].

It is well established that the differentiation of both primary and secondary vessels is induced and controlled by polar movement of auxin [7, 17, 24, 27, 31]. Young leaves are the main source of auxin and the effect of leaves on vessel differentiation can be completely replaced by a source of auxin [16–18, 32]. It is also known that the number of vessel elements formed in a tissue correlates posi-

tively with the amount of auxin applied to the tissue [2, 17, 28]. However, the mechanism by which the patterns of vessel size and density are regulated in the whole plant is not known. In an attempt to explore the mechanism that controls vessel size and density, the following six-point working hypothesis has been set up:

1) Basipetal polar flow of auxin from the growing leaves toward the roots establishes a gradient of decreasing auxin concentration from shoots to roots.

2) Local structural or physiologic obstructions of the flow of auxin, for example at nodes where two flows of auxin merge, raise the auxin concentration locally.

3) The distance from the source of auxin production (the young leaves) to the differentiating cells determines the cells' position in the gradient, thus controlling the amount of auxin flowing through the differentiating cells at a given time.

4) The rate of vessel differentiation is controlled by the amount of auxin; high concentrations cause fast, low concentrations cause slow differentiation. Consequently, the duration of differentiation of individual vessels increases from shoots to roots.

5) The final size of a vessel is determined by the rate of cell differentiation. Cell expansion is stopped when the secondary wall is deposited. Fast differentiation therefore results in narrow vessels, while slow differentiation permits more cell expansion before secondary wall deposition. Slow differentiation therefore results in wide vessel elements. Decreasing auxin concentration from leaves to roots therefore results in an increase of vessel size in the same direction.

6) Vessel density is controlled by the auxin concentration; high concentrations induce greater, low concentrations lower densities. Vessel density therefore decreases from shoots to roots.

Although we do not discount other factors that are involved in vessel differentiation, such as growth regulators (gibberellins, cytokinins, etc.), sugar, water potential, etc., the present working hypothesis is based upon the assumption that a stable and steady polar flow of auxin from leaves to roots is essential for the described pattern of vessel size and density along the plant axis. The work reported here was performed to test our hypothesis. It shows the effects of auxin concentration and of distance from auxin application on vessel differentiation in the secondary xylem of *Phaseolus vulgaris*.

