

SEASONAL CHANGES IN LEVELS OF INDOLE-ACETIC ACID AND ABSCISIC ACID IN STEM TISSUES OF *PINUS RADIATA*

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ABSTRACT

Stem tissue samples from young radiata pine (*Pinus radiata* D. Don) trees growing in the forest were collected at intervals throughout one annual growth cycle, and the amounts of indole-3-acetic acid (IAA) and abscisic acid (ABA) measured. The amount of IAA increased in late winter but was otherwise not well correlated with cambial growth patterns. ABA content fluctuated during the summer in apparent response to seasonal moisture stress and showed some correlation with cambial growth.

INTRODUCTION

According to the auxin theory of cambial growth, renewed cambial activity in spring is initiated by auxin produced in the expanding portion of the crown and transported to the stem (Wareing, 1958; Larson, 1962). After growth initiation, when auxin synthesis is high, large-diameter cells of the earlywood type are produced. A transition to narrow-diameter cells occurs when auxin synthesis declines. A gradient of decreasing cell size down the stem parallels a gradient of decreasing endogenous or applied auxin (Larson, 1969).

Growth substances other than auxin can influence cambial activity. Applied, naturally occurring inhibitors can modify cambial activity, e.g., cuttings of *Picea glauca* (Moench) Voss treated with abscisic acid (ABA) produced fewer and narrower tracheids (Little and Eidt, 1970). Inhibitors supplied in media to explants from conifer stems reduced cambial division (Bonga and Clark, 1965; Brown and Wodzicki, 1969). Balatinez and Kennedy (1968) found that two naturally occurring ether-soluble inhibitors induced a change from earlywood to latewood formation when applied to actively growing larch seedlings.

A controlling role for inhibitors is also indicated by changing inhibitor quantities associated with cell development patterns. Wodzicki (1965), working with young larch trees, reported that a water-soluble inhibitor in cortical tissues increased to a peak at the time of first latewood formation towards the end of the growing season. The inhibitor values then decreased. Balatinez and Kennedy (1968) found an ether-soluble

inhibitor in larch tree stems which increased at the time of latewood formation and was most abundant after cell division. A second ether-soluble inhibitor, similar in Rf to ABA, was found in the foliage and showed a similar increase during latewood formation.

Two important growth substances have been characterised in radiata pine (*Pinus radiata* D. Don) stem extracts: indole-3-acetic acid (IAA) (Shepherd and Rowan, 1967) and ABA (Jenkins and Shepherd, 1972). The former authors found that in late winter (23 August) IAA concentration was greater at the top of the stem than at mid-stem or stem-base, but that the level was high at all three stem heights in spring (8 October). In the present work, stem extracts from young trees growing in the forest were taken at regular intervals throughout a year and both IAA and ABA content measured.

METHODS

Tissue was collected from pine trees in Canberra, Australia, at 2-weekly intervals from 21 July 1969 to 31 March 1970, then at 4-weekly intervals until 21 July 1970. Fifty, 5-year-old, naturally regenerated trees of similar size (about 3 m tall) and apparent vigour were harvested for each collection. Ten-centimetre segments were cut from the main stem formed in the 1968-69 growing season and at least 120 cm below the apices. Tissues external to the wood were peeled off and placed in ice cold methanol, and the stemwood was rinsed in fresh methanol.

The tissues and all methanol washings were combined to minimise the possible effects of tree-to-tree variations. The tissues were homogenised with a blender, stored overnight at 4°C, strained through cheese cloth, rinsed with fresh solvent, strained again, then filtered through Whatman No. 541 paper.

