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Induction of Tension Wood by 2,4-dinitrophenol and Auxins[1]

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With 10 Figures

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Summary

The effect of DNP and auxins on the development of the secondary xylem in erect stems of *Acer rubrum* was studied. DNP affected the development of the secondary xylem only locally in the treated internode. Tension wood is formed in the stem below the DNP treatment site whereas above the application site the development of tracheary elements is altered. In *Acer rubrum* seedlings that were treated with auxin, especially at low concentrations, a thick ring of tension wood is developed in the erect stem below the treatment site. Previous suggestions that the formation of tension wood in arborescent angiosperms is a developmental response to auxin deficiency are discussed in terms of the induction of tension wood in *Acer rubrum* by DNP and auxins.

1. Introduction

The formation of tension wood in variously inclined stems and branches is most probably a developmental response to low auxin level (CRONSHAW and MOREY 1965, 1968, MOREY and CRONSHAW 1966, 1968 a, b). In this regard we have shown that in horizontally placed stems of *Acer rubrum* seedlings auxin readily inhibits the development of tension wood when applied along the upper side of the axis, where tension wood normally develops (CRONSHAW and MOREY 1968). In the erect stems of *Acer rubrum* seedlings, where tension wood is not normally formed, we reported that application of the auxin antagonist, TIBA [3], induces in a localized region the development of a com-

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[3] The following abbreviations will be used: TIBA (2,3,5-tri-iodobenzoic acid); IAA (indole-3-acetic acid); GA (gibberellic acid); NAA (naphthaleneacetic acid); 2,4-D (2,4-dichlorophenoxyacetic acid); DNP (2,4-dinitrophenol).

plete ring of tension wood, and further, that the addition of auxin to the TIBA application site substantially reduces the capacity of TIBA to bring about the development of tension wood (Cronshaw and Morey 1965, Morey and Cronshaw 1968 a, b). We suggested, therefore, that in the stems of erect *Acer rubrum* seedlings treated with TIBA and in the stems of horizontally placed and otherwise untreated seedlings the formation of tension wood is a developmental response to low auxin level.

In most features the tension wood developed on the upper side of the stem of horizontally placed but otherwise untreated seedlings is similar to that formed in erect stems treated with TIBA (Morey and Cronshaw 1968 a).

Table 1. *Concentrations of Plant Growth Substances and DNP which were Applied as Dispersions in Anhydrous Lanolin to the Upright Stems of Six to Eight-Week-Old Acer rubrum Seedlings*

Compound	Concentration (%)
DNP	0.30–0.50
GA	0.10–0.50
IAA	0.50
NAA	0.03–0.10
2,4-D	0.10

However the tracheary elements found in the tension wood induced by TIBA are, in general, smaller in diameter and fewer in number relative to the tracheary elements characteristic of the tension wood of horizontally placed seedlings. In the experiments now reported DNP, which like TIBA is known to interfere with polar auxin transport in other systems (Niedergang-Kamien and Leopold 1957), was applied to the erect stems of *Acer rubrum* seedlings in an attempt to induce the development of tension wood which is anatomically identical to the tension wood characteristic of horizontally placed seedlings. It was anticipated that comparison of the structural details of the tension wood induced by TIBA and DNP would permit deductions to be drawn with regard to the mechanisms regulating the initiation and the differentiation of the various types of xylem elements.

In a previous report we briefly mentioned that when auxin especially at low concentration was applied to the stem of erect *Acer rubrum* seedlings a complete ring of tension wood was formed below the treatment site, and further that the induction of tension wood by auxin was always associated with an accelerated rate of cambial division (Morey and Cronshaw 1968 a). The structural characteristics of auxin induced tension wood are described in this report.

2. Materials and Methods

The procedures followed here have been described previously (MOREY and CRONSHAW 1968 a). Plant growth substances and DNP were used as dispersions in anhydrous lanolin at the designated concentrations (Table 1) and applied laterally as a ring to the mid-point of the first interenode of erect six to eight-week-old *Acer rubrum* seedlings.

