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The Effect of Some Growth Substances
on the Cell Wall Structure

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THE EFFECT OF SOME GROWTH SUBSTANCES ON THE CELL WALL STRUCTURE

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INTRODUCTION

The variability of the cell-wall structure in secondary xylem in a tree stem depends partly on the ontogenetic stage of its development and partly on the environmental conditions under which the individual has been growing. There is plenty of information in the literature about the phaenotypic variability of cell-wall structure, but much less is known about the causal relationships of this variability (V.NEČESANÝ, 1961, V.NEČESANÝ and CETLOVÁ, in litt.).

The number of variable features of cell-wall structure is relatively limited. First of all, there are two features of fine structure, namely the microfibril orientation and, as assessed recently, the microfibril thickness (NEČESANÝ, 1968). Although a feature of chemical composition of the cell wall, cell-wall lignification may also be mentioned here. Its relation to ultrastructure seems to be logical.

From the viewpoint of microscopic structure, three features are characteristic: Cell-wall thickness, cell length and cell width. Under the term cell we understand throughout this paper either a tracheid or a libriform wood fibre.

Research on reaction wood has indicated that auxins influence many of the above-mentioned features of cell-wall structure (WERSHING & BAILEY, 1942; ONAKA, 1949; NEČESANÝ, 1958; CASPERSON, 1967). When new growth substances were discovered we naturally supposed that they also influence structural features of the cell wall. Experiments with tissue cultures of V i n c a r o s e a indicated that this assumption was correct (NEČESANÝ, 1968).

Growth substances may be transported to cambial zone and youngest xylem either as such or in the form of precursors. Here they can be detected

during most part of the day (NEČESANÝ, 1958; NEČESANÝ et al., 1965). In this paper we are describing experiments which had been designed to show if the effect of growth substances in intact plants is the same as in tissue culture. At the same time the plant material was used to analyze additional relationships between submicroscopic and microscopic features of cell-wall structure which could not be elucidated with tissue cultures.

MATERIAL AND METHODS

One-month-old shoots of one-year-old seedlings of fast-growing poplar P o p u l u s x c ana d e n s i s M o e n c h, cultivar I-214 were treated with lanoline pastes containing the following solutions of indoleacetic acid (IAA), gibberellic acid (GA_3), kinetin and 2,3,5-triiodobenzoic acid (TIBA):

IAA	10 ppm.	(5 ppm. in the paste)
IAA	100 ppm.	(50 ppm. "
IAA	1000 ppm.	(500 ppm. "
GA_3	10 ppm.	(5 ppm. "
GA_3	100 ppm.	(50 ppm. "
GA_3	1000 ppm.	(500 ppm. "
kinetin	1 ppm.	(0,5 ppm. "
kinetin	10 ppm.	(5 ppm. "
kinetin	100 ppm.	(50 ppm. "
TIBA	100 ppm.	(50 ppm. "
TIBA	1000 ppm.	(500 ppm. "
TIBA	10000 ppm.	(5000 ppm. "

control with the lanoline paste without any growth substances.

The aqueous solutions of growth substances were mixed 1:1 with lanoline. The content of growth substances in pastes was therefore one half of that of the solution. Considering that lanoline is inert to the organism and that the solution is mechanically bound to it, only concentrations of growth substances in water are used in the text.

Experimental plants were approximately 60 cm tall. Pastes were applied at mid-height (about 30 cm above the soil), where the stem had a diameter of 8-10 mm, to a 25-30 mm wide ring of bark from which the epidermis had been scraped immediately before. The thick layer of paste was then covered with gauze and aluminum foil to protect it against rain and sunlight.

After a one-month incubation time with growth substances all stems were cut off and samples of 50 mm length containing the whole stem diameter were taken from a point 50 mm under the paste ring. Phloem and pith were removed and the wood was cut into small pieces. These pieces were then boiled under a reflux condenser in Franklin reagent and macerated for 30 min.

