

**THE EFFECT OF APPLIED GROWTH HORMONES ON CELL PROLIFERATION IN THE CAMBIAL ZONE OF *THUJA ORIENTALIS* L.**

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**Abstract**

The effect of IAA, GA and Kinetin on cell division in the cambial zone of *Thuja orientalis* L. was studied. IAA was more effective than either GA or Kinetin when used alone. Interaction of growth substances in the combinations used have shown a greater tendency towards cell divisions for the first 15 days of the experimental period. Between 15-30 days period, number of cells decreased in all experiments except where IAA and GA were used together in culture. Maximum number of cells were produced when IAA, GA and Kinetin were used together.

Cell division in the cambial zone responded more to growth hormones used in tissue cultures than applied in lanolin. This has been related to better physical conditions prevailing in cultures.

**Introduction**

A number of workers have reported that cambium is activated as a result of indole-3-acetic acid (IAA) application (Wareing, 1958b; Wareing et al 1964; Belatinecz & Farrar, 1966; Digby & Wareing, 1966a; Digby & Wareing, 1966c; Torrey & Loomis, 1967). Gibberellic acid, (GA) also activates cell division (Lang, 1956; Arney & Ovenden 1965; Digby & Wareing, 1966a; Digby & Wareing, 1966c). Digby & Wareing (1966c) have shown that additive effect of IAA and GA is much more than either IAA or GA alone.

There have been contradictory reports regarding the role of Kinetin. Wareing et. al (1964) have shown that application of Kinetin alone has no promotive effect upon cambial divisions, whereas Torrey & Loomis (1967) noticed proliferation of cambium in the presence of Kinetin. Digby & Wareing (1966c) have also reported promotion of cell divisions in the presence of Kinetin. They found that effects of both GA and Kinetin are additive to those of IAA but the effects of GA and Kinetin are not additive.

A good deal of literature suggests that the concentration, period of treatment and mode of application of growth hormones are important factors which control cell division response in explants or rooted plants. In the present investigation therefore the effects of IAA, GA and Kinetin alone as well as in combinations on cell division of *Thuja orientalis* L. were studied. Different concentrations of hormones were used. The growth hormones were used in cultures as well as in lanolin for 15 and 30 days. No attempt was made to study tissue differentiation.

## Materials and Methods

Lanolin experiments were performed on internodes of branches of living trees. Growth substances were applied in the scar made on the selected internodes. Scars were covered with black paper. Controls were treated with lanolin only.

For tissue culture experiments approximately 3 cm. long pieces of cambial tissue with a thin strip of xylem and phloem on either side, were obtained. This cambial tissue was cut into small pieces of 10 x 4mm; surface sterilized with 0.1% mercuric chloride solution for 30 seconds, washed with three changes of sterile distilled water and transferred to the agar medium under sterile conditions.

Medium was prepared following Earle & Torrey (1965) which contained 8% sucrose and 0.7% agar adjusted to pH5.4 and autoclaved at 15 lbs. pressure for 30 minutes. Amino acids, vitamins and growth hormones were seitz filtered and then added to this medium. Control contained mineral nutrients, amino acids, vitamins, sugar and agar but lacked growth hormones.

Nutrient supplemented medium was poured into 30 ml culture tubes. Inoculated tubes were kept in dark sterile chamber at 25° C. After intervals of 15 days and 30 days tissue pieces from each set were taken and fixed in F.A.A. (3: 6: 91 by volume). Sectioning was done at 30 $\mu$  by clamping tissue pieces on a sliding microtome holder. Sections were dehydrated through ethanol series, stained with safranin and light green and mounted in canada balsam.

## Observations and Results

For each treatment number of cells per row in the cambial zone was counted for 100 rows. Following Newman (1956) and Mahmood (1968) the initials, dividing and enlarging cells in each row were included in the cambial zone. Mean cell number was calculated as percent of control and was plotted against concentration.

### I. GROWTH SUBSTANCES APPLIED IN LANOLIN

Effect of IAA on cell division:

Fig. 1 shows that IAA promoted cell division in the cambial zone. Mean cell number was maximum at 2.5 ppm upto 15 days. This cell number decreased with the increase in IAA concentration. In 30 days experiment cell number also reached optimum at 2.5 ppm, decreased at 5ppm but remained more or less the same between 5-10 ppm.