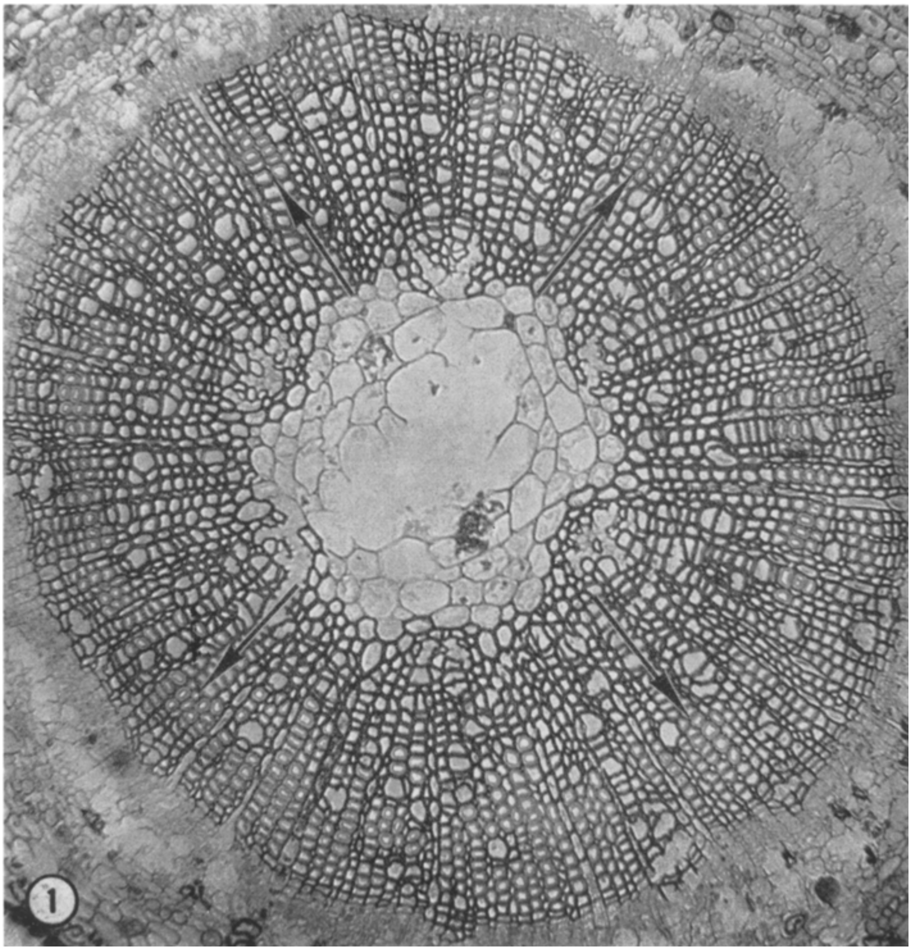


Fig. 1. *Acer rubrum.* Photomicrograph of a transverse section taken from the stem of an eight-week-old seedling that had been treated for two weeks with DNP. The section was cut 1.0 mm below the treatment site. A complete ring of tension wood (arrows) developed during DNP treatment. Safranin light-green stained. ×140

3. DNP Applied as a Single Ring to Erect Seedlings

The leaves developed during treatment appeared normal in all morphological characteristics, although the young leaves of some seedlings had a pronounced orange hue. The rate of cambial division in the stem both above and below the DNP treatment site remained vigorous during the two week treatment period.

3.1. Secondary Xylem below the DNP Treatment Site

In 54% of the seedlings treated with DNP a complete ring of tension wood was developed in the stem locally below the treatment site (Table 2). This is considered significant since rings of tension wood were never formed in lanolin treated controls and untreated controls. Fig. 1 shows a transverse section which was cut from the stem 1.0 mm below the treatment site two weeks after treatment of a six-week-old *Acer rubrum* seedling with DNP. A thick ring of tension wood (Fig. 1, arrows) was developed during DNP application and was restricted to a region of the stem 2.0 to 3.0 mm below the treatment site.

Application of GA with DNP did not alter the frequency at which rings of tension wood were induced in the stem relative to DNP treated controls (Table 2).

