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Auxin in the Cambium and its Differentiating Derivatives

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ABSTRACT

Cambium and differentiating xylem and phloem tissues from the trunks of trees of *Acer pseudoplatanus* L., *Fraxinus excelsior* L., and *Populus tremula* L. were extracted with ether and tested for auxin, which was found on chromatograms of the acidic fraction at an Rf corresponding to that of indol-3yl-acetic acid in five solvent systems. In addition, small amounts of auxin with a higher Rf in ammoniacal isopropanol were found in phloem samples. The amounts of auxin were greatest in xylem samples, less in the cambium, and least in phloem. The differences, which cannot be explained in terms of differential losses during extraction and purification, suggest that auxin is actually formed in differentiating xylem tissue. The significance of these results is discussed.

INTRODUCTION

There is evidence that auxin is produced in stems in which secondary thickening is taking place (Zimmermann, 1936; Söding, 1937, 1940; Gunckel and Thimann, 1949; Sheldrake and Northcote, 1968*a*). In woody plants stems, branches and trunks may be major sites of auxin synthesis, although they are rarely mentioned in standard accounts of the subject. Since auxin is formed as a consequence of cambial activity (Söding, 1937, 1940; Sheldrake and Northcote, 1968*a*), the hormone must be made by the cells of the cambium itself, or by its differentiating derivatives. It is possible to separate differentiating xylem, differentiating phloem and cambium tissue quite easily by a method involving the stripping of the bark from trees in the summer (cf. Thornber and Northcote, 1961; Rubery and Northcote, 1969; Sheldrake, 1970). I have estimated the auxin content of these three tissues from sycamore (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.) and poplar (*Populus tremula* L.) trees, in the hope of shedding light on the cellular site of auxin formation.

MATERIALS AND METHODS

Trees (10–20 years old) growing in Madingley Wood, Cambridge, were felled and sawn into logs which were taken to the laboratory. The bark was stripped off and cambium, xylem, and phloem samples were separated as described by Sheldrake (1970). The xylem and phloem samples contained differentiating and some mature cells. The tissues (4 g) were extracted twice for 2 h with 100 ml peroxide-free ether at 2 °C. The ether extracts were concentrated under reduced pressure at 30 °C and partitioned with sodium bicarbonate (0·1 M) to separate the acidic and neutral fractions (Larsen, 1955). The ethereal solutions containing these

fractions were evaporated to a small volume and applied to the origins of cellulose thin-layer chromatograms, which were developed with isopropanol/ammonia/water (10:1:1 v/v/v). Appropriate zones were scraped off and placed in small tubes for bioassay by the *Avena* mesocotyl bioassay of Nitsch and Nitsch (1956) as described elsewhere (Sheldrake, 1971). Marker spots of indol-3yl-acetic acid (IAA) were also run on chromatograms and revealed by means of a $FeCl_3$/perchloric acid spray (Larsen, 1955). Results are expressed in terms of IAA equivalents on both fresh weight (F.W.) and concentration bases. The latter was calculated by taking the difference between the F.W. and dry weight (determined after drying in an oven at 60 °C for 3 days) as the water content of the tissue.

The recoveries of [1-^{14}C]-IAA (25·6 mCi/mM, Amersham) added to samples before extraction were estimated as described by Sheldrake (1971). All estimations were carried out in duplicate.

RESULTS

In all three tissues auxin activity was almost all confined to the acidic fractions, where it was found on chromatograms at an Rf corresponding to that of IAA (Fig. 1). In most phloem samples a minor zone of auxin activity was also detected at a higher Rf in both acidic and neutral fractions. Zones containing this activity

