A NOTE ON THE STRUCTURE OF MORPHACTIN-INDUCED WOOD IN TWO CONIFEROUS SPECIES

J. E. Phelps, M. Saniewski, M. Smoliński, J. Pieniążek and E. A. McGinnes, Jr.

(Received 1 April 1974)

ABSTRACT

Anatomical features of vertically growing apical shoots of *Pinus sylvestris* L. and *Picea* excelsa Link. trees treated with 0.3% morphactin IT 3456 in lanolin paste were analyzed with light and scanning electron microscopes. These studies indicated that the wood formed above and at the area of treatment was morphologically similar to compression wood. Reasons for production of compression wood after treatment with morphactin in vertically growing shoots are discussed.

Additional keywords: Pinus sylvestris, Picea excelsa, compression wood, auxins, scanning electron microscope, physiology.

INTRODUCTION

Morphactins, consisting of fluorene-9carboxylic acids and their derivatives, are synthetic growth regulators with a unique kind of action that greatly affects various physiological and morphological processes in plants (Schneider 1970). Morphactins are nontoxic in a wide range of concentrations. Their action is slow and systemic. In high concentrations, morphactins inhibit new growth, whereas in low concentrations, they have a transient effect on, among other things, the morphogenesis of new growth (Schneider 1970). Morphactins have also been found to influence photo- and geotropic responses in several herbaceous plants (Khan 1967).

It previously has been shown that vertically growing shoots of *Pinus sylvestris* L. and *Picea excelsa* Link. treated with morphactin IT 3456 (2-chloro-9-hydroxyfluorene-9-carboxylic acid) produced compressionlike wood above and at the area

WOOD AND FIBER

of treatment (Smoliński et al. 1972; Smoliński et al. 1973).

Light microscopic observations and degree of lignification determinations will be reviewed in this note to familiarize the reader with these data. The scanning electron microscope enabled depth-of-focus analyses of anatomical features under conditions that are unavailable with conventional light microscopy. Thus the scanning electron microscope was used to substantiate earlier reported observations on whether or not the newly formed wood possessed characteristics of compression wood.

MATERIALS AND METHODS

Vertically growing apical shoots of sevenvear-old Pinus sulvestris L. and six-vear-old Picea excelsa Link. were treated with 0.3% morphactin IT 3456 in lanolin paste on 25 April 1971, when apical meristematic activity was beginning. Control shoots were treated with lanolin paste. In all, samples from eight shoots were analyzed with the scanning electron microscope, two treated and two control from each species. The shoots were growing in the forest in full sunlight. The paste and/or morphactin was applied as a ring 1.5 cm wide, 7 cm below the apical bud of each shoot. Cylindrical samples from the shoots containing wood formed below, within, and above the areas

SPRING 1974, V. 6(1)

The authors addresses are: J. E. Phelps and E. A. McGinnes, Jr., School of Forestry, Fisheries and Wildlife, University of Missouri, Columbia, MO 65201; M. Saniewski and J. Pieniążek, Institute of Pomology, Skierniewice, Poland; and M. Smoliński, Institute of Physiology and Cytology, Tódź University, Poland. Our appreciation is extended to the University of Missouri, College of Agriculture Electron Microscope facility, for use of their facilities. This study was financed, in part, by McIntire-Stennis funds.

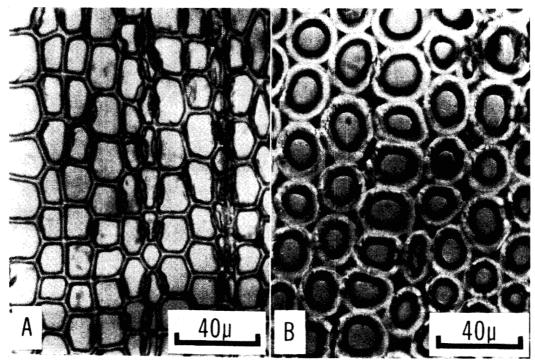


Fig. 1. Transverse sections of *Pinus sylvestris* L. control (A) and treated with morphactin (B). Note rounded cell shape and intercellular spaces in B that are not present in A.

