The possible relation between cytokinins and secondary xylem formation in *Pinus silvestris*

I. Seasonal correlations

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Abstract

The seasonal dynamics of changes in the endogenous cytokinin level was investigated in the tissue of the stem cambial region. The results of the soybean test and *Amaranthus* test show that marked variations occur in the course of the year in cytokinin activity in five fractions obtained from tissue of the cambial region. These variations characterized by a spring maximum and late-summer maximum may be correlated in time with changes in cambial activity and the course of annual ring differentiation in the pine stem during the vegetation season.

INTRODUCTION

Numerous results of investigations indicate that initiation and regulation of cell division in the cambial tissue of trees and the differentiation of cambial derivatives are controlled by a complex of growth regulators (Wareing, 1958; Wodzicki, 1965; Brown, 1970; Roberts, 1976). Many authors suggest that the annual ring differentiation in *Pinus silvestris* L. wood is not solely conditioned by growth factors of the type of auxins and gibberellins. Wodzicki (1971) noted a high auxin activity in the tissue of the cambial region of the pine stem not only in spring, in the period of maximally intensive division in the cambium, but also in autumn and winter, periods when divisions do not occur in the cambium. Neither did he find any distinct relation between the extractable auxin level and changes in the tracheid diameter in the course of the vegetation season. The isolated pine stem segment culture test demonstrated (Zajączkowski, 1973) seasonal changes in the susceptibility of cambial cells

to auxin. The role of gibberellins in maintaining throughout summer or autumn of cambial activity in pine or their role in processes of summer and autumn differentiation of xylem elements could not, however be ascertained.

It is also known that xylem formation in the *Pinus silvestris* stem is stimulated by the substances found in methanol extracts of cambial tissues (Zajaczkowski and Wodzicki, 1975; Zajaczkowski, 1978). Preliminary tests demonstrated that there may be substances with cytokinin activity in these extracts characterised by synergic action with auxin in the regulation of cambium division and differentiation of cambial derivatives. Moreover, the presence has been revealed (Rogosińska, 1967) of substances with cytokinin activity in tissues of the cambial region of the pine stem.

Cytokinins are compounds which so far have been but little studied from the aspect of their role in annual ring formation. Only synthetic cytokinin was found to affect, synergistically with IAA, cambial activity or the differentiation process in the tree stem (Pieniążek, 1968; Pieniążek and Jankiewicz, 1966; Hejnowicz and Tomaszewski, 1969; Robards et al., 1969). A similar effect of synthetic cytokinin was noted in sterile cultures of isolated pine stem segments (Zajaczkowski, 1973). It was, however, also found that kinetin has no influence or inhibits cambial division and the process of differentiation in decapitated shoots of young pines (Wodzicki and Zajaczkowski, 1974). This substance has also an inhibitory effect in the pine hypocotyl test (Zajaczkowski and Wodzicki, 1975; Zakrzewski, 1975).

Since the results of studies on the influence of synthetic cytokinins on wood formation in the stem of *Pinus silvestris* L. proved unequivocal or contradictory, an attempt was made to establish the possible participation of endogenous cytokinins of *P. silvestris* in some phases of annual ring differentiation. This possibility was examined by searching for a correlation in time between the changes in intensity of the process of annual ring differentiation and the changes in the endogenous cytokinin levels in the tissues of the pine cambial region at various stages of annual ring formation.

MATERIAL AND METHODS

The material for determination of the content of cytokinin-like substances was collected from *Pinus silvestris* L. trees aged 74 and 75 years in Rogów, the experimental forest of the Warsaw Agricultural University. Tissue from the stem cambial region was taken after removing the bark layer and the outer phloem layer. Material was

collected from the same 12 trees at the following dates: 11 June, 29 July, 30 August and 29 September, 1975 and on 29 January, 6 April and 22 May, 1976 from the stem region at breast height. The tissues were collected in 1976 from a second group of 12 trees on: 16 June, 13 July, 6 August, 8 September, 14 October, 25 November and 15 December as well as (from a 3rd group of 12 trees) on 12 January, 25 February, 16 March, 15 April and 5 May, 1977.

The tissues were stored at dry ice temperature until extraction. The cooled tissue was homogenised with absolute methanol, extracted overnight and then 3-fold extracted with 80 per cent methanol and water (Wodzicki, 1969). The following steps of purification consisted in separation with ethyl acetate, ion-exchange chromatography and paper chromatography (Fig. 1).

Fig. 1. Scheme of method of purification and testing of extract fractions from cambial region tissue of pine stem

The material from the first groups (years 1975/1976) was extracted according to variant I in Fig. 1. Cytokinin activity of the chromatogram segment with R_F 0.2—0.55 was tested.

Extracts from tissue of the groups II and III collected in 1976/1977 were purified according to variant II in fig. 1. For separation of cytokinin-like substances the technique of fractionation of eluates from

chromatogram strips was applied with the use of Sephadex LH-20 gel (after Armstrong et al., 1969). For this purpose eluates from chromatogram segments with R_F 0.4—0.8 were fractionated on a column 23 cm high with 25 g of Sephadex, which was eluted with 15 per cent ethanol aqueous solution. Fractions of 9 ml were collected and combined in groups (denoted by letters from D to F) on the basis of their optical density measured at wavelength 276 nm. The eluate from the segment with R_F 0.05—0.2 which was not fractionated was denoted by symbol A and the eluate from the segment with R_F 0.2—0.4 was denoted as fraction B+C. The fractions, after evaporation of ethanol and concentration to about 15 ml were tested by two biological tests: *Amaranthus caudatus* and callus tissue from soybean (*Glycine max*). The *Amaranthus* test described by Köhler and Conrad (1966) was applied in 6 replications for each sample. Density was measured according to Bigot (1968). The callus tissue test (after Miller, 1960, 1961, 1968) was carried out in 10 replications for each variant.