The extract was evaporated to the aqueous phase and centrifuged at 3000 rpm. A diethyl ether partitioning schedule was used (Jenkins and Shepherd, 1972) and acidic ether extracts were paper chromatographed on Whatman 3 MM paper, using isopropanol ammonia water (10 : 1 : 1) as the solvent. ABA and IAA zones from paper chromatograms were eluted separately and applied to paper electrophoresis strips (Whatman 3 MM). High-voltage electrophoresis using ammonium acetate buffer (0.05 M pH 7.5) was conducted for 50 minutes at 2200 V and 80 mA on a Shandon L-24 electrophoresis unit. Marker strips were included with each run to indicate the probable position of either IAA or ABA on electrophoretograms loaded with samples.

IAA was assayed by a fluorimetric method (Burnett and Audus, 1964; Shepherd and Rowan, 1967). A 2-cm segment centred on the probable position of IAA was eluted by shaking in buffer (5 ml citrate phosphate buffer pH 5.4) for 30 minutes. Other 2-cm segments were taken from each side of the first segment and treated similarly to ensure that all the IAA on the electrophoretogram was eluted. Duplicate electrophoretograms were assayed for IAA. Fluorescence intensities of these solutions were measured with a Farrand spectrophotofluorimeter Mark 1. The excitation/emission wavelengths of 283/360 μm gave maximum fluorescence for IAA. Fluorescence intensity at this setting was read immediately to avoid the effects of IAA photodecomposition (Shepherd, 1965). The amount of IAA present in each sample was calculated from a standard curve.

For determination of ABA, sections of electrophoretograms, 5-cm wide and centred on the distance run by ABA on a marker electrophoretogram, were eluted in buffer

(phosphate citrate buffer pH 5.4) and bioassayed using the wheat coleoptile bioassay (Nitsch and Nitsch, 1956). Aliquots of 1 ml were transferred to small test tubes and four coleoptile sections added to each tube for incubation at 26°C for 20 hours. Four replicates of each collection were included in each bioassay. The mean length of coleoptiles in the test solutions was expressed as a percentage of control. A standard response curve was obtained using test solutions of authentic ABA. A large number of collections was included in each bioassay and the conclusions reached are based on three repeat bioassays of the purified extracts.

IAA and ABA levels represent relative concentrations present in the tissues before extraction, as the levels were not adjusted for losses known to occur in purification of extracts (Shepherd, 1965; West, 1969). Synthetic ABA used was a 50/50 mixture of *cis*, *trans* and *trans*, *trans* isomers.

The experimental site was between two weather stations 5 km apart. Weather data collected on the site showed close correlation with recordings of the Watershed Research Unit of the Forestry and Timber Bureau, Canberra for the two stations.

RESULTS

The seasonal pattern of IAA concentration (Fig. 1) shows that:

1. Estimated IAA content increased in late winter, from 21 July to 18 August in 1969 and from 22 June to 21 July in 1970;
2. During the growing season IAA content fluctuated between consecutive collections to the end of March;
3. The midsummer collection on 2 February had the greatest IAA content;
4. In autumn and winter, from 31 March to 22 June, IAA content was uniform though not markedly lower than at other times.

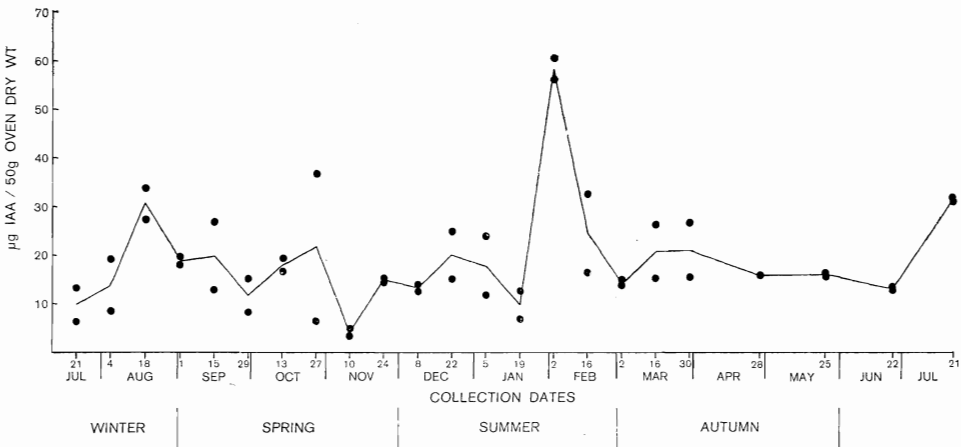


FIG. 1—Estimates of IAA levels in stem tissue collected over a full year from young radiata pine.