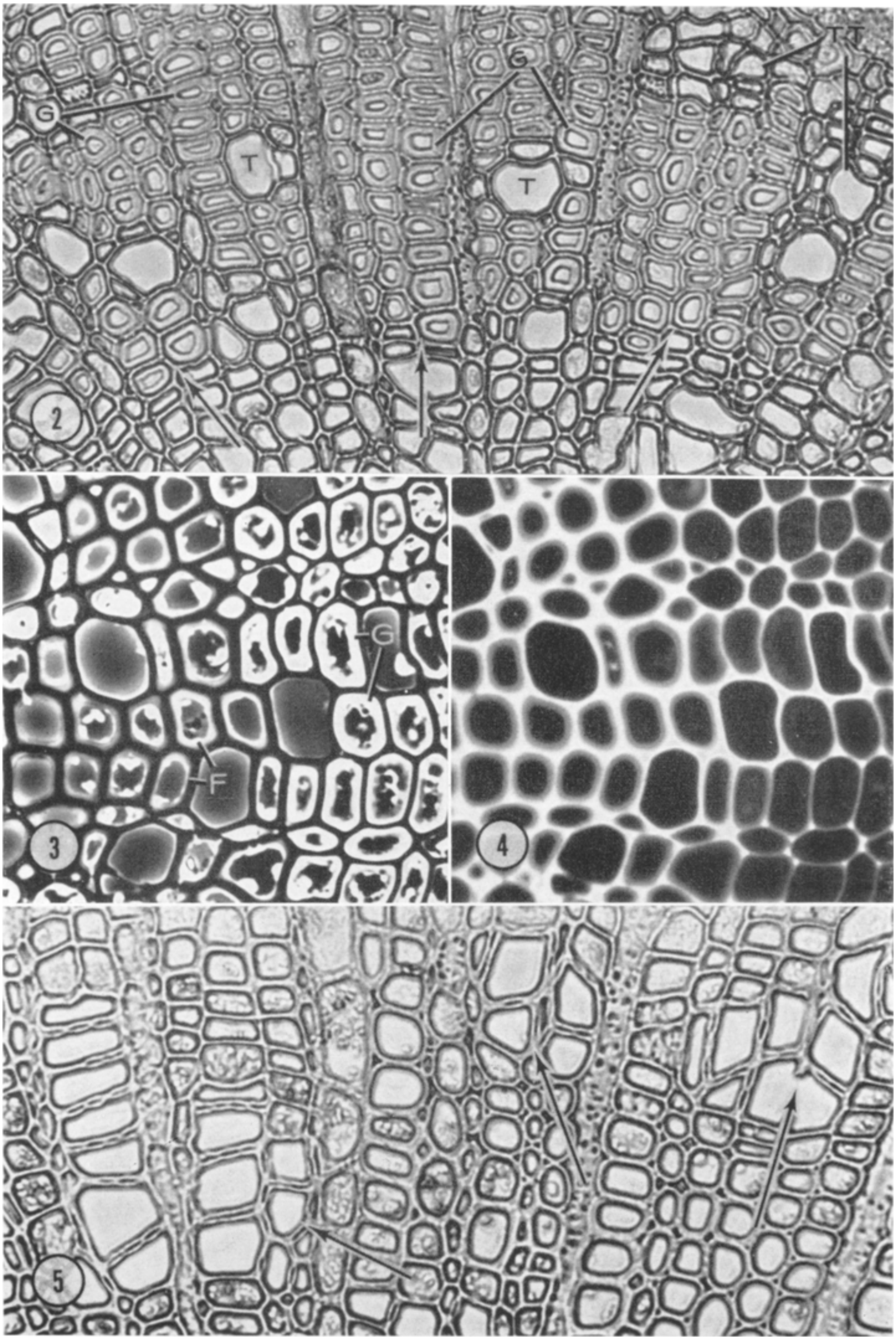
The number of tracheary elements present in the tension wood induced by DNP was in some seedlings equivalent and in others slightly reduced relative to the number of tracheary elements found in the secondary xylem developed before treatment. Fig. 2 shows part of a transverse section of a specimen similar to that in Fig. 1 showing details of the tension wood induced by DNP (Fig. 2, arrows). Fibers with a thick gelatinous wall layer (Fig. 2, *G*), wide diffusely distributed tracheary elements (Fig. 2, *T*), and tracheary elements of variable breadth in grouped arrangement (Fig. 2, *TT*) are found in the tension wood induced by treatment with DNP.

Fig. 2. *Acer rubrum.* Photomicrograph of part of a transverse section of a specimen similar to that in Fig. 1. Tension wood (arrows) developed during DNP treatment is characterized by fibers with a thick gelatinous wall layer (*G*), wide diffusely distributed tracheary elements (*T*), and tracheary elements in grouped arrangement (*TT*). Safranin light-green stained. ×400

Fig. 3. *Acer rubrum.* Phase-contrast micrograph of an unstained transverse section of a specimen similar to that in Fig. 1 showing details of xylem formed before and during treatment with DNP. The xylem developed during DNP treatment is characterized by fibers with a thick gelatinous wall layer (*G*). Normal fibers (*F*) are found in the xylem formed before DNP treatment. ×600

Fig. 4. *Acer rubrum.* The same specimen as in Fig. 3 photographed in a fluorescence microscope. The thick gelatinous wall layer (Fig. 3, *G*) of tension wood fibers is characterized by a very weak fluorescence. The secondary wall of normal fibers (Fig. 3, *F*) exhibits a relatively high level of fluorescence. ×600

Fig. 5. *Acer rubrum.* Photomicrograph of part of a transverse section which was cut from the stem of an erect seedling 1.0 mm above the treatment site showing details of the xylem formed during DNP treatment. In response to DNP application xylem is developed which is characterized by groups of densely pitted tracheary elements (arrows) with a conspicuous angular appearance. Safranin light-green stained. ×500



Figs. 2–5

The lignin distribution in the various wall layers of tension wood fibers differentiated in response to DNP treatment was established by observation of the intrinsic fluorescence of the walls. Figs. 3 and 4 show part of an unstained transverse section of xylem which was taken from an eight-week-old *Acer rubrum* seedling that had been treated with DNP for two weeks. The section was cut from the stem 1.0 mm below the treatment site. The thick gelatinous wall layer (Fig. 3, *G*) of the tension wood fibers induced by treatment with DNP is characterized by a very weak fluorescence (Fig. 4) whereas fluorescence is relatively intense in the compound middle lamella. The lignin distribution is thus similar to that described for tension wood fibers induced in erect stems by TIBA and for tension wood fibers developed on the upper side of stems during geotropic reorientation (MOREY and CRONSHAW 1968 a). The secondary wall of the normal fibers (Fig. 3, *F*) formed in the stem before DNP treatment is characterized by a relatively high level of fluorescence (Fig. 4).