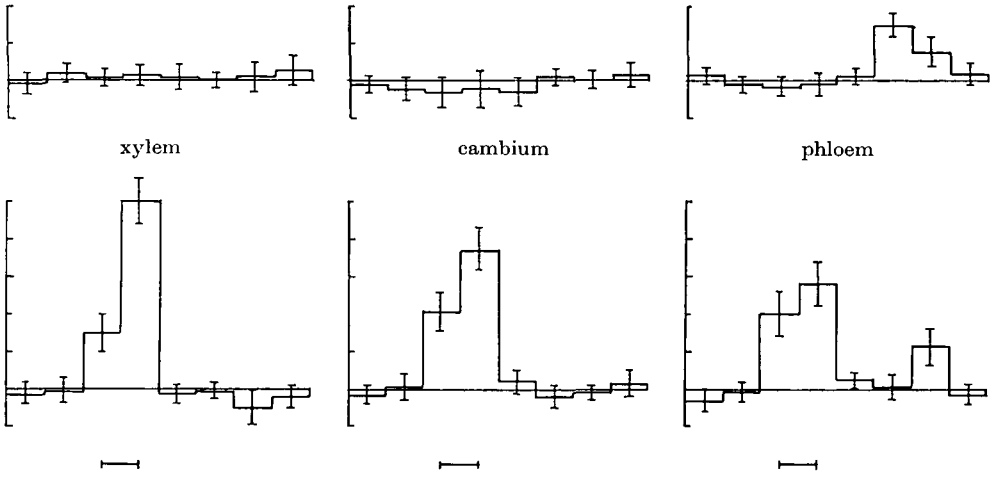

FIG. 1. Auxin activity on chromatograms developed with ammoniacal isopropanol of neutral (upper) and acidic (lower) fractions of ether extracts of 4 g of xylem, cambium, and phloem tissue of sycamore. The extensions of 4 mm mesocotyl sections are shown, with mean deviations. Each scale division on the vertical axis represents an extension of 0·2 mm. The origin is at the left of each diagram, the solvent front at the right; the positions of IAA marker spots are indicated.

were eluted and heated (100 °C for 5 min) and subjected to alkali (0·1N NaOH at 25 °C for 30 min), re-extracted with ether and re-chromatographed. Auxin activity was detected only in the same regions as before. This auxin is therefore neither heat-labile nor an alkali-labile complex of IAA.

Zones corresponding to the Rf of IAA were removed from chromatograms of all three tissues and eluted with ether. The eluates were chromatographed on cellulose thin-layer plates developed with four different solvent systems: *n*-butanol:ethanol:water (4:1:1 v/v/v; IAA Rf 0·90), pyridine:ammonia (4:1 v/v; IAA Rf 0·57), chloroform:acetic acid (95:5 v/v; IAA Rf 0·98) and ethanol:water

(7:3 v/v; IAA Rf 0·74). In all cases auxin activity was detected in zones corresponding to the Rf of IAA, indicating that the auxin activity was due to IAA.

In Table 1 it can be seen that the amount of auxin was greatest in the xylem, less in the cambium and least in the phloem in all samples except the sycamore of 24/7, where the concentrations of auxin in the xylem and cambium were about equal. However, by this time there was little cambial activity and it was

TABLE 1. *The auxin content of xylem, cambium and phloem samples*

Zones corresponding to the Rf of IAA from chromatograms of the acidic fractions of ether extracts were assayed; the results are expressed in terms of IAA equivalents

Tree	Date		Xylem	Cambium	Phloem
Poplar	19/6/70	μg/kg F.W.	7·1	5·0	2·5
		μg/l	8·5	5·5	3·1
Ash	9/6/70	μg/kg F.W.	8·8	5·6	3·0
		μg/l	10·9	6·4	4·9
Ash	24/7/70	μg/kg F.W.	32·3	30·1	6·6
		μg/l	39·6	34·7	9·5
Sycamore	9/6/70	μg/kg F.W.	13·8	6·9	5·4
		μg/l	16·8	7·3	6·8
Sycamore	19/6/70	μg/kg F.W.	11·8	7·7	5·8
		μg/l	15·8	8·3	7·7
Sycamore	24/7/70	μg/kg F.W.	24·2	27·2	6·2
		μg/l	29·8	29·5	7·8
Sycamore	29/7/70	μg/kg F.W.	26·2		9·5
		μg/l	28·2		11·6

difficult to separate the cambium and differentiating xylem tissue; a microscopical examination showed contamination of the sample with secondarily thickened and partly lignified xylem cells. By 29/7 collection of separate cambial and differentiating xylem samples was not possible.

The average recoveries of [1-^{14}C] IAA added to the samples before extraction were: xylem 53 per cent, cambium 57 per cent, and phloem 61 per cent.

DISCUSSION

Considerable losses of auxin can occur at various stages of extraction and purification (Hamilton, Bandurski, and Grigsby, 1961; Mann and Jaworski, 1970). A comparison of the auxin content of different tissues could be complicated by differential losses during these procedures. The percentage recoveries of IAA found here are indeed slightly different for the different tissues, but if corrections are applied to the results shown in Table 1 the differences in auxin content between the different tissues are magnified rather than diminished. Thus the relative amounts of auxin detected, greatest in xylem and least in phloem, probably reliably reflect the relative amounts which are actually present in the tissues.

