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NATURAL GROWTH SUBSTANCES AND WOOD DIFFERENTIATION IN BLUE PINE (*PINUS WALLICHIANA*, A.B. JACKS.).

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Abstract

Twenty-five trees selected in a large Blue pine stand in Burban Forest in Murree Forest Division were studied for xylem cell formation and seasonal variation in the amount of natural growth substances. Auxin extracted during different periods of the season showed significant correlation with the radial diameter of tracheid, but no significant correlation was found between the rate and duration of the radial enlargement of differentiating tracheid and the auxin level. There was no significant correlation in seasonal changes in natural auxin and cell wall thickness of tracheid. Significant correlation was found between cell wall thickness of tracheid and the duration of phase of maturation. Water soluble inhibitors showed significant correlation with the duration of phase of maturation.

Introduction

Literature dealing with the stimuli controlling the activity of cambial cells to change from large diameter thin walled cells (typical of earlywood) to comparatively narrow diameter thick walled cells (typical of latewood) in an annual ring is enormous. This change has been attributed to seasonal variation or to endogenous factors such as auxin and carbohydrate supply (Wershing & Bailey, 1942; Wareing, 1958; Larson, 1964; Richardson, 1964; Whitmore & Zahner, 1966; Balatinecz & Kennedy, 1968; Gordon & Larson, 1968). Interaction of various growth promoters in the control of xylem differentiation has also been reported (Brown, 1970; Torrey, *et al.*, 1971; Khalida & Mahmood, 1972).

Although the role of auxin in the differentiation of cambial derivatives to xylem elements is well established, it is not specific. Auxin failed to stimulate cambial division under dormancy (Reinders – Gouwentak, 1941). It can therefore be said that beside auxin some other growth regulating substances may also be involved. Wodzicki (1965) has shown that differentiation of annual ring in *Larix decidua* was due to interaction between growth promotors and inhibitors. The present paper describes the effect of interaction between natural growth promoting and growth inhibiting substances on wood differentiation in Blue pine.