The resulting fibre material was used to measure lengths and widths of libriform wood fibres and thickness of their cell walls with a light microscope. Thicknesses of primary wall microfibrils were measured on electron micrographs after preparation of the material by a method published recently (NEČESANÝ, 1968). These values of microfibril thickness, measured in electron micrographs at a magnification of 29'000, should not be regarded as absolute ones. They are relative only, but since all of them are subject to the same systematic error they are still useful for the study of the effect of growth substances on microfibril thickness.

RESULTS

Results are given in Tab.1 and for better clarity plotted in Figs. 1-4. They are more similar to tissue-culture experiments than we anticipated and do not require, therefore, very much comment.

The average thicknesses of primary wall microfibrils and the average lengths of libriform wood fibres decrease both with increasing concentrations of IAA and GA_3, but increase with increasing concentration of TIBA and show a peak with a concentration of 10 ppm.kinetin. Cell-wall thickness is influenced by different concentrations of IAA and GA_3 in the same way as microfibril thickness and cell length. The effect of kinetin and TIBA on the formation of cell-wall thickness, on the other hand, is rather irregular in comparison with those of the other growth regulators.

In spite of all these effects, the cell width does not seem to be regularly limited by growth substances at the concentrations used in our experiments. Only the effect of TIBA corresponds to the known antiauxin activity of this substance.

DISCUSSION

The results must be evaluated from two different points of view, namely the

effects of growth substances as such, as well as effects of their interaction. Recently, the effect of IAA, GA_3 and kinetin was investigated with crown-gall tissue cultures of V i n c a r o s e a L.(NEČESANÝ, 1968), but at concentrations three orders of magnitude lower than those used in the present study. Even though the two plant species differ taxonomically and morphologically, their curves of average microfibril thickness and average tracheid length possess the same characteristics though at two different levels of concentration. The same relationship can be observed with average cell-wall thickness except with kinetin treatment. The shifting of curves between 0.01 and 1.0 ppm in V i n c a and between 10 and 1000 ppm. in P o p u l u s looks very similar. Comparison of this effect with the double peaked curve showing the effect of IAA concentration on the average velocity of plasma streaming (NEČESANÝ, 1956) indicates that other growth substances have effects similar to that of IAA. The difference in relative hight of the two peaks may possibly be explained by the different characteristics of the two investigated plants.

The effect of TIBA on xylem formation is that of an antiauxin (GALSTON, 1947; CRONSHAW and MOREY, 1965; KENNEDY and FARRAR, 1965). Our results support this view: the effect of low doses of TIBA is identical to that of high doses of IAA and vice versa.

This paper is particularly concerned with the relationship between various pairs of features of microscopic and submicroscopic cell-wall structure. With the information given in Figs. 1-4 we can construct the relationship between individual pairs of features for each of investigated growth substances (Tab.2). Microfibril thickness, cell-wall thickness and cell length can be considered as features of mutual direct proportionality. The relationship between cell length and cell-wall thicknesss as well as between microfibril thickness and cell length has recently been assessed (NECESANY, 1968). The appearance of some of these features is so closely correlated that the effects are brought about simultaneously by growth regulators of very different mode of action. The effect of kinetion of cell-wall thickness is an exception. An inversely proportional relationship between cell length and microfibril orientation is well known (WARDROP and PRESTON, 1947; WARDROP and DADSWELL, 1951; NEČESANÝ, 1961, in litt.). Since the same relationship between cell-wall thickness and microfibril orientation has been found (NEČESANÝ, in litt.), it can be assumed that a similar inverse proportional relationship of microfibril orientation and microfibril thickness propably exists. The

relation of lignin content to the above-mentioned structural features is interesting. According to our unpublished data, an inverse proportional relationship between microfibril thickness and lignin content seems to be evident. We may therefore also assume a similar indirect proportional relationship of lignin content and cell-wall thickness as well as cell length. The existence of a relationship between lignin content and cell-wall thickness has been found in hardwoods (NEČESANÝ, in litt.), another one - between lignin content and cell length - is indicated by chemical and structural characteristics of hardwood reaction wood. In the same manner a direct proportional relationship between cell wall lignification and microfibril orientation in secondary wall seems to be indicated also by the characteristics of reaction wood. Last of analysed cell-wall features - the cell width- does not seem to be correlated with the other features analysed here. This is in contrast with the finding that the formation of wider or shorter cells is supported by an abundance of IAA. Our results with IAA do not show this relationship, although the effect of TIBA and partly that of kinetin indicate the possibility of its existence. One of the possible explanations of this failure could be that too low concentrations of IAA had been applied for obtaining such effect.