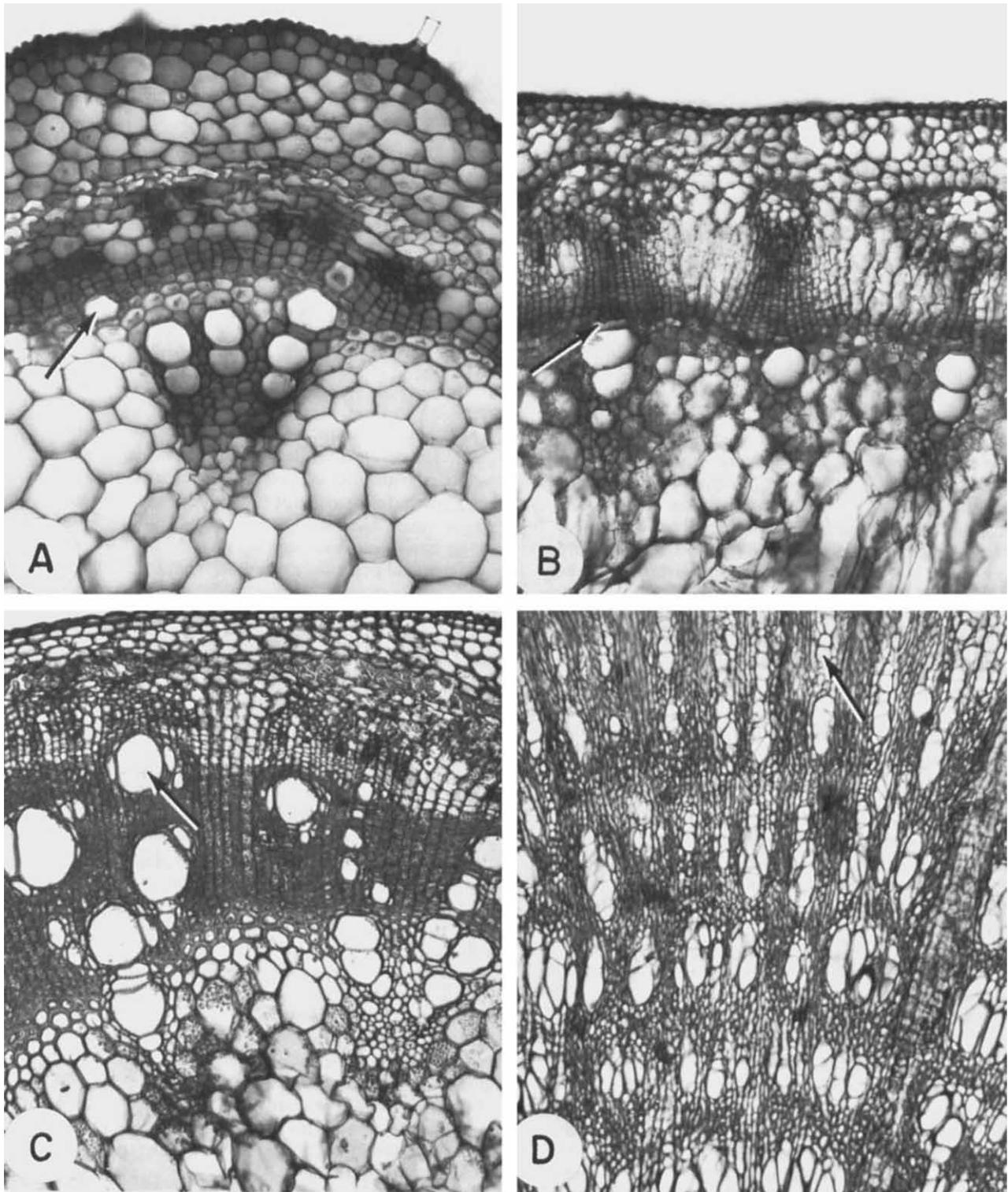


Fig. 1. Transverse sections from the center of the second internode above the cotyledons of *Phaseolus vulgaris*. All photographs were taken from the same experiment and are presented in the same direction. **A** The zero-time control shows an early stage of secondary xylem differentiation. **B** The 20-day lanolin control with no additional xylem differentiation. **C** The intact control shows the typical secondary xylem formed during a 20-day period of growth. **D** Treatment with 0.1% NAA, section taken immediately below the site of auxin application. *Arrows* mark secondary vessels. **A** $\times 200$; **B, C, D**, $\times 100$

Methods

Sixteen-day-old, greenhouse-grown bean seedlings (*Phaseolus vulgaris* L.) were selected for uniformity according to

blade length of their third leaf, i.e., the first trifoliate leaf, situated on the second node above the cotyledons, which was about 40 mm long. The distal stem portion of the seedling was excised with a razor blade by a cut across the