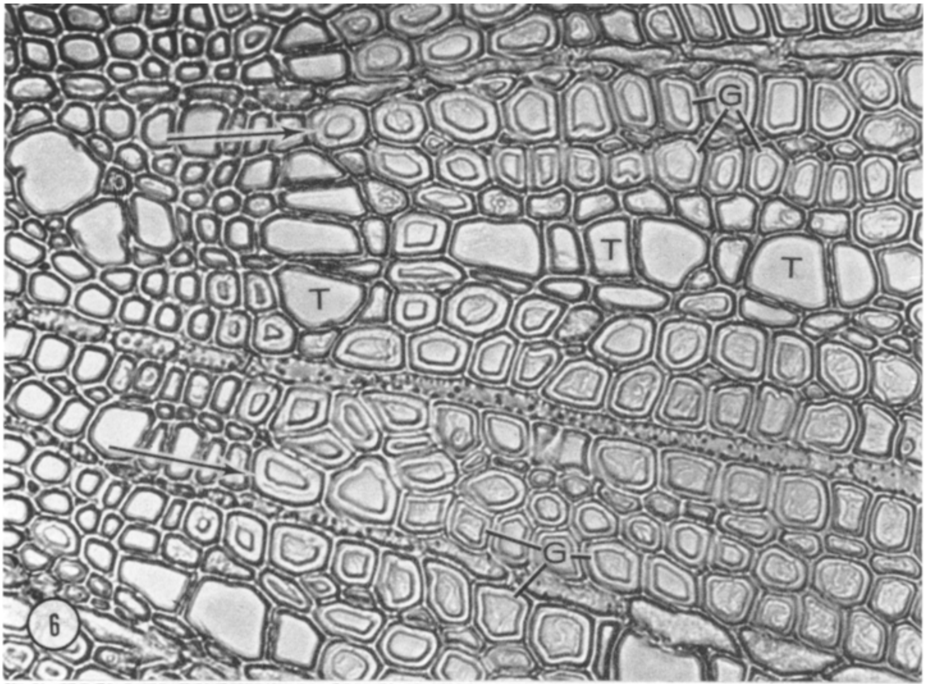
Table 2. *The Effect of DNP on the Development of the Secondary Xylem in Stems of Erect Acer rubrum Seedlings*[1]

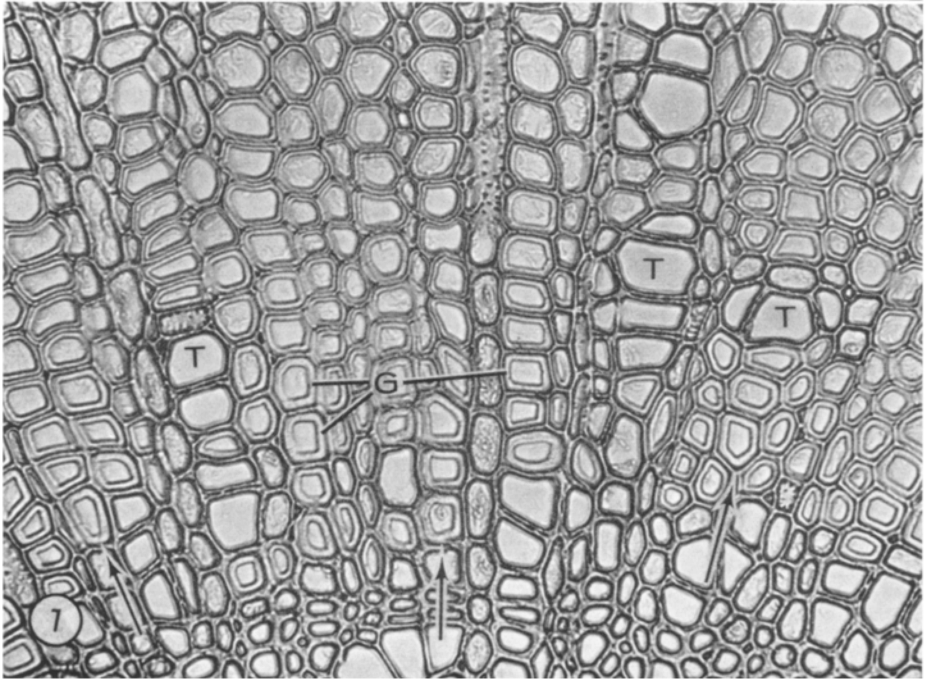
Treatment	Seedlings in which a ring of tension wood was developed		Seedlings in which an arc of tension wood was developed		Seedlings in which only normal fibers were differentiated	
	No.	%	No.	%	No.	%
DNP	13	54	3	13	8	33
DNP—GA	13	52	7	28	5	20
Lanolin treated controls		0		9		91
Untreated controls		0		0		100