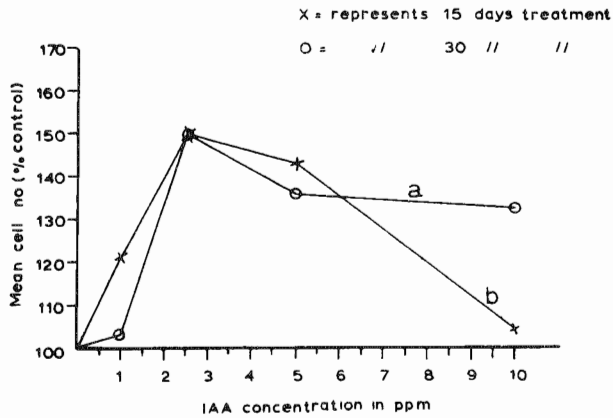


Fig. 1. The effect of IAA in lanolin on cell division in the cambial zone of *Thuja orientalis*.

Effect of GA on cell division:

GA promoted cell division at all concentrations except 50 ppm used for 15 days. Maximum number of cells was found at 25 ppm (fig. 2). Cell divisions continued for more than 15 days in the 30 days experiment.

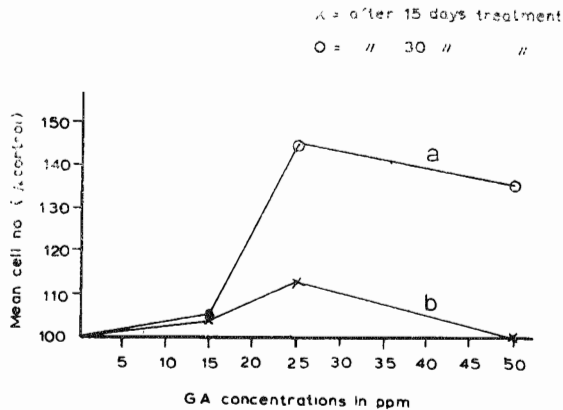


Fig. 2. The effect of GA in lanolin on cell division in the cambial zone of *Thuja orientalis*.

Effect of Kinetin on cell division:

Kinetin promoted cell division (fig. 3). Maximum number of cells was found at 2.5 ppm. At higher concentrations number of cells decreased.

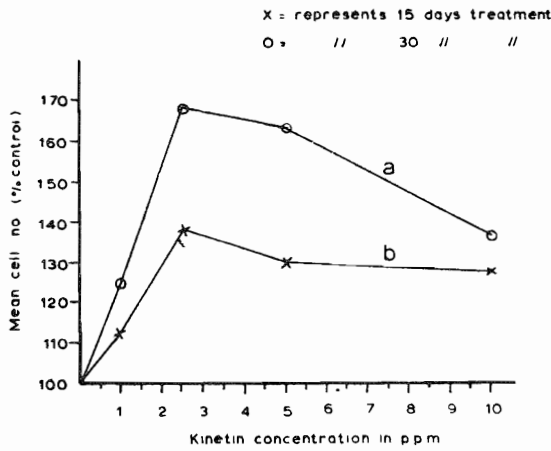


Fig. 3. The effect of Kinetin in lanolin on cell division in the cambial zone of *Thuja orientalis*.

Effect of IAA and GA on cell division:

Fig. 4 shows that mean cell number is more when IAA at 1 ppm (fig. 1) was used alone but is less when GA was used alone at 25 ppm (fig. 2).

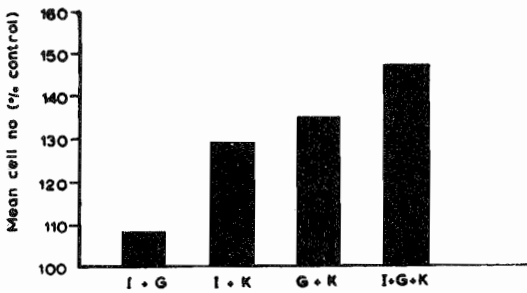


Fig. 4. The interaction of IAA, GA and Kinetin in lanolin on cell division in the cambial zone of *Thuja orientalis* noted after 15 days.

I=IAA 0.75 ppm.  
 G=GA 25 ppm.  
 K=Kinetin 2.5 ppm.