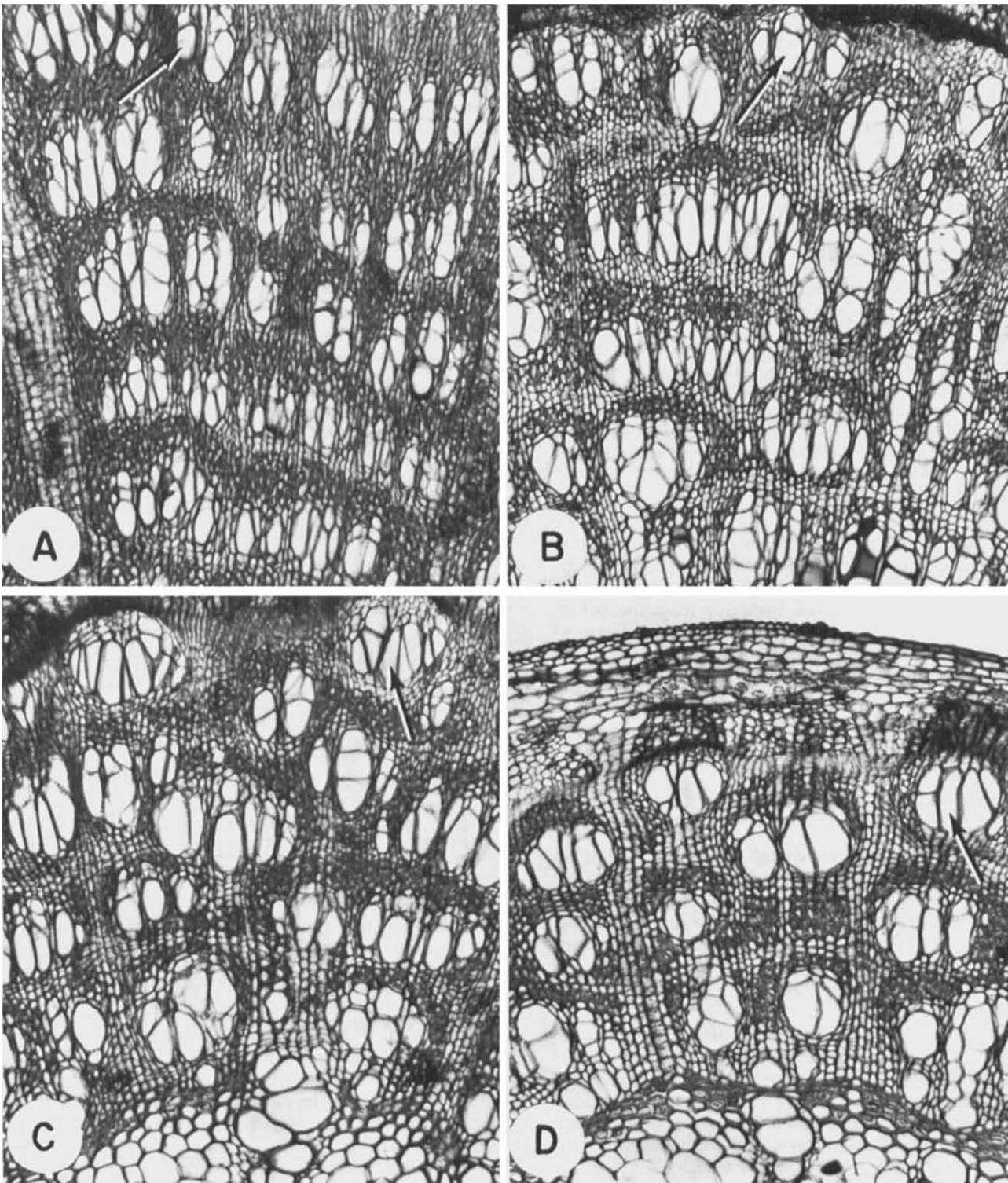


Fig. 2. Transverse sections, taken along the same internode as the one shown in Fig. 1D, treated with 0.1% NAA. **A** Half a centimeter below the site of auxin application, **B** 1.0 cm, **C** 2.0 cm, and **D** 4.0 cm below the site of NAA application. The photographs show an increase in vessels diameter and a decrease in vessel density with increasing distance from the auxin source, as well as the changes of vessel pattern from layers (**A**) to bundles (**D**). *Arrow* marks a late formed secondary vessel. All $\times 100$

middle of the second internode above the cotyledons, i.e., the one immediately below the first trifoliate leaf. Axillary buds were removed from the remaining plant which thus consisted of a rooted stem portion containing cotyledons and two simple leaves. Naphthaleneacetic acid (NAA) was

applied in the form of a lanolin paste [1] in three concentrations: 0.03%, 0.1% and 1.0% (w/w), and reapplied every three days to the cut surface of the lower half of the internode.