The seasonal pattern of ABA concentration (Fig. 2) shows that:

1. ABA levels increased in late winter and early spring (August and September), then fluctuated up until December (early summer);
2. From midsummer to early autumn ABA content fluctuated, e.g., small content on 5 January, 16 February, 30 March and pronounced peaks on 2 February and 17 March;
3. ABA decreased steadily from the end of March to low values in late autumn and winter.

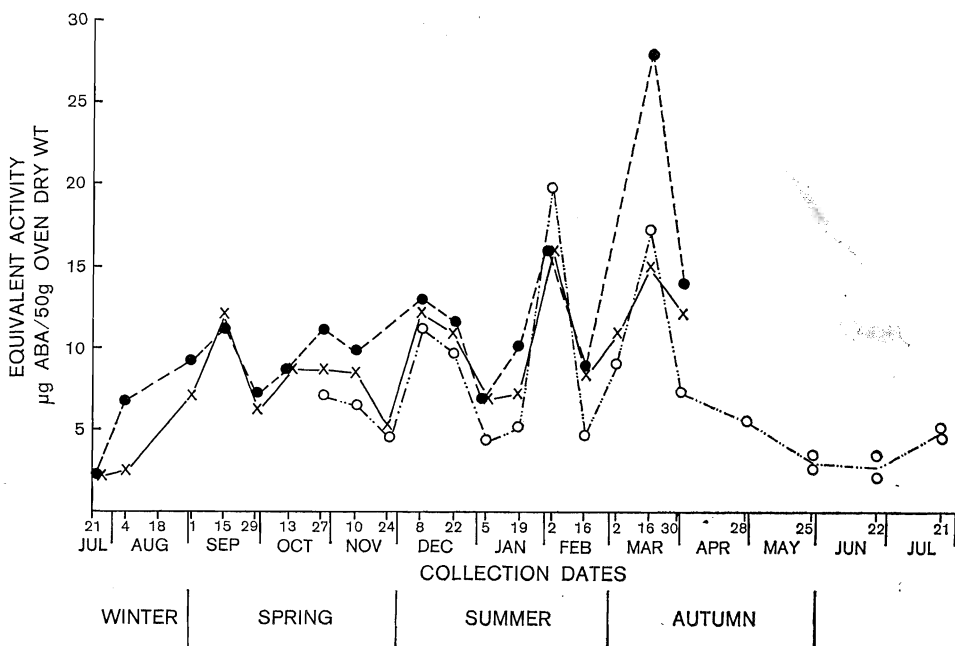


FIG. 2—Estimates of ABA levels in stem tissue collected over a full year from young radiata pine. Each point represents an estimate. Estimates were made from three different assays represented by the three lines shown.

DISCUSSION

Many tree species have only small concentrations of IAA during winter. In radiata pine, however, IAA synthesis and use apparently continue in winter and concentrations are not markedly less than at other times. Radiata pine has a remarkable capacity for active growth, given suitable light, temperature, and moisture conditions. The continued presence of IAA in winter supports a theory of growth quiescence, probably because of only moderately low ambient temperatures (usually between 0° and 12°C), rather than the forms of physiological dormancy known in many other trees of the temperate zone. Lack of physiological dormancy of young radiata pine in Rotorua, New Zealand has been shown by continued cell division in winter, with differentiation of the derivative cells to phloem rather than xylem (Barnett, 1971). In southern Australia, cell wall

deposition in the xylem was observed up to about 10 days before renewed active division (late July) in 15-year-old radiata pine (Skene, 1969).