SUMMARY

The effect of growth substances IAA, GA_3, kinetin and TIBA (three different concentrations each) on the thickness of primary wall microfibrils and on the length of libriform wood fibres of fast-growing poplar P o p u l u s x c a n a d e n s i s, I-214, has been shown to be the same as in tissue culture of the V i n c a r o s e a crown-gall, but at concentrations higher by three orders of magnitude. The possible relationship between structural features and the velocity of plasma streaming is briefly discussed.

The analysis of the effect of some growth substances has been extended to two other cell-wall features, i.e. cell-wall thickness and cell width. Cell-wall thickness, but not cell width was controlled in the same way as the two other features.

Direct proportionality exists between thickness of primary microfibrils, cell-wall thickness and cell length. An inverse proportional relationship between these features on the one hand, and lignin content and microfibril orientation in secondary walls on the other hand, seems probable. Accordingly,

a directly propotional relationship between the microfibril orientation and the lignin content is also possible.

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Tab. 1: The values of feature dimensions characterising the effect of increasing concentrations of different growth substances on the cell wall formation in poplar xylem P o p u l u s x c a n a d e n s i s I-214

growth substance	concentration/ppm.	microfibril thickness n=50 m/A	s/A	v/%	cell wall thickness n=50 m/um	d/um	v/%	fibre cell length n=50 m/um	s/um	v/%	fibre cell width n=50 m/um	s/um	v/%
IAA	10	367.6	48.2	12.80	1.51	0.33	25.19	491	64	13.09	3.61	0.63	17.34
IAA	100	363.6	52.3	14.39	1.18	0.30	25.08	481	73	15.08	3.45	0.73	20.75
IAA	1000	286.2	50.3	17.66	1.14	0.32	27.81	477	62	12.97	3.59	0.71	19.64
GA_3	10	289.8	41.7	14.39	1.37	0.39	28.10	607	69	14.38	4.17	0.92	22.06
GA_3	100	276.7	40.0	14.47	1.31	0.33	25.19	567	75	13.16	3.57	0.86	24.01
GA_3	1000	263.6	45.3	17.19	1.28	0.32	24.90	559	71	12.70	3.82	0.80	21.05
kinetin	1	283.8	33.2	11.72	1.24	0.30	24.52	609	102	16.67	3.89	0.70	18.05
kinetin	10	324.3	37.1	11.44	1.13	0.26	23.09	636	94	14.72	3.73	0.70	18.71
kinetin	100	280.8	33.7	12.01	1.23	0.29	23.29	513	69	13.40	3.72	0.72	19.22
TIBA	100	302.9	30.7	10.15	1.09	0.37	33.87	511	54	10.64	3.94	0.98	24.95
TIBA	1000	332.9	32.2	9.65	1.13	0.33	29.09	542	69	12.69	3.85	0.84	21.77
TIBA	10000	354.0	42.9	11.82	1.28	0.35	27.38	584	68	11.62	3.51	0.71	20.20
control	0	289.8	26.5	9.14	1.15	0.32	27.83	500	65	12.98	3.73	0.70	18.81

Tab. 2: Approximative relationships between various pairs of cell-wall features - if different growth substances applied - in accordance to Tab. 1 and Fig. 1-4

+ a tendency to the proportionality

- a tendency to the inverse proportionality of the relationship

	IAA	GA_3	kinetin	TIBA
microfibril thickness/cell wall thickness	+	+	-	+
microfibril thickness/fibre cell length	+	+	+	+
microfibril thickness/fibre cell width	?	?	-	-
cell wall thickness/fibre cell length	+	+	-	+
cell wall thickness/fibre cell width	?	?	+	-
fibre cell length/fibre cell width	?	?	?	-

LEGENDS TO FIGURES

Fig. 1 - The effect of increasing concentration (ppm.) of growth substances on the average microfibril thickness (Å) in primary wall of wood fibre cells. Note the comparison level at 200 Å.

