Seasonal variation of auxin in stem cambial region of *Pinus sylvestris* L.

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(Received: April 13, 1978)

ABSTRACT

Investigation of auxin from the stem cambial region of adult *Pinus sylvestris* trees by diffusion to agar, and by extraction revealed both seasonal variation and gradients along the stem. Variation in dynamics of auxin efflux and the effect of the size of tissue blocks are used as arguments in the critical discussion of the concept of auxin regulation of cambial activity by way of simple seasonal concentration changes.

INTRODUCTION

The concept of auxin regulation of seasonal changes in the activity of cambium arose very soon after the discovery of auxin in plants. This concept has been discussed in detail by several authors during the past two decades (Wareing 1958, Larson 1962, 1964, Reinders-Gouwentak 1965, Brown 1970). Stimulation of cambial activity by synthetic auxin, and seasonal variation of natural auxin concentration in stems of woody plants are the main arguments supporting this theory. Such seasonal variation was not, however, evident in early experiments with *Pinus sylvestris* when extracts from tissues of the stem cambial region and phloem were bioassayed (Wodzicki 1971). This fact stimulated investigation, which resulted in finding an important seasonal change in cambial sensitivity to auxin in *Pinus sylvestris* (Zajaczkowski 1973). Simultaneously, experiments with ring-barked, and decapitated adult trees (Wodzicki, 1973) and culture of isolated sections of pine stem (Zajaczkowski, Romberger (1978) indicated the significance of basipetal transport of auxin for

stimulation of cambial activity. All these facts, stimulated reinvestigation of the seasonal variation of natural auxin content in the stem cambial region of *Pinus silvestris*. The results are presented in this paper.

MATERIAL AND METHODS

Stems of mature *Pinus silvestris* L. trees (60-70-year-old) in a forest stand were sampled at several intervals during the year. Two methods of investigating auxin in the cambial region were employed:

a) auxin translocation was studied by direct contact of the tissue with agar, followed by Went's modified Avena (variety Seger I) coleoptile curvature test;

b) extraction of homogenized tissue (young phloem and cambial zone) with MeOH followed by ether partitioning paper chromatography (with isopropanol, NH_3 , H_2O , 10:1:1 v/v), and straight growth tests using sections of oat mesocotyl or pine hypocotyl. Details of the methods used are given elsewhere (Nitsch and Nitsch 1956, Wodzicki 1968, Witkowska-Żuk and Wodzicki 1970).

Small blocks of tissue (comprising phloem, cambial zone, differentiating xylem, and 2 mm of mature xylem) for auxin investigation by Went's method were cut from large (20×15×5 cm) pieces of the outer part of tree stems brought to the laboratory. Axial and tangential diameters of the blocks prepared for investigation varied according to the requirements of the experiments. The blocks were first placed on agar with either their basal or apical ends for 10 min. to remove the substances from the cells severed at the cut surface, and only afterwards transferred to fresh agar plates to study auxin efflux. After removal of tissue agar cubes (2×2×1.2 mm) were cut from the area of the agar plate that had been opposite to the cambial region. Concentration curves prepared with authentic IAA were used for quantifying the result of bioassays.

RESULTS AND DISCUSSION

Efflux of auxin from small blocks of stem tissue (10×20×5 mm) to agar applied for 1 hour at basal and apical ends confirms the hypothesis of seasonal variation (Fig. 1). The maximum in the oat coleoptile curvature test was observed in May and June. More auxin always diffused from tissue blocks collected at the tree crown level of the

stem. This basipetal gradient with respect to auxin efflux to agar was also confirmed by two experiments performed with 4 groups of 4 trees sampled in April and August 1975 and 1976 (Fig. 2). On the other hand a reverse gradient was revealed by the oat mesocotyl section straight growth tests of the chromatographed ether fraction of MeOH extracts of tissues collected from stems of the same trees (Fig. 2).

Fig. 1. Stimulation of oat coleoptile curvature by pine auxin collected in agar by diffusion from the stem cambial region at various dates of the year.