The experiment extended over a 20-day period and was

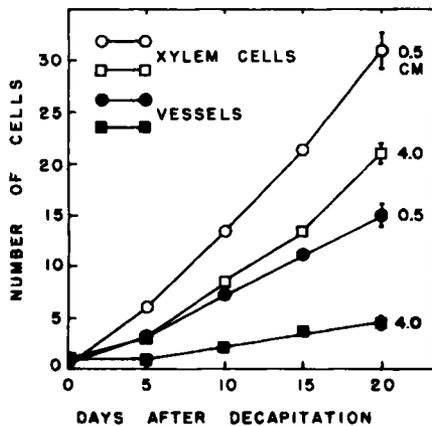


Fig. 3. Effect of distance (0.5 and 4.0 cm shown) from 0.1% NAA application site on the rate of formation of secondary vessel (filled circles and squares) and total number of secondary xylem cells (open circles and squares) along a radius in the second internode above the cotyledons of *Phaseolus vulgaris*. Each point represents the average of ten measurements (two for each internode). Vertical bars indicate standard errors which are comparable at all points

repeated three times, with five to ten plants per treatment. Internodes were collected throughout the time of the experiment and the differentiation of vessels was studied in transverse sections taken at various distances from the site of auxin application. Sections were stained with a mixture of safranin and alcian green [30] which gave a red stain to the xylem cells. Vessels were counted and measured under a light microscope.

Results

Effect of distance from auxin source on rate of vessel differentiation

At the beginning of the experiment all plants showed an early stage of secondary xylem differentiation (Fig. 1A). This remained unchanged in the control plants that were decapitated and treated with lanolin paste only (Fig. 1B). Intact control plants produced a well-developed secondary xylem during the same time (Fig. 1C), but there was no detectable gradient in vessel size along the lower half of the internode studied.

Auxin treatments produced wide bands of secondary xylem containing numerous vessels (Figs. 1D, 2A-D, 3 and 5). The rate of formation of vessels and other secondary xylem cells decreased basipetally with increasing distance from the point of the auxin source. The rate of vessel differentiation and that of xylem formation remained constant at any one distance from the source (Fig. 3).

Effect of auxin concentration and distance from the site of application on vessel size

All three auxin concentrations used induced a substantial gradient of increasing vessel diameters basipetally from the auxin source (Figs. 1D, 2A-D, and 4). The highest auxin concentration (1.0% NAA) yielded the narrowest vessels immediately below the site of application (Fig. 4). NAA (0.1%) resulted in wider vessels (Figs. 1D, 2A, and B) which were somewhat smaller than those formed under

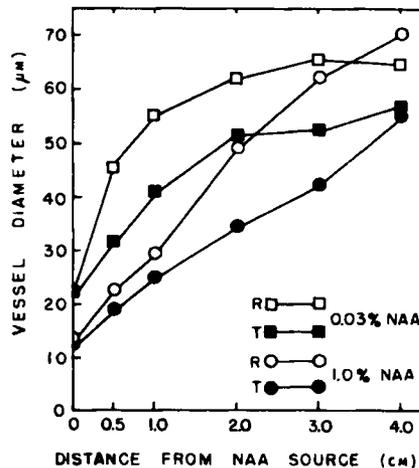


Fig. 4. Effect of 0.03% NAA (squares) and 1.0% NAA (circles) on the radial (R) and tangential (T) diameter of the late-formed vessels, along the studied internodes of *Phaseolus vulgaris*. Each point represents an average of 25 vessels (five vessels from five internodes)

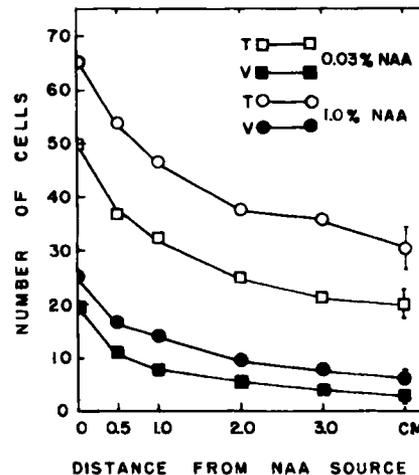


Fig. 5. Effect of 0.03% (squares) and 1.0% NAA (circles) on the total (T) number of secondary xylem cells and the number of secondary vessels (V) counted along a radius in *Phaseolus vulgaris*. Each point represents the average of five measurements, each from a different internode. Vertical bars indicate standard errors which are comparable at all points

0.03% NAA (Fig. 4). In all auxin treatments the average radial diameters were wider than the tangential diameters (Figs. 2 and 4).

