The effects of 2,4-dichloro-phenoxy-acetic acid, indol-3yl-acetic acid and kinetin on the activity of auxin-destroying enzymes of Sycamore cell suspension cultures[1]

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Summary. Sycamore cell suspension cultures were grown on medium containing either 2,4-dichloro-phenoxy-acetic acid (2,4-D), kinetin (K) or indol-3yl-acetic acid (IAA). Cells were harvested on days 2 and 11 of the growth cycle and the IAA-destroying (IAA-D) activity of the cytoplasmic fraction analysed. IAA-D activity was highest in the 2,4-D-grown cells, next highest in IAA-grown and lowest in K-grown cells. The levels of compounds inhibitory to IAA-D activity were similar in cells grown in the presence of 2,4-D or IAA and highest in K-grown cells.

Suspension cultures of Sycamore (*Acer pseudoplatanus* L.) cells are normally maintained on medium containing 2,4-D. They show little growth if 2,4-D is omitted and they have, therefore, been considered to be auxin-requiring cultures. The presence of an IAA-like compound has been demonstrated in 2,4-D-grown cells[4] and it is possible that growth in 2,4-D-free medium is limited by the endogenous level of IAA. It has been observed, however[5], that the cells grow well in 2,4-D-free medium if 10 mg of K/l is provided instead. Further, Sycamore cells synthesize cytokinins when grown in the presence of 2,4-D[6]. This raises the question of whether the primary hormonal requirement of the cells is for auxin(s) or cytokinin(s).

The endogenous level of IAA may be changed by altering both the rate of breakdown and the rate of synthesis. The rate of biodestruction of IAA appears to depend on the activity of IAA-D enzymes[7] and the level of phenolic inhibitors[8].

The general methods for the growth of Sycamore cell suspension cultures and for counting cell numbers were previously described[9]. Stock 2,4-D-grown cells and second-passage IAA-grown (10 mg/l) and K-grown (10 mg/l) cells were harvested by filtration at the indicated times. They were frozen in liquid nitrogen, ground in a mortar and pestle then extracted with 0.1 M phosphate buffer (pH 7.0). After a 15-min incubation (277 K) cell debris was removed by centrifugation ($10^4 \times$ g, 30 min). The pellet was washed with the same buffer and the supernatants combined. Sufficient poly-N-vinylpyrrolidone (PVP, Polyclar AT, General Aniline Film Company) was added to make a thin slurry and the mixture maintained at 277 K for 15 min. PVP was removed by centrifugation and the supernatant used as the source of enzymes. Assay conditions for IAA-D activity were optimised as follows: 0.1 ml of the enzyme solution in a reaction mixture containing 3.3×10^{-4} M IAA, 6.67×10^{-4} M H_2O_2 and 10^{-4} M p-coumaric acid in 5×10^{-2} M acetate buffer (pH 5.0). Activity was determined[10] by the change in absorbance at 250 nm (310 K) and the rate reported is from that part of the curve at which it was proportional to enzyme used. Levels of inhibitory compounds were determined by a method similar to that described previously[11] using the PVP-treated enzyme solution from 11-day-old 2,4-D-grown cells as a stock enzyme. Boiled non-PVP-treated samples of enzyme solution were used as the source of inhibitors and the effect of an amount equivalent to 10^5 cells was determined.

Table 1 shows the IAA-D activity of cells grown in the presence of IAA, 2,4-D and K. As can be seen, in cells cultured for 11 days - which is the middle of the most active part of the growth cycle[9] - the IAA-D activity is significantly larger for the 2,4-D treatment than that for IAA and K. From 2 to 11 days, IAA induced a slight decrease in IAA-D activity; in contrast 2,4-D and K caused a strong increase. However, assays to determine the in vitro effect of these regulators on IAA-D activity showed that it was not significant for 2,4-D (0.1 and 1.0 mg/l) or for K (1.0 and 10.0 mg/l).

The amount of inhibitor was originally determined directly by a comparison between PVP-treated and non-PVP-treated samples of enzyme. While the non-PVP-treated samples showed proportionally less IAA-D activity at high enzyme levels, on dilution to the part of the curve at which activity was proportional to enzyme concentration no inhibitory effect occurred. An alternative method was, therefore, used as previously described. The results, given in table 2, show that: a) inhibitor activity was similar in cells grown in the presence of IAA and of 2,4-D but much higher in K-grown cells; b) inhibitor activity was lower at 11 than at 2 days and the decrease of inhibitor activity was not significantly different in the 3 treatments.