[1] DNP and GA were applied at the concentrations designated in Table 1 laterally as a ring to the mid-point of the first internode and after two weeks a transverse section was cut from the stem 1.0 mm below the treatment site.

Fig. 6. *Acer rubrum.* Photomicrograph of part of a transverse section which was cut from the stem of an erect seedling 1.0 mm below the treatment site showing tension wood developed during NAA (0.10%) application. Fibers with a thick gelatinous wall layer (*G*) and small groups of tracheary elements (*T*) are present in the tension wood (arrows) formed during NAA treatment. Safranin light-green stained. ×400

Fig. 7. *Acer rubrum.* Photomicrograph of part of a transverse section cut from the stem of an erect seedling 1.0 mm below the treatment site showing tension wood developed during 2,4-D (0.10%) application. Tracheary elements (*T*) of variable breadth and fibers with a moderately thick gelatinous wall layer (*G*) are present in the tension wood (arrows) formed during 2,4-D treatment. Safranin light-green stained. ×350





Figs. 6 and 7

3.2. Secondary Xylem above the DNP Treatment Site

In *Acer rubrum* seedlings rings of tension wood were never developed above the DNP treatment site. DNP does, however, modify the development of tracheary elements that are formed locally in the stem above the treatment site; the secondary xylem here is characterized by groups of densely pitted tracheary elements (Fig. 5, arrows) with a conspicuous angular appearance in transverse section. The development of the secondary xylem in the second and more apical woody internodes of seedlings treated at the mid-point of the first internode with DNP is unaffected; the secondary xylem here appears to be identical to that of erect lanolin treated and untreated *Acer rubrum* seedlings (MOREY and CRONSHAW 1968 a).

4. Auxin Applied to Erect Seedlings

When IAA (0.50%) and NAA (0.20%) are applied to the mid-point of the first internode of erect *Acer rubrum* seedlings and especially when at low concentrations NAA (0.03 to 0.10%) and 2,4-D (0.10%) are similarly used, a complete ring of tension wood is in some seedlings formed locally in the stem below the treatment site (Table 3). Whereas NAA and 2,4-D at low concentrations are highly effective in inducing tension wood in erect stems of *Acer rubrum* seedlings (Table 3) we have previously reported that in the same

Table 3. *The Effect of Auxins on the Development of the Secondary Xylem in Stems of Erect Acer rubrum Seedlings* [1]

Treatment	Seedlings in which a ring of tension wood was developed		Seedlings in which an arc of tension wood was developed		Seedlings in which only normal fibers were differentiated	
	No.	%	No.	%	No.	%
IAA	2	18	1	9	8	73
NAA (0.03%)	3	50	3	50	0	0
NAA (0.05%)	4	66	1	17	1	17
NAA (0.10%)	4	66	2	34	0	0
NAA (0.20%)	2	40	0	0	3	60
NAA (0.50%)	0	0	0	0	2	100
2,4-D (0.10%)	5	83	0	0	1	17
2,4-D (0.50%)	0	0	0	0	6	100
Lanolin treated controls		0		9		91
Untreated controls		0		0		100

[1] Auxins in lanolin paste were applied laterally as a ring to the mid-point of the first internode and after two weeks a transverse section was cut from the stem 1.0 mm below the treatment site.

system tension wood is not induced by treatment with NAA and 2,4-D at high concentration, but that the development of tracheary elements is considerably modified (MOREY and CRONSHAW 1968 a).

Figs. 6, 7, and 8 show transverse sections of xylem which were cut from the stem 1.0 mm below the treatment site two weeks after treatment of six-week-old *Acer rubrum* seedlings with NAA (0.10%), 2,4-D (0.10%), and IAA (0.50%) respectively. The wide zone of tension wood that was formed during auxin treatment (Figs. 6, 7, and 8, arrows) is characterized by fibers with thick gelatinous wall layers (Figs. 6, 7, and 8, *G*) and by tracheary elements (Figs. 6, 7, and 8, *T*) which are variable in breadth. The number of tracheary elements present in the tension wood zone is in some seedlings equivalent and in others somewhat reduced relative to the number of tracheary elements that are present in the xylem formed before treatment.