Fig. 2 - The effect of increasing concentration (ppm.) of growth substances on the average cell-wall thickness (µm) of wood fibre cells. Note the comparison level at 1.00 µm.

Fig. 3 - The effect of increasing concentration (ppm.) of growth substances on the average fibre-cell length (µm). Note the comparison level at 400 µm.

Fig. 4 - The effect of increasing concentration (ppm.) of growth substances on the average fibre cell width (µm). The comparison level at 20 µm.

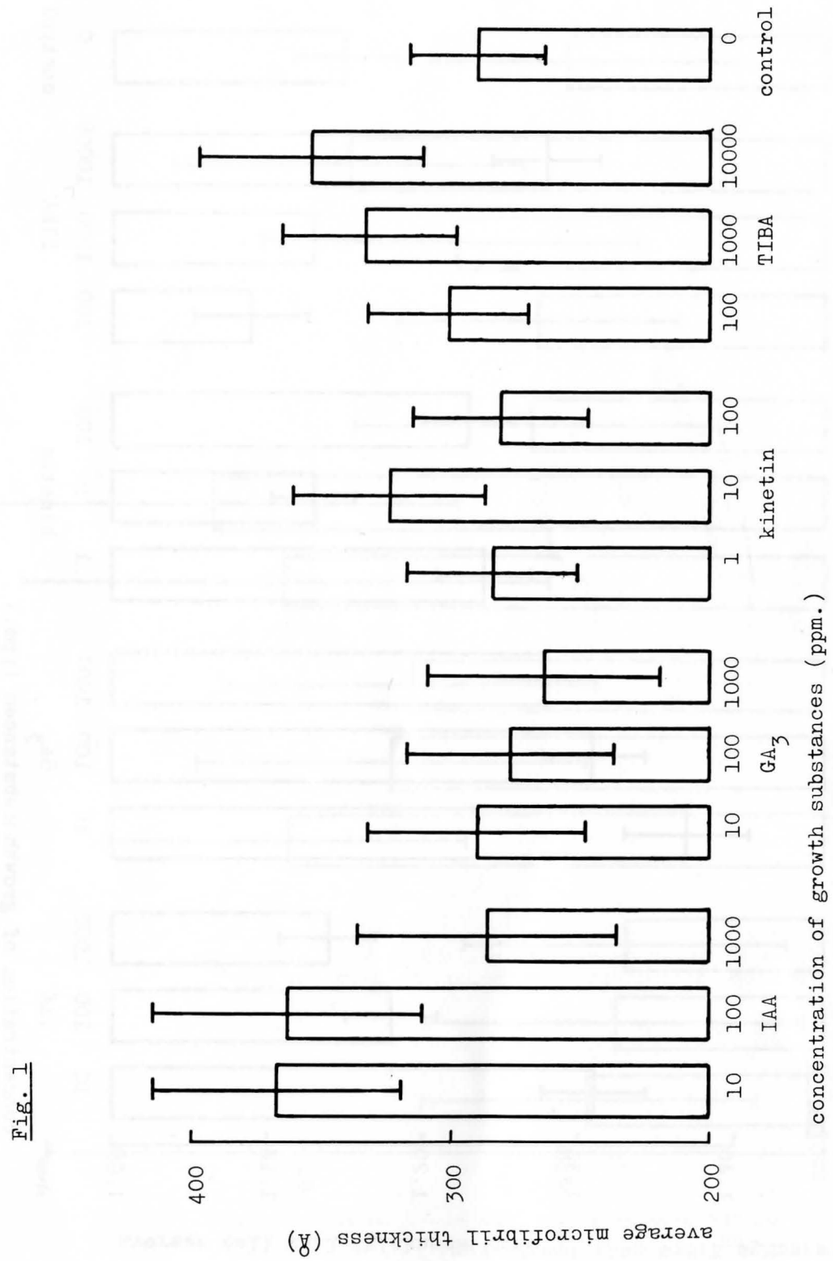


Fig. 1

Fig. 2

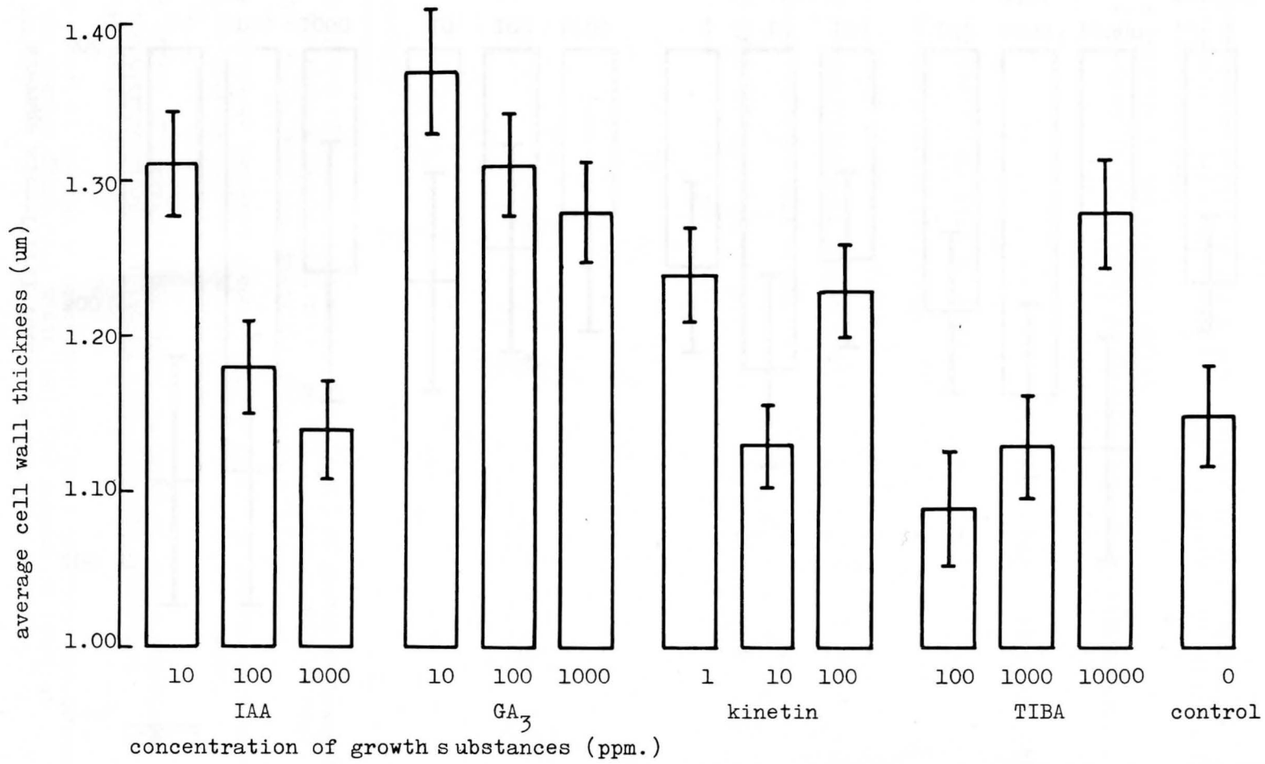

average cell wall thickness (um)
1.40
1.30
1.20
1.10
1.00
10
100
1000
IAA
10
100
1000
GA3
1
10
100
kinetin
100
1000
10000
TIBA
0
control
concentration of growth substances (ppm.)