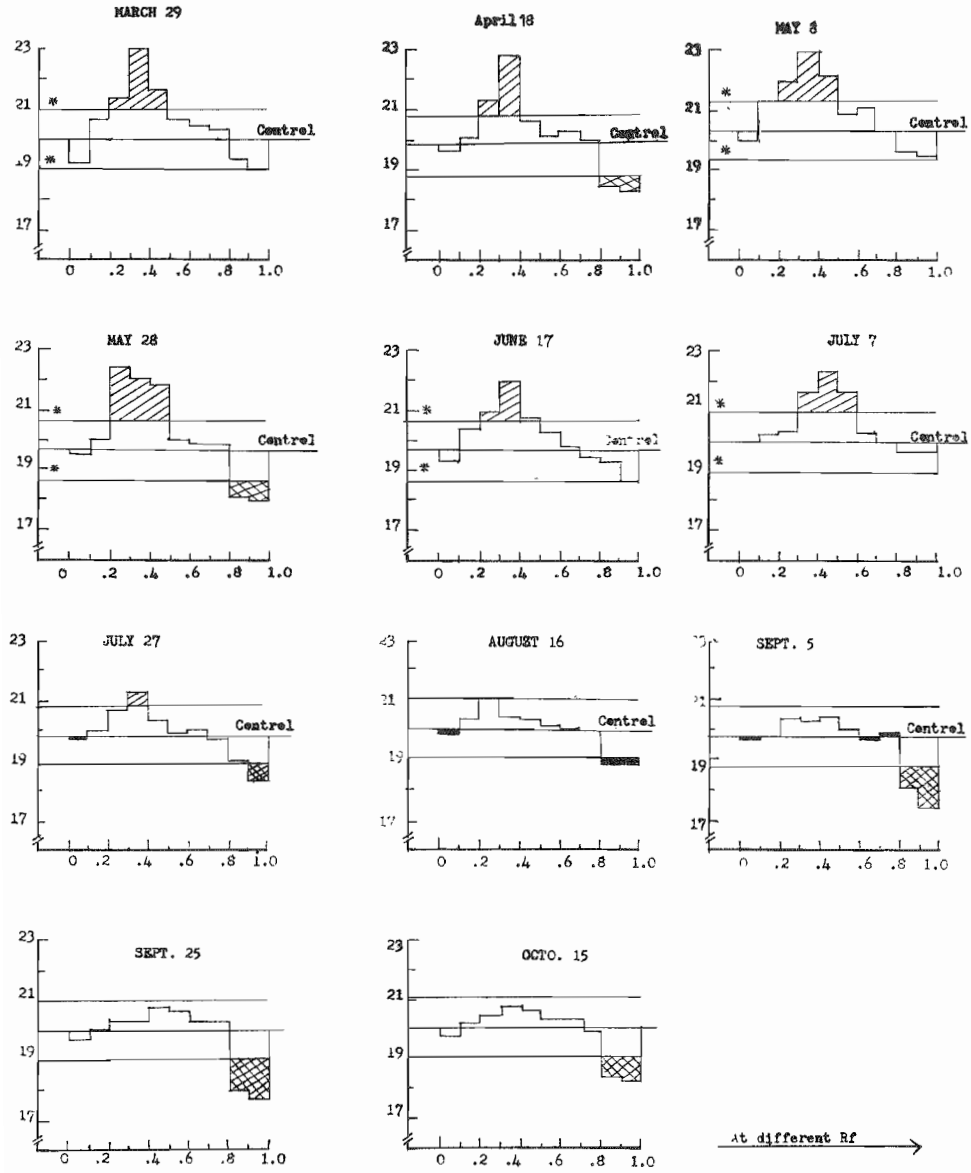

Fig. 1. Ether fraction of pine cortical tissues extract containing auxin. Average results of two replicate tests of the substances which migrated to all 20 Rf strips of chromatogram developed with isopropanol: 1, ammonia and water (10: 1, w/v). Tissues were extracted and bioassayed immediately or stored at dry ice temperature till extracted. Each disgram represents the growth response of wheat coleoptile sections to the concentration of 2.5 g fresh weight of tissue per ml.

*LSD 0.1% level.

Materials and Methods

Twenty five trees were selected from a large Blue pine stand in Burban Forest of Murree Forest Division. Samples were collected 11 times at 20 days interval during March–October growing season 1979. Samples were collected from the main trunk by the method of Newman (1956). Each sample consisted of a strip containing bark cambium and xylem. It was divided into two equal halves. One portion was used for anatomical investigation and the other half with xylem removed was used for bioassay. Extraction was done with absolute and 80% aqueous methanol by the method of Philips & Wareing (1958). Further separation of 80% aqueous methanol and ether fraction was done after Wodzick (1968). The aqueous and ether fraction concentrated to known volume was loaded separately on Whatman paper No. 3 and the chromatograms developed with isopropanol, ammonia (sp. gr. 880) and water (1: 1: V/V) by descending method to 26 cm. in darkness at 27 ± 1°C. Rf strips of chromatograms were eluted with a mixture of phosphate buffer (pH 5.6) and 20% sucrose solution for 20 h at constant temperature immediately before assay. Wheat coleoptiles cv. Pak. 70, 10 mm long were used for straight growth test as described by Bentley & Housley (1954). Tests were carried out in duplicate.

Results

a) *Ether soluble substances extracted from cortical tissues:*

Substances stimulating the growth of wheat coleoptile section found in the ether fraction of the cortical tissues are presented in Fig. 1. The contents of promoters varied between Rf 0.3 to 0.5 throughout the vegetative season with significant increase in March the beginning of radial growth. It remained highly significant till early July and became insignificant in the samples collected in the middle of August. It again increased in the middle of September but dropped to insignificant level at the end of the season. The contents of inhibitors at Rf 0.9 to 1.0 which are known to be due to the accumulation of resinous substances (Wodzicki, 1969), were also found in all the samples of ether fraction. Its significant increase was first observed in the samples collected in early April which remained insignificant till early May but increased to significant level towards the end of May. It became insignificant from early July till the middle of October. The displacement of auxin level from 0.3 to 0.5 could be due to slight variation in chromatographic conditions.

b) *Water soluble substances extracted from cortical tissues:*

The results presented in Fig. 2 reveal that the amount or activity of growth inhibiting substances found in aqueous fraction of cortical tissue varied throughout the vege-