In seedlings in which tension wood is induced by application of auxin (NAA 0.03–0.10%, 2,4-D 0.10%, and IAA 0.50%), a strong swelling, which is the result of an acceleration of the rate of cambial division, occurs in the stem below the treatment site. Fig. 9 shows an eight-week-old *Acer rubrum* seedling that had been treated for two weeks with NAA (0.10%) at the mid-point of the first internode (Fig. 9, arrow). The woody axis is swollen in the lower half of the first internode as well as in the hypocotyl (Fig. 10).

5. Discussion

DNP blocks the polar transport of IAA in *Avena* coleoptile sections (DU BUY and OLSON 1940) and in sunflower stem segments (NIEDERGANG-KAMIEN and LEOPOLD 1957). The formation of tension wood which in horizontally placed *Acer rubrum* seedlings occurs along the upper side of the stem is suppressed by application of auxin on the same side (CRONSHAW and MOREY 1968) and in the stems of erect *Acer rubrum* seedlings the induction of tension wood by TIBA and TIBA-GA is largely prevented by the application of auxin to the treatment site (MOREY and CRONSHAW 1968 a, b). Thus, it seems probable that the thick ring of tension wood which is developed only in a restricted region of the erect stem below the DNP treatment site is a developmental response to low auxin level that is most probably brought about by blockade of polar auxin transport. That DNP and DNP-GA are equally effective in inducing tension wood in the upright stems of *Acer rubrum* seedlings (Table 2) is in agreement with the suggestion made previously (MOREY and CRONSHAW 1968 b) that the pathway of differentiation of cambial derivatives is regulated by an auxin level mechanism.

The xylem that is formed locally in the stem above the DNP treatment site is characterized by tracheary elements which occur in a grouped arrangement. A similar effect on the development of tracheary elements in erect stems of *Acer rubrum* is induced by application of relatively high concentrations of auxin, especially 2,4-D and NAA (MOREY and CRONSHAW 1968a). We suggest that the formation of grouped tracheary elements locally above the DNP treatment site, may thus be a developmental response to an accumulation of endogenous auxin brought about by blockade of polar auxin transport by DNP.

TORREY (1953) reported that the maturation of primary xylem in pea root tips grown *in vitro* is completely inhibited by DNP. Direct application of DNP to stem wounds in *Coleus blumei* prevents wound xylem formation (ROBERTS 1960). TORREY (1953) suggested that the DNP induced inhibition of xylem element maturation, especially secondary wall thickening, may reflect an interference with cellulose biosynthesis resulting from lowered levels of phosphorylated intermediates. The capacity of DNP to inhibit the maturation of primary xylem elements no doubt results from a direct effect of the inhibitor on the metabolism of the differentiating cell. On the other hand in *Acer rubrum*, the induction by DNP of tension wood, the xylem elements of which are fully differentiated, indicates that DNP in this system must have an indirect effect on the differentiation of the cambial derivatives.

The number of tracheary elements found in the tension wood induced by DNP is in some seedlings equivalent and in others slightly reduced relative to the number of tracheary elements present in the xylem formed before treatment. In the *Acer rubrum* system it is probable that the relative frequency at which tracheary elements are initiated from the fusiform initials is causally related to the level of auxin in the cambial initial zone (MOREY and CRONSHAW 1968a). This implies that the level of auxin in the stem below the DNP treatment site where tracheary elements are initiated from the dividing initials or adjacent cambial derivatives is largely uneffected by treatment with DNP. It seems inconsistent, on the other hand, that the capacity of DNP to induce the formation of tension wood in the same region of the stem is explained in terms of a developmental response to auxin deficiency. However, the cambial derivatives undergoing secondary wall development, namely the xylem elements in the wall thickening phase of development, are spatially segregated from the cambial initials by a more or less arbitrary