The results are presented as arithmetical means.

RESULTS AND DISCUSSION

The results of investigation of extracts from tissues of the Scot's pine stem cambial region have shown the presence in pine of cytokinin-like compounds as demonstrated by two specific biological tests. This confirms the earlier results of Rogozińska (1967) with water extracts obtained in the tobacco callus tissue test and also the results of experiments with other tissues and organs of *P. silvestris* L. (Rogozińska and Legocki, 1969; Michniewicz et al., 1976; Kopicewicz et al., 1977) with the use of other methods. The finding of cytokinin activity in the extract from cambial region tissue of the pine stem has added to the number of reports started by Nitsch and Nitsch (1965) concerning the presence of cytokinin-like compounds in the cambial region tissue of trees. The procedure applied in the present investigations for extraction, purification and isolation demonstrated the presence of several (6—7) cytokinin-like compounds in the tissue of *P. silvestris* L. stems. Differences in the endogenous substances extracted from tree tissues have been noted repeatedly (Engelbrecht, 1971; Hewett and Wareing 1973a, b, c; Taylor and Dumbroff 1975; Henson and Wheeler, 1977b; Letham et al., 1976; van Staden 1976, 1977 and others).

The results of biological tests indicate that there occur in the course of the year distinct variations in the activity level of the cytokinin fraction obtained from cambial regions of the stem. Two maxima were observed in this activity: in spring and in late summer, both in the

Fig. 3, a-d. Seasonal changes in cytokinin activity of five extract fractions from cambial region tissue of *P. silvestris* L. stem, collected at 12 dates in years 1976/1977. Continuous line — *Amaranthus* test, dashed line — soybean callus test

tissue collected in the period 1975/1976 (Fig. 2) and in the 5 fractions from the period (1976/1977 (Fig. 3a-d). As a rule the cytokinin activity peak was higher in spring than in summer. Both tests from all sample groups demonstrated that the cytokinin activity maxima were separated by a deep depression appearing between June and July. A second less pronounced or equal depression was noted in the late autumn fractions. Some fractions exhibited a third maximum of cytokinin activity in winter.

Fig. 2. Seasonal changes in cytokinin activity in extracts from cambial region tissue of *Pinus silvestris* L. stem, collected in the years 1975/1976. Results of *Amaranthus caudatus* test (A) and soybean callus test (B)

So far complete studies of seasonal changes in cytokinin activity of the cambial region tissue have not been performed in woody plants. Only the experiments of Dumbroff and Brown (1976) with shoots of *Acer saccharum* also indicate seasonal changes in the level of cytokinin compounds in whole shoots or, in other species — in the xylem sap (Luckwill and Whyte, 1968; Hewett and Wareing 1973a; Alvim et al., 1976).

The above mentioned papers concerning seasonal studies of cytokinin activity in various materials from woody plants, supplied data on the occurrence of a spring maximum, but did not mention another in summer. An exception are the studies of Henson and Wheeler (1977a) on the root nodules of alders in which, beside the spring rise of cytokinin activity another peak was found in mid summer, falling to August. The autumn depression of cytokinin activity has by now been reported in several woody plant species (Davison, 1965; Alvim et al., 1976). Winter maxima have also been detected in other plant material (Alvim et al., 1976; Dumbroff and Brown 1976).

The present results indicating variations in cytokinin activity in cambial region tissue may be correlated in time with changes in cambial activity in the stem of *P. silvestris* L. described by Wodzicki (1962). Changes in cytokinin activity level in cambial region tissue may be related also in time with the course of annual ring differentiation in the pine stem during the vegetation season. The depression in cytokinin activity in mid summer, corresponds, namely, to the depression of cambial activity in June, and the period of transition from early wood production to that of late wood. The late autumn depression falls to the period of inhibition of all annual ring differentiation processes in pine. Only the December-January cytokinin peak falling to the winter cambium resting period shows no relation with the occurrence of division processes in the cambium. It is possible that this lack of correlation is due to winter accumulation of cytokinins, when external conditions render meristematic activity impossible.

The correlation found between the dynamics of changes in cytokinin activity in the pine stem and the process of annual ring formation in wood points to the possibility of participation of endogenous cytokinins in the system of factors regulating annual ring differentiation in the course of the vegetation season.

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*Możliwość udziału cytokinin w różnicowaniu rocznego słoja drewna u *Pinus silvestris* L.*

I. Sezonowe zmiany

Streszczenie

W pracy badano dynamikę sezonowych zmian poziomu endogennych cytokinin w tkance regionu kambialnego pnia sosny zwyczajnej. Na podstawie wyników z testu tkanki kalusowej soi i testu *Amaranthus* stwierdzono, że w ciągu roku występują wyraźne wahania poziomu aktywności cytokininowej w pięciu frakcjach uzyskanych z ekstraktów tkanki regionu kambialnego. Wahania te, charakteryzujące się maksimum wiosennym i późno-lętnim można skorelować w czasie ze zmianami aktywności kambialnej i przebiegiem różnicowania słoja drewna w pniu sosny w ciągu sezonu wegetacyjnego.