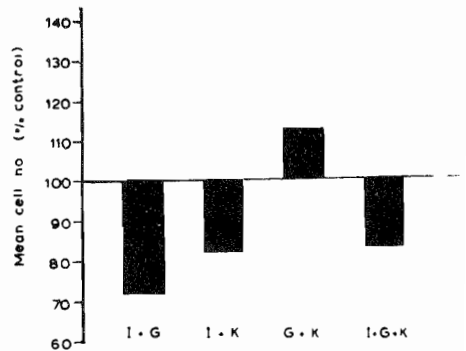


Fig. 5. The interaction of IAA, GA and Kinetin in lanolin on cell division in the cambial zone of *Thuja orientalis* noted after 30 days.

I=IAA 0.75 ppm.  
 G=GA 25 ppm.  
 K=Kinetin 2.5 ppm.

Effect of IAA and Kinetin on cell division:

Kinetin with IAA promoted cell divisions upto 15 days of treatment (fig. 4). Between 15-30 days there was marked decrease in cell number and at the end of 30 days cell number in the cambial zone was less than the control (fig. 5).

Effect of GA and Kinetin on cell division:

GA with Kinetin induced more cell divisions in the cambial zone than IAA and Kinetin (fig. 4). The cell number after 30 days was more than the control, (fig. 5) but less than 15 days treatment (fig. 4).

Effect of IAA, GA and Kinetin on cell division:

IAA, GA and Kinetin produced maximum number of cells in the cambial zone. Results of 15 days treatment (fig. 4) show that mean cell number was greater than when IAA; GA or Kinetin were used alone or in combinations of two. Between 15-30 days there was decrease in cell number (fig. 5).

## II. TISSUE CULTURE STUDIES

Effect of IAA on cell division:

Fig. 6 shows that IAA promoted cell divisions. Optimum number of cells were recorded at 5 ppm. At 10 ppm cell number decreased. This decrease was very rapid and in 15 days treatment the number of cells was slightly less than the control.

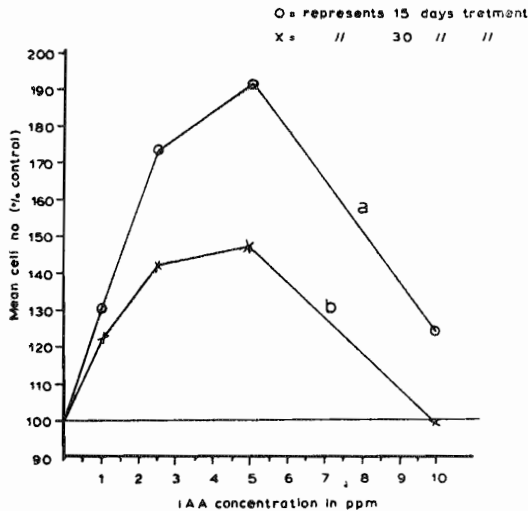


Fig. 6. The effect of IAA on cell division in the cambial zone of *Thuja orientalis* grown in culture.

## Effect of GA on cell division:

There was an increase in mean cell number. Maximum cell divisions occurred at 25 ppm. At 50 ppm cell number declined sharply. Cell divisions increased from 15-25 ppm but decreased at 50 ppm (fig. 7).

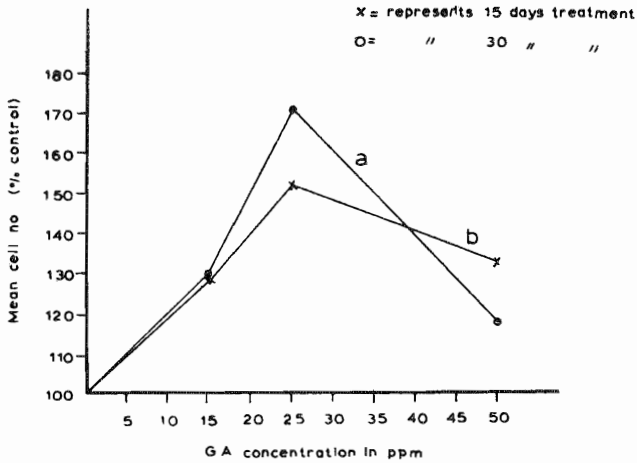


Fig. 7. The effect of GA on cell division in the cambial zone of *Thuja orientalis* grown in culture.