Söding (1937, 1940) investigated the auxin content of xylem, cambium, and phloem tissues and found that the great majority of auxin activity was present in the cambium. He detected practically none in xylem or phloem tissue. But the

conflict with the results presented here is only apparent; his 'cambium' fractions in fact contained differentiating xylem and phloem tissue (Söding, 1940), and the xylem and phloem tissues he analysed were not differentiating but mature. Furthermore, the auxin was not extracted but collected by diffusion methods of dubious reliability for comparing different tissues. Söding admitted the possibility that the auxin might be present in differentiating vascular tissue, but rejected it as being 'plainly improbable'. ("Außer der handgreiflich unwahrscheinlichen Annahme, daß nicht das Kambium, wohl aber das jüngste Holz und die jüngste Rinde wuchsstoffhaltig seien, bleibt also nur der Schluß, daß das Kambium selbst ein Hauptsitz des Wuchsstoffes ist" (Söding, 1940, p. 133).) He had previously (Söding, 1937) pointed out that except in coleoptile tips, auxin is in general formed in meristematic and growing regions, and implied that it is made by the meristematic or growing cells themselves. There is as yet no direct evidence that auxin is produced by meristematic cells. There is, on the other hand, evidence that auxin is formed by autolysing cells and that in higher plants it is normally produced as a consequence of cell death (Sheldrake and Northcote, 1968*a*, *b*, *c*). The high levels of auxin associated with developing embryos could be produced as the nutritive tissues surrounding them regress and die, and the production of auxin in other areas of meristematic activity could be explained by the association of meristematic activity with vascular differentiation; the differentiation of xylem cells involves their death. In contrast to Söding's expectations, this hypothesis predicts that higher amounts of auxin should be detected in differentiating xylem tissue than in the cambium. The auxin found in the xylem cannot be ascribed to its presence in xylem sap travelling within mature xylem vessels since the xylem sap of trees contains practically no auxin (Sheldrake and Northcote, 1968*d*). So unless there is an unlikely lateral transport system for auxin against a concentration gradient, the results reported in this paper strongly suggest that auxin is formed in differentiating xylem cells, and support the dying-cell hypothesis of auxin production.

The reactivation of the cambium in the spring is known to depend on the presence of expanding buds, which are rich in auxin, or if these are removed, on a supply of exogenous auxin (Reinders-Gouwentak, 1965). This requirement for auxin does not imply that a supply of auxin from the shoots is necessary for the maintenance of cambial activity once it has been initiated. The auxin formed by the developing buds in the spring could have a 'priming' effect on the cambium. If in general auxin is produced as a consequence of cambial activity in quantities sufficient to stimulate further cambial activity, in the intact plant the subsequent control of the cambium must be brought about by factors other than the supply of auxin from the shoots and leaves. In many species cambial activity continues after shoot growth has ceased (Priestley, 1930; Wareing and Roberts, 1956). Wareing and Roberts (1956) found that the maintenance of cambial activity in *Robinia pseudacacia* depended on the exposure of the leaves to long days; they suggested that either mature leaves under long days produce a cambium stimulus, or that short-day treatments lead to the formation of an inhibitor of cambial activity. If stimulation rather than inhibition is involved,

auxin seems unlikely to be responsible since mature leaves produce little auxin and differences in auxin production by such leaves under long and short days have not been detected (Chailakhyan and Ždanova, 1938; Bonner and Liverman, 1953). In some diffuse porous trees cambial activity ceases with the termination of extension growth. Again the available evidence does not make it possible to decide whether the cessation of cambial activity is due to inhibition or to the lack of a stimulus which the mature leaves of the tree are presumably unable to provide. But even if an influence from leaves or shoots is necessary for the maintenance of cambial activity in some species, at least in the case of tobacco no such stimulus is required: in the absence of exogenous hormones cambial activity continues in excised internodes in sterile culture for many months (Sheldrake and Northcote, 1968*a*).

The gradient of auxin from xylem to phloem across the cambium means that cambial derivatives must be in different hormonal environments on different sides of the cambium. Moreover, on the phloem side the presence of mature, functional sieve elements is likely to mean that cells in lateral and longitudinal proximity to them are exposed to relatively high concentrations of sucrose and other translocated metabolites. Both sucrose and auxin are necessary for organized vascular differentiation; a high sucrose to auxin ratio leads to phloem differentiation, and other combinations of these two factors stimulate xylem differentiation (Wetmore and Rier, 1963; Jeffs and Northcote, 1967; Rier and Beslow, 1967), which in turn leads to further auxin production. This could help to explain how the cambium and its derivatives can form a stable pattern of differentiation which is self-catalysing and self-perpetuating (Sheldrake and Northcote, 1968*a*).

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