Fig. 3

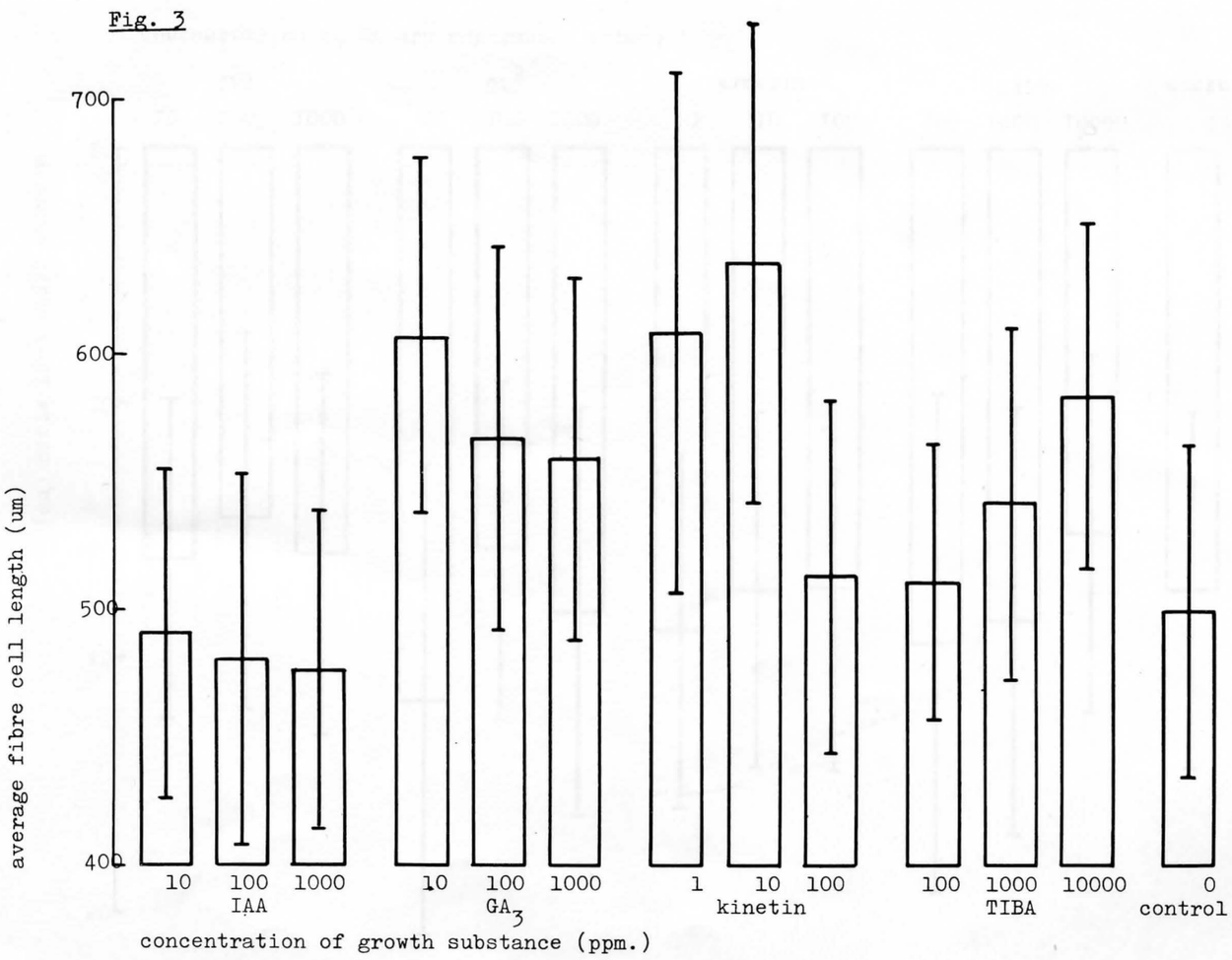
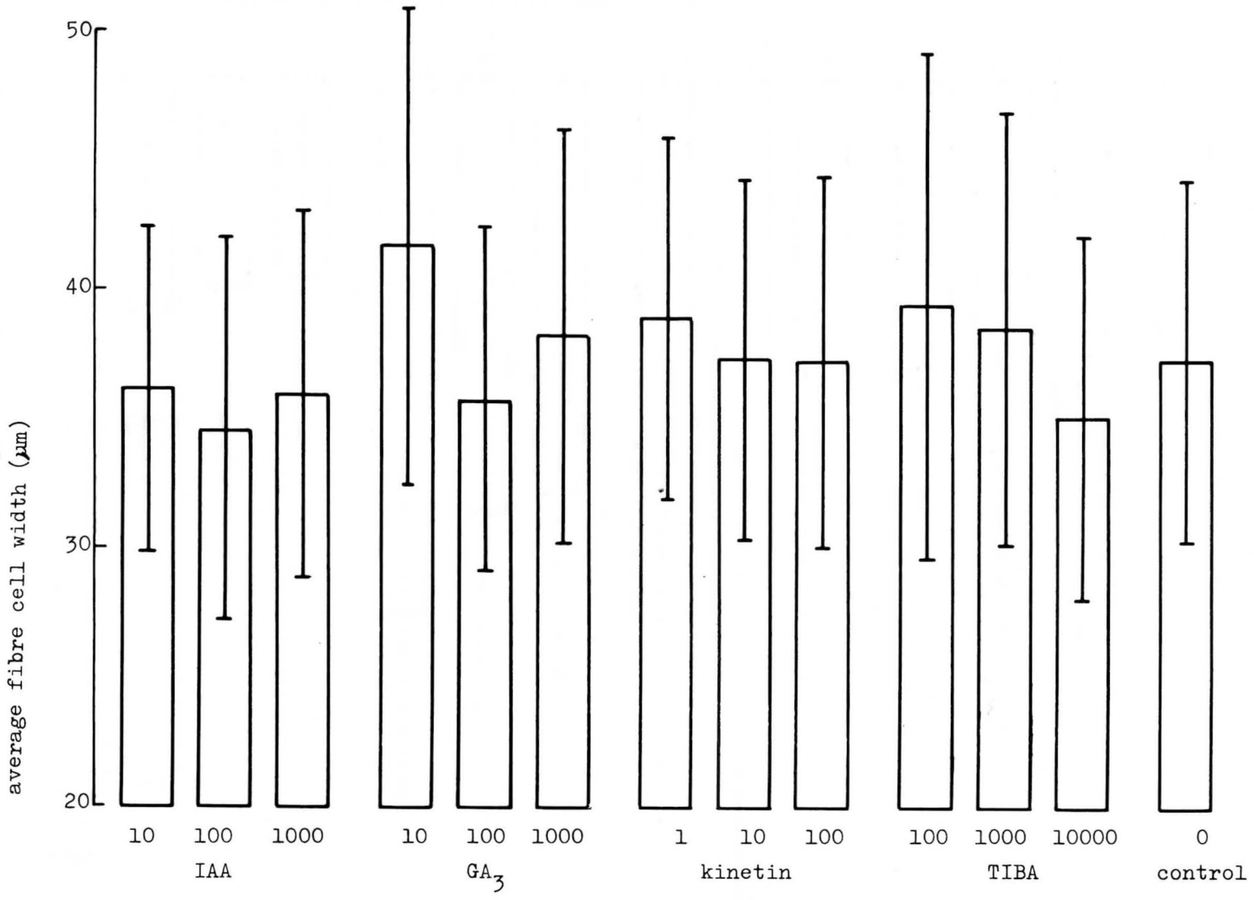

average fibre cell length (um)
700
600
500
400
10
100
1000
IAA
10
100
1000
GA3
1
10
100
kinetin
100
1000
10000
TIBA
0
control
concentration of growth substance (ppm.)

Fig. 4


50
40
30
20
average fibre cell width (µm)
10
100
1000
10
100
1000
1
10
100
100
1000
10000
0
IAA
GA3
kinetin
TIBA
control
concentration of growth substances (ppm.)