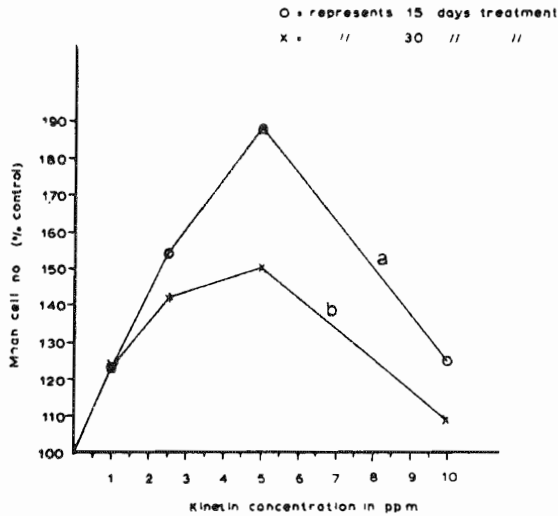


Fig. 8. The effect of Kinetin on cell division in the cambial zone of *Thuja orientalis* grown in culture.

## Effect of Kinetin on cell division:

Fig. 8 shows that Kinetin promoted cell divisions in the cambial zone. Number of cells increased from 1-5 ppm. Maximum number of cells was found at 5 ppm. Between 5-10 ppm cell number decreased sharply. In 30 days experiment there was a slight increase in cell number at 1 ppm, reached to a maximum at 5 ppm, then decreased (fig. 8).

## Effect of IAA and GA on cell division:

IAA with GA increased cell divisions in the cambial zone. Up to 15 days of treatment cell number was less than GA alone at 25 ppm. (fig. 7). Between 15-30 days mean cell number increased markedly (fig. 10). This cell number was greater than induced by GA alone at 25 ppm for 30 days (fig. 7).

## Effect of IAA and Kinetin on cell division:

Kinetin with IAA promoted cell divisions upto 15 days (fig. 9). Between 15-30 days cell number decreased markedly (fig. 10).

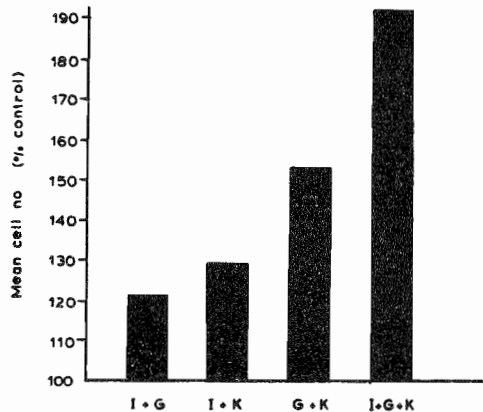


Fig. 9. The interaction of IAA, GA and Kinetin on cell division in the cambial zone of *Thuja orientalis* grown in culture for 15 days.

I=IAA 0.75 ppm.  
G=GA 25 ppm.  
K=Kinetin 2.5 ppm.

## Effect of GA and Kinetin on cell division:

GA with Kinetin induced more cell divisions than IAA and Kinetin (figs. 9 and 10). Cell number decreased between 15-30 days.

Effect of IAA, GA and Kinetin on cell division:

IAA, GA and Kinetin increased the proliferative activity of cambial zone markedly. Maximum cell number was recorded after 15 days treatment (fig.9). Between 15-30 days cell number decreased (fig. 10).

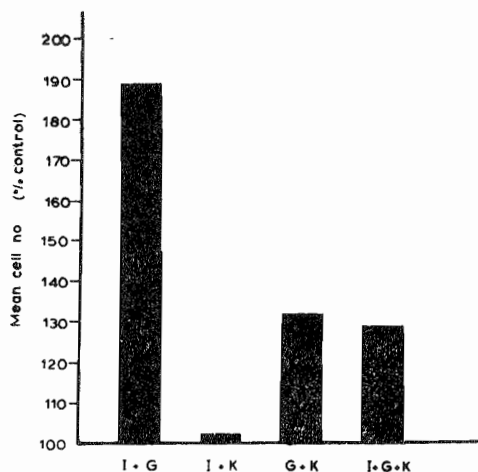


Fig. 10. The interaction of IAA, GA and Kinetin on cell division in the cambial zone of *Thuja orientalis* grown in culture for 30 days.