Cultures of Sycamore cells have been considered to be auxin-requiring. Although they can synthesize an IAA-like compound[4], the growth rate is considered to be limited by the rate of IAA biosynthesis; thus, growth may be enhanced by the provision of IAA or an alternative, e.g. 2,4-D. The observation that K supports the growth of cells in auxin-free medium can be interpreted in at least 2 ways. K may exert a sparing action on auxin either by enhancing its synthesis or reducing its breakdown[12]. Alternatively, Sycamore cells may not be auxin-requiring but cytokinin-requiring. On the 2nd hypothesis, which is considered less likely, the ability of auxins to support growth is interpreted in terms of an enhanced production of cytokinins[6]. Present results show that K-grown cells have lower IAA-D activity than IAA-grown or 2,4-D-grown cells. The similarity in the relative reduction between day 2 and 11 of the 2,4-D-grown and K-grown cells is very striking. Further, the level of inhibitory compounds is highest in the K-grown cells and, again, comparisons of the relative reduction between days 2 and 11 show distinct similarities. Thus, although the values for IAA-D activity and inhibitor level are different in 2,4-D-grown as compared with K-grown cells, the pat-

Table 1. IAA-D activity (in terms of changes in absorbance at 250 nm per 5 min and per 10^6 cells) in Sycamore cells grown (2 and 11 days) in the presence of IAA, 2,4-D and K

	2 Days	11 Days	%*
IAA	2.41	1.78	−26.1
2,4-D	3.51	5.65	+60.9
K	0.85	1.54	+80.0

*Change in % (+: stimulation; −: inhibition) from 2 to 11 days.

Table 2. Inhibitor activity (in percent inhibition of activity of a stock enzyme prepared from 11 day cells grown in 2,4-D) in Sycamore cells grown (2 and 11 days) in the presence of IAA, 2,4-D and K

	2 Days	11 Days	%*
IAA	14.5	2.4	83.4
2,4-D	17.4	5.4	68.9
K	73.7	17.6	76.1

*% Decrease from 2 to 11 days.

tern of their behaviour in non-dividing as opposed to actively-dividing cells (i.e. 2 days versus 11 days) is quite similar. If the inhibitory compounds are in the same cell compartment as the IAA-D enzymes, the level of IAA-D activity in K-grown cells would be approximately $^1/_{10}$ the level in IAA-grown and 2,4-D-grown cells. But if the 2 components are in separate compartments, IAA-D activity of K-grown cells is still only $^1/_3$ that of 2,4-D-grown cells. Under both circumstances there would be a tendency for higher endogenous levels of IAA to be achieved in cells grown in the presence of K. Experiments are underway to measure endogenous levels of IAA, and to determine both the isozymic pattern of IAA-D enzymes and the structure of the inhibitory compounds.

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Sterilising activity of methoprene and hydroprene in *Tribolium castaneum* (Herbst)[1]

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Summary. The productivity of *T. castaneum* adults, previously reared in flour incorporating either the IGR methoprene or hydroprene, was found to be impaired depending on the concentration of the IGR in the flour, whether or not the individual was morphologically deformed, and its sex.

Insect growth regulators (IGRs) possessing juvenile hormone activity disrupt the life functions of insects which may result in a reduction or suppression of productivity, or in the production of lethal morphogenetic effects[2]. The IGRs methoprene and hydroprene show considerable potential as commodity protectants[3-6], but little attention has been given to sub-lethal effects that they may have on insect pests[7]. Some degree of sterility induced by a sub-lethal dose of an IGR may be an advantage particularly when it is used as a commodity protectant because such sub-lethal effects would extend the period of protection by retarding or even preventing a build-up of an infestation.

Using a malathion susceptible strain of *T. castaneum* (Herbst)[8], sub-lethal concentrations of methoprene and of hydroprene were tested for sterilising activity. Flour (wholemeal) incorporating either methoprene or hydro-

Table 1. Viability and productivity of adults of *T. castaneum* previously reared in flour containing either methoprene or hydroprene

IGR and concentration (ppm)	Adult type (N or D)* crossed with control adult	Male Number of crosses set up	Number of crosses producing progeny	Mean number of progeny****	Female Number of crosses set up	Number of crosses producing progeny	Mean number of progeny****
Methoprene							
0.001	D	14	11	142.7^{ab}	26	15***	131.6^{a}
	N	30	27	129.8^{ac}	30	30	138.9^{a}
0.01	D	18	14	173.9^{b}	22	15***	138.9^{a}
	N	28	27	132.6^{a}	30	25	121.3^{a}
0.1	D	28	3***	69.3^{c}	30	5***	99.6^{a}
	N	30	16***	80.8^{c}	30	27	119.7^{a}
Hydroprene							
0.001	D	4**	4	112.0	3**	0	–
	N	30	24	121.2^{a}	29	27	138.1^{a}
0.01	D	29	19***	145.8^{a}	28	8***	126.4^{ab}
	N	30	27	146.5^{a}	30	26	143.3^{a}
0.1	D	30	20***	130.1^{a}	30	16***	98.4^{b}
	N	30	28	161.0^{a}	29	27	135.0^{a}
Control		30	29	135.6^{a}	30	29	135.6^{a}

* N denotes morphologically normal, and D morphologically deformed, adults bred in flour containing either methoprene or hydroprene. ** Too few crosses set up to permit any statistical analysis. *** Significantly different from control (X^2-test). **** The mean number or progeny produced per viable cross; means followed by a different letter are significantly different at $p = 0.05$, Duncan's Multiple Range Test.