The amount of IAA in stem tissue of young radiata pine increased in early August 1969 and late July 1970, when temperatures were close to mid-winter values (Fig. 3). A similar increase in ABA values lagged behind the IAA increase by 1 or 2 weeks. No significant increase in mean daily temperature occurred until mid-August in both years. Later studies of cambial activity in the same area (Shepherd and Drielsma, unpubl. data) have shown that renewed cambial activity in 17-year-old radiata pine occurred at the same time of year as the rises in IAA level shown in this study.

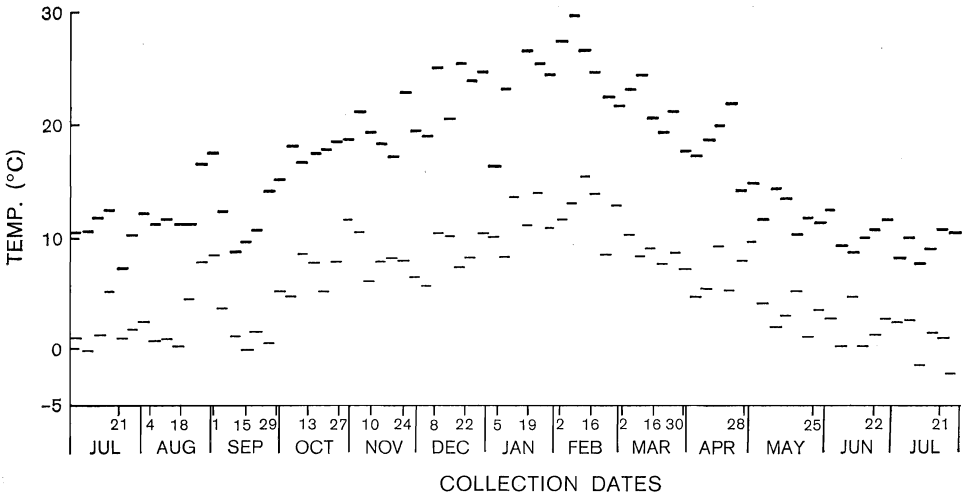


FIG. 3—Five-day averages of maximum and minimum temperatures (°C) recorded for the period of the collections (1969/1970).

Such increases in auxin content in spring have been observed previously and are believed to initiate the return to active cambial division (Wareing, 1958). Bonga and Clark (1963) and Balatinez and Kennedy (1968) observed increased IAA content in conifer cambial extracts at about the commencement of cambial division. However, the initiation of cambial activity could be triggered by other growth substances not measured in this and other studies, e.g., cytokinins are known to occur in radiata pine stem extracts (Jenkins, 1971) and gibberellins have been found in other conifers (e.g., Ruddat *et al.*, 1968).

A marked fluctuation in IAA content occurred in midsummer, as on 19 January the concentration was small but on 2 February was at the greatest value recorded during the year. This large concentration occurred over only a short period associated with high temperatures, little rain (Fig. 4), and very low air humidity. These conditions would have markedly reduced cambial activity (Shepherd, 1964). By 16 February, the IAA content had decreased with a return to conditions more favourable to growth after heavy rain and reduced temperatures.