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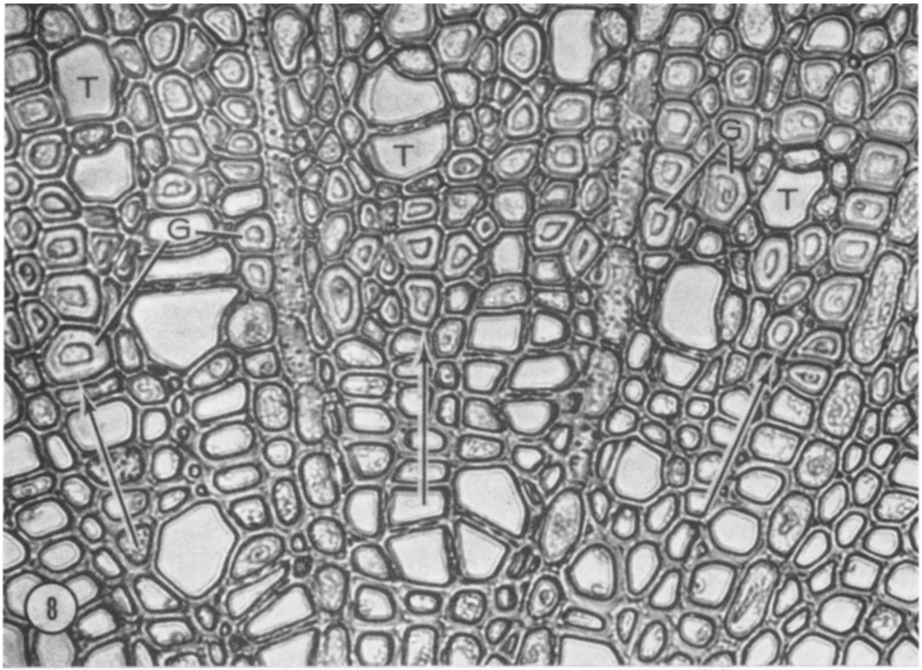
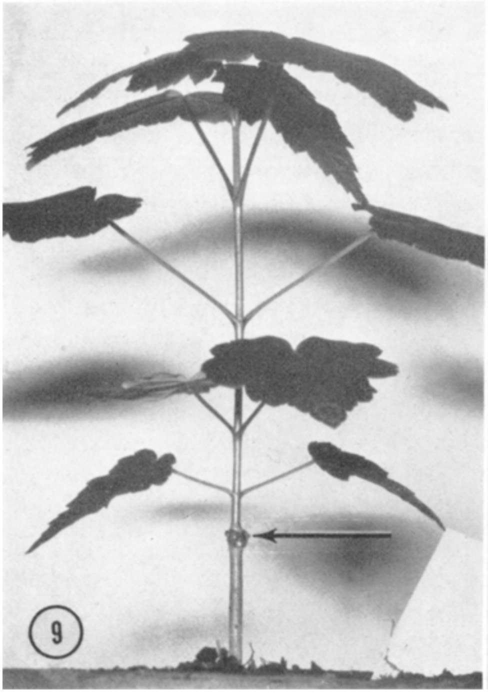
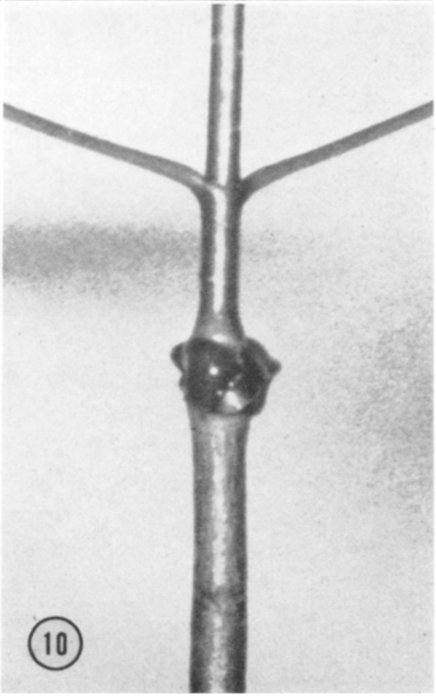
Fig. 8. *Acer rubrum.* Photomicrograph of part of a transverse section which was cut from the stem of an erect seedling 1.0 mm below the treatment site showing xylem formed before and during application of IAA. In response to IAA treatment tension wood (arrows) is developed which is characterized by fibers with a thick gelatinous wall layer (*G*) and by diffusely distributed tracheary elements (*T*). Normal fibers are present in the xylem formed before IAA application. Safranin light-green stained. ×460

Fig. 9. *Acer rubrum.* Photograph of an eight-week-old seedling which had been treated for two weeks with NAA (0.10%) at the mid-point of the first internode (arrow). ×0.85

Fig. 10. *Acer rubrum.* Same seedling as that in Fig. 9 showing swelling of the woody axis in the lower half of the first internode and in the hypocotyl. The rate of cambial division in the stem below the NAA treatment site is accelerated relative to that of erect lanolin treated controls and untreated controls. ×3.7







Figs. 8–10

zone of cells in which the walls are expanding by surface growth. In this regard DNP may be more effective in lowering the auxin level in the more centripetal zones of the stem than in the peripheral meristematic region.

The presence of some wide tracheary elements in the tension wood induced by DNP indicates that the latter has no pronounced effect on the normal surface growth of differentiating tracheary elements. By contrast, in the tension wood that is induced in stems of erect *Acer rubrum* seedlings by treatment with TIBA and TIBA-GA, tracheary elements are narrow, and this is interpreted as an effect of TIBA itself on the differentiation of these cells (Morey and Cronshaw 1968 a, b).

Whereas the development of tracheary elements is modified throughout the woody stem in *Acer rubrum* seedlings treated with TIBA (Morey and Cronshaw 1968 a), DNP has a similar effect only in a localized region of the stem above the treatment site. This suggests either that DNP is immobile or that it has no direct influence on the development of tracheary elements.