Auxin efflux from basal end of tissue block — continuous lines, from apical end — dotted lines. Tree crown level —○—, stem base —●— Averages of 4 trees

On the basis of unpublished data concerning stimulation produced by extracts of cambial region tissues collected simultaneously from stems of 28 trees every two weeks during the 1968 season, as quantified by IAA concentration, curves a seasonal variation can be demonstrated (Fig. 3). The broad maximum of stimulation occurred from May through the end of July when a higher concentration (eqv. to 0.5 gm fresh wt.) was tested. At a lower concentration (eqv. 0.05 gm fresh wt.) a sig-

nificant increase of stimulation was discovered in the extract collected as late as July 5. This maximum was followed by a deep depression two weeks later and a second maximum in August. This seasonal pat-

Fig. 2. Amount of pine auxin diffused to agar (I) or extracted (II) from cambial region tissues collected from pine stem at tree crown level (A), below crown (B) and at 1.3 m above ground (C).

April — open bars, August — dashed bars. Averages of two years study, four trees in each experiment. The results were quantified by Went's *Avena* coleoptile curvature tests (I) and Nitsch's *Avena* mesocotyl section straight growth tests (II).

tern was not revealed previously (Wodzicki 1971) when data concerning higher concentrations of the extract (1.0 gm fresh wt.), expressed as percentage of a single IAA concentration (0.1 ppm) were analysed.

Fig. 3. Stimulation of oat mesocotyl section straight growth by pine auxin eluted from R_f 0.25—0.5 of the chromatographed ether fraction of methanolic extract from tissues of the stem cambial region. Equivalent to 0.05 gm o-o and ●-● 0.5 gm fresh wt.

The results obtained with the two methods as presented in Figs. 1 and 3 reveal more or less similar seasonal variations. Neither method, however, provides fully reliable data concerning concentration of natural auxin in the cambial region of the pine stem. First of all, each test

Fig. 4. Basipetal efflux of auxin from pine stem tissue blocks, -o-- 5 mm, -●-- 10 mm, -x-- 40 mm high.

Averages of 2 trees (A), 3 trees (B) and (C). Trees were sampled on July 1 (1), August 16 (2), October 14 (3). Vertical bars in (A) indicate the differences between two replicate trees

method responds to free and bound auxins to a different extent. The results obtained when the extracts are tested are, of course affected by changes produced during extraction (destruction or synthesis), but above all they may also represent the modifying effects of other growth regulators not separated from auxin by simple methods of purification.

When the regulatory consequences of seasonal variation of auxin content established by the extraction method are discussed, one should take into account that the extracted substances are obtained from two functionally different tissues such as the meristematic region of cambium and the phloem. Seasonal variation of at least some of the above mentioned interfering factors is possible.

The interpretation of measurements of the diffusion of natural auxin to agar is still more complex. It is seen (Fig. 4AB) that auxin efflux to agar is related both to time of exposure and especially to the length of the exposed block of tissue. The efflux from short blocks (5 mm) was always greater than from longer ones (40 mm).

The dynamics of auxin efflux to agar from tissue blocks of similar size (10 mm height) also change during the season (Fig. 4C). Later in summer the efflux starts with lower amounts and ends earlier.

It is not possible to decide whether the seasonal changes observed in this study result from seasonal changes of velocity of auxin transport, changes of its concentration, or interactions of other growth substances. The latter explanation seems possible at least in the case of differences between results determining the gradient of auxin along the stem by the two methods employed.

Seasonal variation of cambial activity and xylem differentiation determining the specific stem morphology of woody plants depends upon variation of auxin action and upon changes of sensitivity of responsive cells. The first results concerning the mechanism of regulation in such an integrated system are being published in a separate papers (Zajączkowski, Wodzicki 1978a,b). The information transmitted by moving auxin in this system is not necessarily related only to its concentration. The tissue as an organized unit of polar cells regulating the mobility of auxin, may condition the morphogenic response at the cellular level.