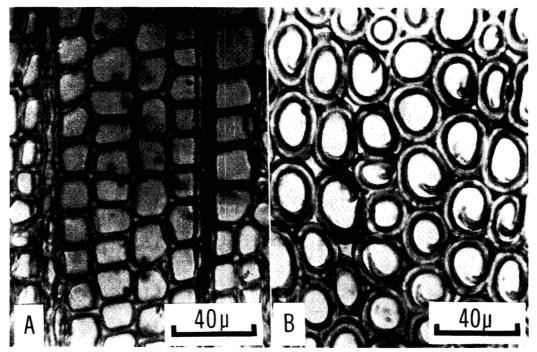


FIG. 2. Transverse sections of *Picea excelsa* Link. control (A) and treated with morphactin (B). Note rounded cell shape and intercellular spaces in B that are not present in A (*from* Smoliński et al. 1972).

of treatment were collected on 29 May. 20 June, and 1 August 1971 and stored at that time in 75% ethyl alcohol. Light microscopic analyses of sections from shoots collected on these various dates indicated that similar changes in wood morphology occurred regardless of sampling date (Smoliński et al. 1972). Scanning electron microscopic observations were confined to those areas where the most significant changes occurred, that is just above areas of the application. Degree of lignification determinations were made after the sections were stained with malachite green and acid fuchsin, phloroglucinol in HCl and with iodine in zinc chloride.

Scanning electron microscopic observations were made using longitudinal sections taken from the above samples stored in ethyl alcohol. Since light microscopic observations had already been made, the authors felt that scanning electron microscopic observations of longitudinal sections would probably give the best indication of differences in cell-wall characteristics. Longitudinal sections, 3 mm by 5 mm by 1 mm, were dehydrated in ethyl alcohol (75 to 95 to 100%), transferred through a graded series of isoamyl acetate (30, 50, 70, 90, 100%) and critical point dried in carbon dioxide following a procedure by Anderson (1951) modified for scanning electron microscopy. This technique enables samples to be dried while reducing drying stresses to a minimum. The dried samples were coated with gold in a vacuum evaporator and examined with a JEOL JSM-S1 scanning electron microscope operating at 10 kV.

RESULTS

The following changes in cell structure were observed with a light microscope. Analyses of transverse sections of *Pinus sylvestris* L. wood treated with morphactin indicated that the wood had round tracheids with abnormally thick, highly lignified cell walls. The degree of lignification was determined by observing the relative intensities of the dyes malachite green and acid fuchsin in the control and treated samples. This wood also had distinct intercellular spaces in transverse section (Fig. 1B). Other analyses indicated that the tips of these tracheids were distorted and the tracheids were shorter in length than the untreated samples. Similar changes were observed in wood of *Picea excelsa* Link. shoots after treatment with morphactin (Fig. 2, taken from Smoliński et al. 1972).

A scanning electron microscopic study of control and morphactin-treated wood of the two species indicated that helical checks were present in the S_2 layer of the wall of those samples treated with morphactin (Figs. 3B and 4B). Both control and morphactin-treated wood came from the same intraincremental positions.

DISCUSSION AND CONCLUSION

The results of this study indicate that wood formed above an area treated with morphactin IT 3456 is morphologically similar to compression wood as described by Côté and Day (1965) and Jutte and Levy (1972).

It is known that compression wood is formed on the lower side of inclined stems and branches as a response to normal geotropic stimuli. Compression wood can also be obtained artificially by an application of auxins, IAA and NAA, to the stem (Westing 1965, 1968). Large amounts of auxins cause compression wood to occur by stimulating cell division and also cause excessive lignification within the cell wall (Kennedy and Farrar 1965). Differentiation of cambial cells forming compression wood comes about by an increase in the number of anticlinal divisions, and this differentiation reportedly is complete in less than the usual 20 or so days that are typical of more normal tracheid differentiation (Scurfield 1973).

The formation of compression wood in vertically growing shoots of *Pinus sylvestris* L. and *Picea excelsa* Link. as a result of morphactin treatment may be due to a blockage of the basipetal transport of auxin (Kaldewey 1973; Parups 1970) and conse-

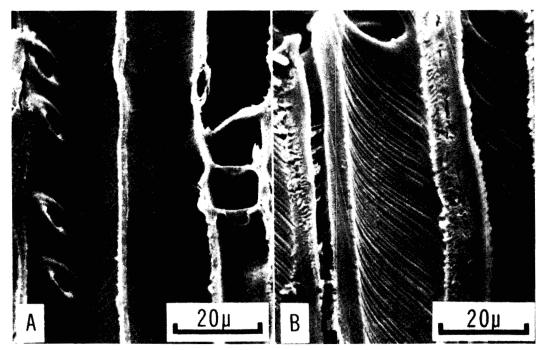


FIG. 3. Longitudinal sections of earlywood zones of *Pinus sylvestris* L. control (A) and treated with morphactin (B). Note helical checks and thick cell walls in B that are not present in A.