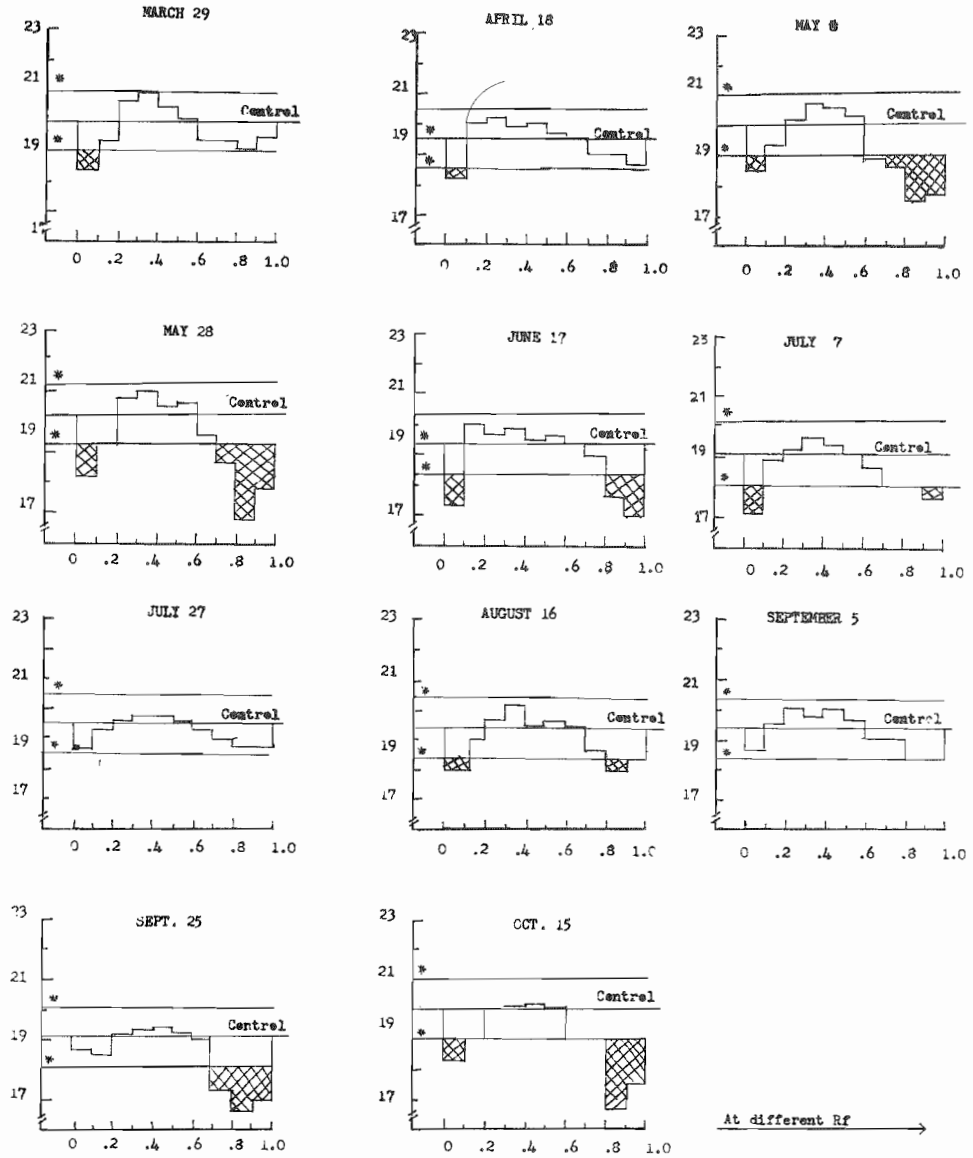

Fig. 2. Aqueous fraction of pine cortical tissues extract containing inhibitors. Average results of chromatograms developed with isopropanol, ammonia and water (10: 1, v/v. Tissues were extracted immediately after collection or stored at dry ice temperature till extracted. Extract bioassayed immediately after collection.

*LSD at 0.1% level.

tative season and were located at Rf 0.9 to 1.0. It was low at the beginning of the radial growth, i.e., end of March till the middle of April, and then increased significantly in the month of May till the middle of June. In early July it passed the level of least significant difference (LSD 0.001) and suddenly dropped at the end of July. In the sample collected in the middle of August and early September it again showed an increase and continued to show significant increase from the end of September till the middle of October (end of the season).

No significant increase in growth regulating substances was observed in aqueous fraction throughout the vegetative season except at the start of the season's growth, where it reached the level of significance ($p = 0.001$). In few cases there was displacement of the inhibitors from their particular Rf position which may probably be due to the slight variation in chromatographic conditions.

Discussion

The occurrence of auxin (promotors) which is presumed to be IAA (Wodzicki, 1968) showed significant correlation with the radial diameter of tracheid at all Rf values from 0.3 to 0.5. The level of auxin remained significantly high till the middle of August, the period during which earlywood was continuously being formed. However, auxin level dropped in early September till the end of the season which correlates with the initiation of latewood cells. As IAA is known to affect cell division and cell enlargement of newly differentiated tracheids (Balatinecz & Kennedy, 1968), it is reasonable to hypothesise that auxin may also be responsible for regulation of cell division and cell enlargement in the Blue pine. Since no significant correlation was found between seasonal changes in natural auxin and cell wall thickness, interpretation of cell wall thickness on the basis of auxin hypothesis is ruled out. However, a significant correlation was found between auxin level and radial diameter of tracheid. This is in agreement with the hypothesis of earlier workers (Larson, 1964; Denne & Wilson, 1977). As the prolongation of the phase of maturation results in the formation of thick walled tracheids (Wodzicki, 1969), it is natural that any factor which prolongs the phase of maturation may be responsible for transition of thin walled earlywood type of cells to thick walled latewood type of cells. In this regard a significant correlation was found between the cell wall thickness of tracheid and the duration of the phase of maturation.

In the present study water soluble inhibitors were found to have a significant correlation with the duration of phase of maturation which is in accordance with the finding of Wodzicki (1965), who correlated cell wall thickness with water soluble inhibitors present in the cortical tissues. It seems likely that seasonal variation in the duration of the phase of maturation which in turn is affected by water soluble inhibitors is responsible for the transition of thin walled earlywood tracheid to thick walled latewood tra-

cheid in *P. wallichiana*. This conclusion substantiates the findings of earlier workers (Wodzicki, 1969; Skene, 1969; Denne & Wilson, 1977).

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