Acknowledgments

This work was supported in part by a grant from the U.S. Department of Agriculture (No. FG-Po-317).

The skilfull assistance of my wife Alina B. Wodzicki in doing the bioassays and auxin extractions is sincerely acknowledged. The author owes special thanks to Dr. John A. Romberger for critical reading of the paper before submission to the journal.

REFERENCES

- Brown C.L., 1970. Physiology of wood formation in conifers. *Wood Science* 3(1): 8-22.
- Larson P.R., 1962. Auxin gradients and the regulation of cambial activity. In: *Tree Growth* (T.T. Kozłowski, ed.), pp. 97-117. The Ronald Press Co. New York.

- Larson P.R., 1964. Some indirect effects of environment on wood production. In: The Formation of Wood in Forest Trees (M. H. Zimmermann ed.); pp. 345—365. Academic Press, New York.
- Nitsch J.P., Nitsch C., 1956. Studies on the growth of coleoptile and first-internode sections. A new, sensitive, straight growth test for auxin. *Plant Physiol.* 31: 94-111.
- Reinders-Gouwentak C., 1965. Physiology of the cambium and other secondary meristems of the shoot. In: *Encyclopedia of Plant Physiology* (W. Ruhland ed.) 15(1): 1076-1105.
- Wareing P. F., 1958. The physiology of cambial activity. *Journ. Inst. Wood Sci.* 1: 34-42.
- Witkowska-Żuk L., Wodzicki T. J., 1970. *Pinus silvestris* L. hypocotyl section straight growth test for auxin. *Acta Soc. Bot. Pol.* 39: 175-184.
- Wodzicki T. J., 1968. On the question of occurrence of indole-3-acetic acid in *Pinus silvestris* L. *Amer. J. Bot.* 55(5): 564-571.
- Wodzicki T. J., 1971. Mechanism of xylem differentiation in *Pinus silvestris* L. *J. Exp. Bot.* 22: 670-686.
- Wodzicki T. J., Wodzicki A. B., 1973. Auxin stimulation of cambial activity in *Pinus silvestris* L. II. Dependence upon basipetal transport. *Physiol. Plant.* 29: 288-292.
- Zajaczkowski S., 1973. Auxin stimulation of cambial activity in *Pinus silvestris* L. I. The differential cambial response. *Physiol. Plant.* 29: 281-287.
- Zajaczkowski S., Romberger J. A., 1978. Polarity of xylem formation in isolated stem segments of *Pinus silvestris* L. *Physiol. Plant.* (in press).
- Zajaczkowski S., Wodzicki T. J., 1978a. On the question of stem polarity in respect to auxin transport. *Physiol. Plant.* 44: 122-126.
- Zajaczkowski S., Wodzicki T. J., 1978b. Auxin and plant morphogenesis — a model of regulation. *Acta Soc. Bot. Pol.* 47 (3): 233—243.

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Sezonowe wahania auksyny w regionie kambialnym pnia Pinus silvestris L.

Streszczenie

Aktywność naturalnej auksyny w tkankach regionu kambialnego pnia 60—70-letnich drzew *Pinus silvestris* L. badano przy zastosowaniu metody dyfuzji do agaru i testu wygięciowego koleoptile owsa oraz metod ekstrakcji i chromatografii bibułowej i testów prostoliniowego wzrostu odcinków mezokotyłu owsa i hypokotyłu sosny. Przeprowadzone badania wykazały różnice w aktywności auksyny zarówno w ciągu sezonu, jak też gradienty wzdłuż pnia. Stwierdzony przebieg tych zmian uzależniony był jednak od rodzaju wybranej metody i koncentracji ekstraktu. W przypadku zastosowania metody dyfuzji aktywność auksyny zmniejszała się wraz ze zwiększeniem rozmiarów badanego odcinka pędu. Na podstawie uzyskanych wyników przedyskutowano zasadność teorii uznającej, że aktywność kambium w ciągu sezonu uzależniona jest bezpośrednio od zmian koncentracji auksyny.