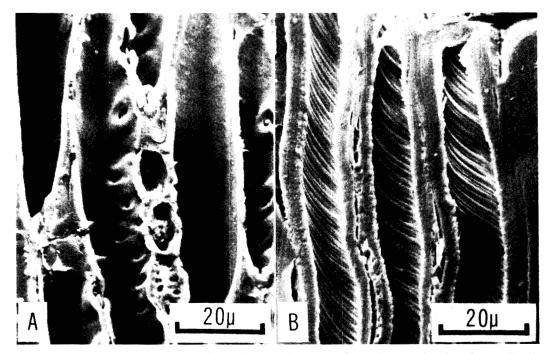


FIG. 4. Longitudinal sections of earlywood zones of *Picea excelsa* Link. control (A) and treated with morphactin (B). Note helical checks and thick cell walls in B that are not present in A.

quently the accumulation of auxins above the treated area takes place. The thickening of shoots after treatment with morphactin was probably due to a synergistic effect of endogenous auxin, cytokinins, and applied morphactin on the rate of cambial divisions. Similar interactions between the above growth regulators have been demonstrated previously in *Malus* spp. shoots (Pieniążck et al. 1970).

In summary, morphactin IT 3456 apparently blocked the basipetal transport of auxins, causing an accumulation of auxins above the treated area. This resulted in the formation of compression wood above the treated area. This investigation is being continued to study the influence of morphactin IT 3456 on wood formation in *Aesculus hippocastanum* L.

REFERENCES

- ANDERSON, T. F. 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N.Y. Acad. Sci. 13:130–134.
- Côté, W. A., JR., AND A. C. DAY. 1965. Anatomy and ultrastructure of reaction wood. Pages 391–418 in W. A. Côté, Jr., ed. Cellular ultrastructure of woody plants. Syracuse Univ. Press, Syracuse, N. Y.
- JUTTE, S. M., AND J. F. LEVY. 1972. Compression wood in *Pinus ponderosa* Laws.—a scanning electron microscopy study. IAWA Bull. 1972/ 2:3–7.
- KALDEWAY, H. 1973. Transport and metabolism of indoleacetic acid as influenced by morphactins. Third Symposium on Accumulation and Translocation on Nutrients and Regulators in Plant Organisms (Abstracts),

Warszawa, Jablonna, Brzezna, Krakow (Poland), May 14–18, Pp. 35–36.

- KENNEDY, R. W., AND J. L. FARRAR. 1965. Tracheid development in tilted seedlings. Pages 419-453 in W. A. Côté, Jr., ed. Cellular ultrastructure of woody plants. Syracuse Univ. Press. Syracuse, N. Y.
- KHAN, A. A. 1967. Physiology of morphactins: effect on gravi- and photo-response. Physiol. Plant. 20:306-313.
- PARUPS, E. V. 1970. Effect of morphactin on the gravimorphism and the uptake, translocation, and spatial distribution of indol-3 yl-acetic acid in plant tissues in relation to light and gravity. Physiol. Plant. 23:1176– 1186.
- PIENIĄŻEK, J., M. SMOLIŃSKI, AND M. SANIEWSKI. 1970. Induced structural changes in anatomy of apple shoots after treatment with morphactin IT 3456 and other growth regulators/NAA, GA, BA/. Acta Agrobotanica 23:387–396.
- SCHNEIDER, G. 1970. Morphactins: physiology and performance. Ann. Rev. Plant Physiol. 21:499–536.
- SCURFIELD, G. 1973. Reaction wood: its structure and function. Science 179:647-655.
- SMOLIŃSKI, M., M. SANIEWSKI, AND J. PIENIĄŻEK. 1972. The effect of morphactin IT 3456 on cambial activity and wood differentiation in *Picea excelsa*. Bull. Acad. Polon. Sci., Ser. Sci. Biol. 20:431–435.
- SMOLIŃSKI, M., J. PIENIĄŻEK, AND M. SANIEWSKI. 1973. Induction of compressionlike wood by morphactin in vertically growing shoots of some coniferous species. Third Symposium on Accumulation and Translocation of Nutrients and Regulators in Plant Organisms (Abstracts). Warszawa, Jablonna, Brzezna, Krakow (Poland). May 14–18. Pp. 74–75.
- WESTING, A. H. 1965. Formation and function of compression wood in gymnosperms. Bot. Rev. 31:381-480.
- WESTING, A. H. 1968. Formation and function of compression wood in gymnosperms. II. Bot. Rev. 34:51-78.