These observations are contrary to the generally accepted concepts of the auxin

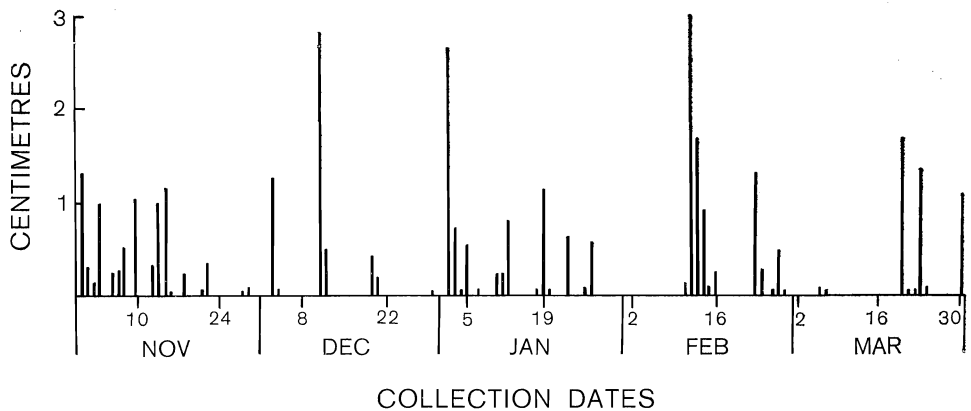


FIG. 4—Recorded rainfall for the summer months of 1969/1970.

theory of cambial control. There is little published work on the effects of moisture stress on IAA concentrations in trees. Hatcher (1959) assayed diffusible IAA in apple shoots in 2 successive years. In the first year, when 125 mm of rain fell in August, growth continued throughout the northern summer and auxin level declined steadily during the growing season. However, in the second year auxin increased in the lower part of the young shoot during "a completely rainless August" which caused "a marked interruption to growth". As in the present study, increased auxin concentration was associated with a dry spell and not with active growth.

Other studies have given variable results. In young trees of *Pinus resinosa* Ait., IAA decreased during drought and recovered partially after watering (Larson, 1963). Unpublished work on droughted and irrigated *P. resinosa* trees did not confirm these findings (see Whitmore and Zahner, 1966). There was no significant decrease in auxin concentrations in the droughted trees despite growth differences. Shepherd (1965) sampled radiata pine seedlings growing in pots in a phytotron after cessation of watering. With progressive reduction of soil moisture, IAA concentrations were less than in unstressed controls on the first day, greater on the third and fourth days, and less again on the sixth and eighth days.

Mer (1969), in analysing plant-growth relationships, postulated that extractable IAA represents the balance between synthesis and utilisation, so that the concentration may not be directly related to growth. This interpretation could explain the observed trends in IAA content in the present study and other published results. The sequence of events could be as follows:

- (i) During winter both synthesis and use of IAA are small. Any variations would have only slight effects on concentrations because turnover of IAA is low.
- (ii) In late winter IAA synthesis increases in the expanding apices. The IAA stimulus moves to the stem but use here remains small, so therefore stem IAA content increases.
- (iii) With the initiation of cambial growth, due either to increased IAA or to

some other cause, IAA use increases as the accumulated IAA is used in growth.

- (iv) A balance is reached between IAA synthesis and production, with a large turnover of IAA during active growth.
- (v) The variations in IAA values during the growing season could reflect independent changes in either rates of synthesis or use of IAA. For example, the large auxin value of 2 February could result from a rapid decline in growth due to moisture stress and a similar decline in the use of IAA.

The observed changes in ABA are more closely correlated with seasonal temperature and rainfall patterns. A general increase in ABA content occurred as temperatures rose and rainfall became inadequate for continued growth. The sharp decrease in ABA content on 5 January, 16 February, and 30 March corresponded with rainfall ending short drought periods (Fig. 4). After 30 March, the ABA content remained small, declining steadily during late autumn and winter.

The increased ABA content may have been a response to severe moisture stress in the trees, as this has been established for other plants (e.g., Wright and Hiron, 1969; Zeevaart, 1971; Hoad, 1973). The moisture stress could be due to soil moisture deficits, high atmospheric temperatures, and low humidity, or a combination of these circumstances.

Moisture stress is known to be associated with increased wood density (Zahner and Oliver, 1962; Zahner *et al.*, 1964; Shepherd, 1964; McKinnell and Shepherd, 1971). In the present study, rainfall interrupted the hot, dry summer weather on three occasions. These conditions produced minor bands of wood of contrasting density within the annual ring and were associated with increases and marked decreases in the ABA content as noted above.