That tension wood is induced in erect stems of *Acer rubrum* by treatment with auxin appears inconsistent with the evidence which suggests in *Acer rubrum* (Cronshaw and Morey 1965, 1968, Morey and Cronshaw 1968 a, b) as well as in other arborescent angiosperms (Nečesaný 1958, Casperson 1963, 1965, Morey and Cronshaw 1966, Kennedy and Farrar 1965) that the development of tension wood is a response to auxin deficiency. However, as the induction by auxin of tension wood in *Acer rubrum* is associated with an accelerated rate of cambial division, it may be argued that the auxin level on the xylem side of the cambial initials is actually lowered in response to the growth of an increased number of cambial derivatives, thereby switching the pathway of differentiation from that forming libriform fibers to that of tension wood fibers. Thus, in *Acer rubrum* seedlings treated with auxin, especially with low concentrations of NAA and 2,4-D (Table 3), we envisage that although auxin penetrates into the stem in sufficient quantity to stimulate the rate of cambial division, the amount of auxin present in the more centripetal zones of the stem is insufficient to prevent the differentiation of cambial derivatives as tension wood fibers. In this regard it is significant that in seedlings treated with high concentrations of NAA (0.50%) and 2,4-D (0.50%), and in which the rate of cambial division is also accelerated, tension wood fibers are not developed (Morey and Cronshaw 1968 a), and we suggest that this is due to the presence in the centripetal zones of the stem of a substantial amount of exogenous auxin.

Casperson (1965) reported that the application of IAA or 2,4-D unilaterally to one side of erect epicotyls of *Aesculus hippocastanum* induces the formation of tension wood on the side of the axis directly opposite the treatment area. He suggested that tension wood is induced in response to an auxin gradient, always in the region of the stem characterized by the lowest auxin concentration.

We have suggested that the development of tension wood in the erect stems of *Acer rubrum* seedlings treated with IAA or with low concentrations of NAA or 2,4-D is due to an auxin deficiency induced indirectly by an accelerated rate of cambial division. In erect *Aesculus hippocastanum* seedlings the formation of tension wood on the side of the epicotyl opposite the site of application of IAA or 2,4-D (CASPERSON 1965) may be explained also by this mechanism assuming that unilateral application of auxin is effective in stimulating the rate of cambial division on all sides of the axis and that on the treated side of the axis, IAA or 2,4-D are present in sufficient concentration to prevent the differentiation of tension wood fibers from the cambial derivatives.

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References

BUY, H. G. DU, and R. A. OLSON, 1940: The relation between respiration, protoplasmic streaming and auxin transport in the *Avena* coleoptile, using a polarographic microrespirometer. Amer. J. Bot. **27**, 401—413.

CASPERSON, G., 1963: Über die Bildung der Zellwand beim Reaktionsholz II. Zur Physiologie des Reaktionsholzes. Holztechnologie **4**, 33—37.

— 1965: Über endogene Faktoren der Reaktionsholzbildung I. Wuchsstoffapplikation an Kastanienepikotylen. Planta **64**, 225—240.

CRONSHAW, J., and P. R. MOREY, 1965: Induction of tension wood by 2,3,5-tri-iodobenzoic acid. Nature **205**, 816—818.

— — 1968: The effect of plant growth substances on the development of tension wood in horizontally inclined stems of *Acer rubrum* seedlings. Protoplasma **65**, 379—391.

KENNEDY, R. W., and J. L. FARRAR, 1965: Induction of tension wood with the anti-auxin 2,3,5-tri-iodobenzoic acid. Nature **208**, 406—407.

MOREY, P. R., and J. CRONSHAW, 1966: Induced structural changes in cambial derivatives of *Ulmus americana*. Protoplasma **62**, 76—85.

— — 1968 a: Developmental changes in the secondary xylem of *Acer rubrum* induced by various auxins and 2,3,5-tri-iodobenzoic acid. Protoplasma **65**, 287—313.

— — 1968 b: Developmental changes in the secondary xylem of *Acer rubrum* induced by gibberellic acid, various auxins and 2,3,5-tri-iodobenzoic acid. Protoplasma **65**, 316—326.

NEČESANÝ, V., 1958: Effect of β-indoleacetic acid on the formation of reaction wood. Phyton (Buenos Aires) **11**, 117—127.

NIEDERGANG-KAMIEN, E., and A. C. LEOPOLD, 1957: Inhibitors of polar auxin transport. Physiol. Plant. **10**, 29—38.

ROBERTS, L. W., 1960: Experiments on xylem regeneration in stem wound responses in *Coleus*. Bot. Gaz. **121**, 201—208.

TORREY, J. G., 1953: The effect of certain metabolic inhibitors on vascular tissue differentiation in isolated pea roots. Amer. J. Bot. **40**, 525—533.

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