Effect of distance from the auxin source on vessel density

The highest auxin concentration induced the widest secondary xylem band, containing the largest number of vessels (Fig. 5). The total number of cells and the total number of vessels in the xylem decreased basipetally. The density of vessels decreased basipetally with increasing distance from the point of NAA application, regardless of concentration. At the end of the experiment the ratio of number of secondary vessels per total number of secondary xylem

cells dropped from 0.4 (or more) at the site of application to 0.2 (or less) 4 cm below 1.0% and 0.03% NAA (Fig. 5). As the rate of secondary xylem cell formation was higher than that of secondary vessel differentiation, the density of vessels slowly decreased during the experiment from 0.54 vessels/xylem cell 10 days after decapitation to 0.47 vessels/xylem cell 20 days after decapitation at 0.5 cm below 0.1% NAA, and from 0.27 (10 days) to 0.21 (20 days) at 4 cm (Fig. 3).

Effect of auxin concentration on vessel pattern

The pattern of vessels, as seen on a transverse section, varied along the treated internode with increasing distance and depending upon the applied concentration. Immediately below the point of application, vessels were typically arranged in layers (Figs. 1D and 2A), further down they were grouped in bundles (Figs. 2C and D). The layer pattern extended over 2–3 cm below the 1.0% NAA application point. It was limited to about 1 cm or less under the 0.03% application point.

Discussion

The present findings confirm our general hypothesis that auxin controls size and density of vessels along the plant axis. The results show that a substantial gradient in vessel size is induced by the hormonal signal from the site of its application toward the roots, manifested by a transition from numerous narrow vessels to fewer and wider ones. The present findings are in accordance with previous reports that other auxins (used as herbicides), induce a significant increase in the number of vessels and a reduction in diameter, i.e., 2,4 dichlorophenoxyacetic acid (2, 4-D) and 2, 4, 5 trichlorophenoxyacetic acid (2, 4, 5-T) in *Prosopis* [22], and 2, 4 dichlorophenoxybutyric acid (2, 4-DB) in soybean [20].

The second point of our hypothesis was not investigated in the present study. However, we were able to show its significance in a study of vessel differentiation in which strips of cambium and bark were removed from *Acer rubrum* [5]. Interestingly, other cell types differentiate in a pattern very similar to vessels along the plant axis. Fibers [4, 11, 13] and tracheids both appear wider and longer with increasing distance from the leaves [6, 13, 26, 35]. Recent studies have shown that fiber differentiation is controlled by auxin as well as gibberellic acid [1] and cytokinin [3]. Accordingly, we propose to apply our working hypothesis to the study of fiber and tracheid differentiation as well.

The first point of our hypothesis proposes that the basipetal polar flow of auxin from leaves to roots results in a gradient of decreasing auxin concentration from shoots to roots. This is a fundamental feature of cell differentiation along the plant axis. Goodwin and Cohen [14] proposed that a signal which travels along the organism axis in the form of waves might subject the cells to intermittent gradients. Such waves could convey detailed positional information [14]. There is evidence that auxin moves in waves or oscillations of transport rates along corn coleoptiles [15] and along pine stems [33, 34].

Finally, Sachs and Cohen [25] have recently shown that the orientation of vessel differentiation is determined by a flux of auxin which moves through the differentiating

cells. The present study further shows that vessel size and vessel density along the plant axis is controlled by a gradient of decreasing auxin concentration from leaves to roots.

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