I=IAA 0.75 ppm.  
G= GA 25 ppm.  
K=Kinetin 2.5 ppm.

## Discussion

The results of IAA application confirm the findings of a number of workers that IAA promotes cell division (Wareing, 1958b; Clutter, 1960; Digby & Wareing, 1966a; Digby & Wareing 1966c and Torrey & Loomis, 1967).

Rate of cell division in the cambial zone of *Thuja orientalis* was greater upto 15 days when IAA was applied in lanolin or used in culture. Between 15-30 days period rate of cell divisions decreased (figs. 1 and 6).

GA promoted cell divisions except at 50 ppm in culture medium (fig. 7). Increase in cell number in the cambial region of *Populus robusta* has been reported by Wareing et. al (1964), and Bostrack & Struckmeyer (1967) in the cambial region of *Coleus blumei* and *Salvia splendens* as a result of GA application. Digby & Wareing (1966c), have reported an increase in the number of cambial cells in liquid suspension cultures



of *Acer pseudoplatanus* supplied with GA. They found that GA promoted cell divisions upto 50 ppm.

Kinetin showed varied responses in culture and lanolin. In culture experiments number of cambial cells increased upto 15 days and then decreased (fig. 8). In lanolin experiments on the other hand increase in cell number was noticed upto 30 days (fig. 3). These results are at variance with the results of Digby & Wareing (1964) in *Acer pseudoplatanus* and *Populus robusta*. They applied Kinetin at 1,000 ppm which is supraoptimal concentration. This could have inhibited cell divisions. Sorokin *et al* (1962) found that in pea internodes Kinetin induced a more active cambial development than IAA. Torrey & Loomis (1967) provided Kinetin to the cut roots of *Raphanus sativus* grown in sterile culture and observed proliferation of vascular cambium. In the absence of Kinetin cell divisions did not occur. Digby & Wareing (1966c) observed in *Acer pseudoplatanus* grown in liquid suspension cultures a gradual increase in cell divisions when Kinetin concentration was raised from zero to 10 ppm. The optimum response was found at 5 ppm.

IAA with GA increased cell division response. Upto 15 days of treatment rate of cell divisions was low. It increased markedly between 15-30 days in culture medium but decreased in lanolin experiments. These results lend support to the hypothesis that in the presence of IAA and GA tissues are capable of synthesizing some growth factors which increase growth response after a period of initial slow growth. In lanolin experiments very few new cells were formed with IAA and GA upto 15 days (fig. 4). Between 15-30 days cell number was even less than the control.

Kinetin used with IAA or with GA whether in culture or applied in lanolin promoted cell divisions upto 15 days of treatment (figs. 4 and 9). After 15 days decrease in cell number was observed (figs. 5 and 10). It can be said therefore that interaction of Kinetin with IAA or with GA is not inhibitory to cell divisions. A slightly inhibitory effect of IAA and Kinetin in lanolin, is reported on shoots of *Acer pseudoplatanus* by Wareing *et al* (1964). Kinetin stimulated cell divisions when used with IAA or GA but its additive effect was more with GA than IAA.

IAA, GA and Kinetin together produced maximum number of cells in the cambial zone upto 15 days. This cell number was greater than the number of new cells obtained as a response to any single growth substance or any two growth substances used for the same time period. These results support the findings of Digby & Wareing (1966c).

In the present study, response to cell divisions was more in culture experiments than in lanolin experiments though concentration and period of treatment of growth hormones in both sets of experiments was the same. This discrepancy in the activity

of the cambial zone, can be explained on many grounds. The first important factor may be the diffusion of growth hormones in rooted plants. Since a rooted plant is expected to undergo active physiological processes, a part of applied growth hormones might be translocated from the place of their application, thus decreasing their concentration at the place of application. Secondly in culture, tissues were receiving all essential minerals, amino acids and vitamins in the optimum concentrations. The third important factor was the temperature. Lanolin and culture experiments were performed during May-September, 1969. Tissue cultures were maintained at 25°C throughout the experimental period. Growth substances were applied in lanolin to the living trees of *Thuja orientalis* during May-September, 1969. Mean monthly temperature for these months was 86.4, 87.4, 86.8, 82.5, 82.7°F. respectively. The high temperature of summer months could effect the vital physiological processes of the plant, including diffusion of growth substances.

### Acknowledgements

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