Results in this study reveal an association, during summer, between moisture stress, ABA content, and the pattern of wood production. Recently, ABA applications to radiata pine seedlings have produced changes in xylem cell dimensions and production similar to drought-induced changes indicated in Fig. 5 (Jenkins, 1973). The evidence and the results from other studies cited suggest that, at times of severe moisture stress, ABA is a growth substance with a major influence on cambial activity.

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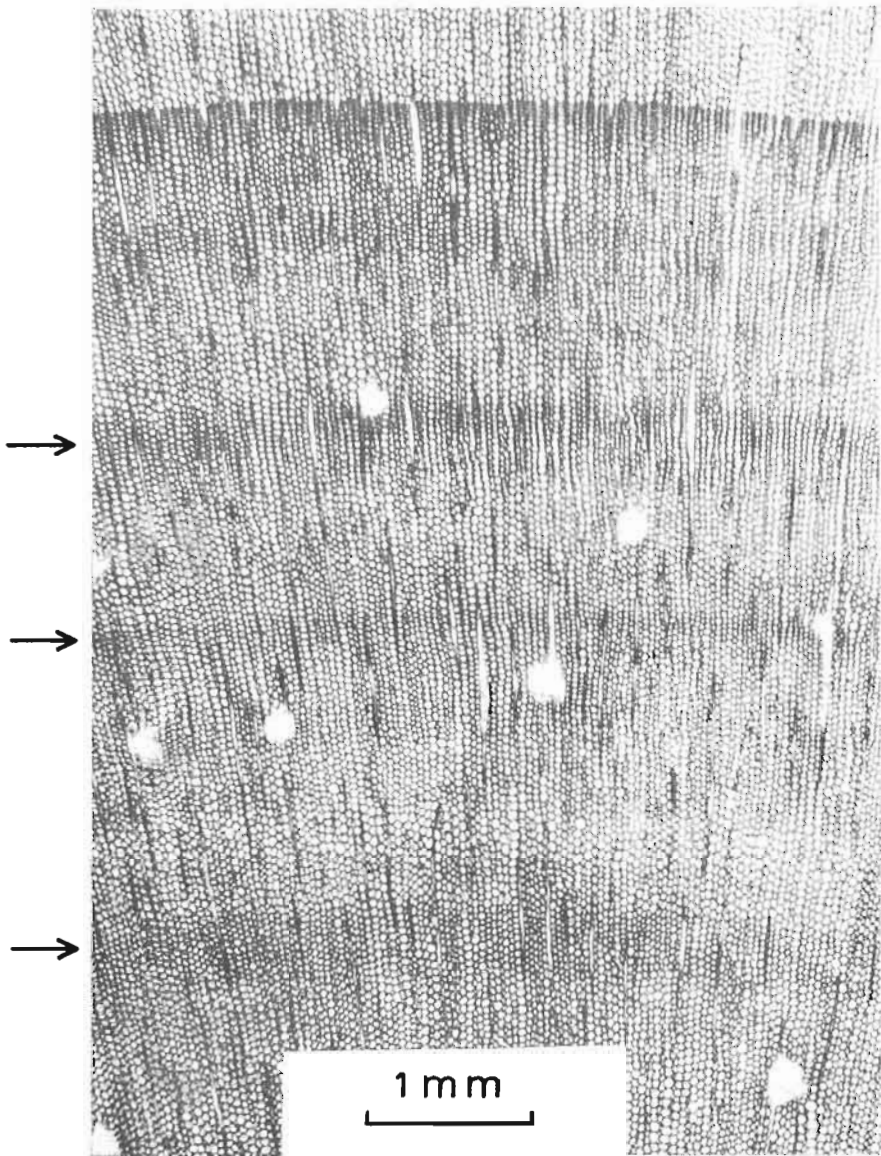


FIG. 5.—Transverse section of a stem showing the second half of the annual ring of wood formed at the sampling position in 1969/1970. Three false rings of denser wood (arrowed) are discernible before the final latewood band.

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