A Further Contribution to the Question of Cytokinin-Like Activity of 8-Quinolinol Sulphate

Some months ago CHUA[1] reported that 8-quinolinol sulphate, a compound known for its antifungal and antibacterial properties, acts cytokinin-like in retarding the senescence of excised oat leaves when added to the incubation media used in this bioassay to prove cytokinin activity. CHUA showed 8-quinolinol sulphate to be inactive in retarding oat leaf senescence at pH 4.0, but to possess maximum activity at pH 7.0 at concentrations between 160 and 180 mg/l.

More recently RAMESHWAR and STEPONKUS[2] doubted the results, emphasizing that the effect obtained with the bioassay in non-sterile conditions is far from being specific for cytokinins, since bacterial contamination hastens the senescence process and, therefore, the effect on the retarded senescence found by CHUA might be an indirect one, due to a mere inhibition of micro-organism growth. In fact, when tested in sterile conditions applying the soybean callus bioassay[3], a very sensitive test for cytokinin activity, 8-quinolinol sulphate was proved by RAMESHWAR and STEPONKUS[2] not to possess any cytokinin activity at concentrations ranging from 1 to 200 mg/l.

As reported in a previous paper[4], the regeneration of flowers in *Begonia* species is strictly dependent on a cytokinin present in the culture medium. Hence the regeneration of buds may be regarded as an indicator for cytokinin activity of a hitherto unknown compound. Accordingly, we performed experiments with 8-quinolinol[5] and 8-quinolinol sulphate[5] using the flower formation capacity of excised *Begonia* fragments cultured in vitro as qualitative test for cytokinin action.

When segments of about 10 mm long, from the first internode of partial florescences of *Begonia x richmondensis*, are isolated and implanted into culture tubes on a defined basal medium[6] supplemented with $10^{-6}M$ IAA[7] and $5 \times 10^{-6}M$ BA[8], the *Begonia* tissue brings forth some vegetative shoots and regularly organized male flowers as is shown in Figure 1. In contrast, media lacking the BA supply but containing instead 8-quinolinol sulphate at concentrations of 40, 80, 160 or 320 mg/l at different pH values (pH 4.5, 5.0, 6.0, 7.0; see CHUA[1], who reported maximum activity at pH 7.0) do not allow any regeneration of buds (Figure 2). Rather, the implanted fragments quickly lose their red colour resulting from anthocyanins in the epidermal cells and underlying tissues, turn greyish-brown in a few days and subsequently wither. On the control media without added BA, as well as on the media supplemented with 40 and 80 mg/l of 8-quinolinol or 8-quinolinol sulphate, hair-like structures grow out of superficial cells near the cut surfaces of the segments; at the higher concentrations their formation is suppressed.

Thus the results of the experiments reported do not ascertain that 8-quinolinol or 8-quinolinol sulphate may substitute for a cytokinin; and we therefore assume that these compounds do not possess cytokinin-like activity.

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[7] IAA = Indole-3-acetic acid; E. Merck AG, Darmstadt (Germany).
[8] BA = Benzyladenine; Nutritional Biochemicals Corporation, Cleveland (Ohio, USA).

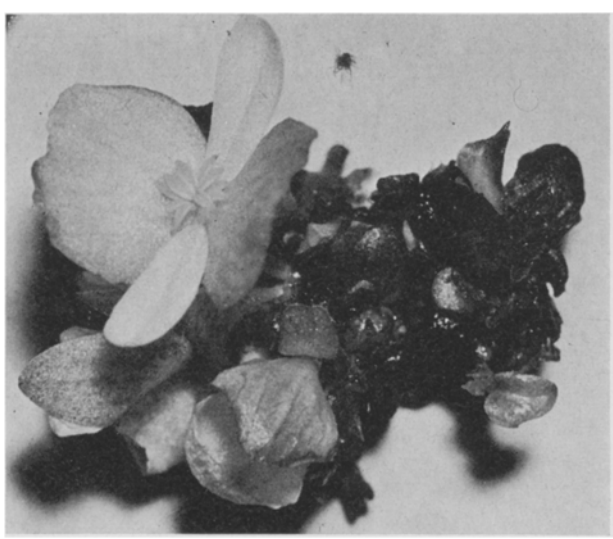

Fig. 1. Aspect of regenerated vegetative and flower buds on an explant taken from the first internode of a partial florescence of *Begonia x richmondensis*. The picture is taken after 9 weeks of sterile culture in vitro on the basal medium supplemented with IAA ($10^{-6}M$) and BA ($5 \times 10^{-6}M$).

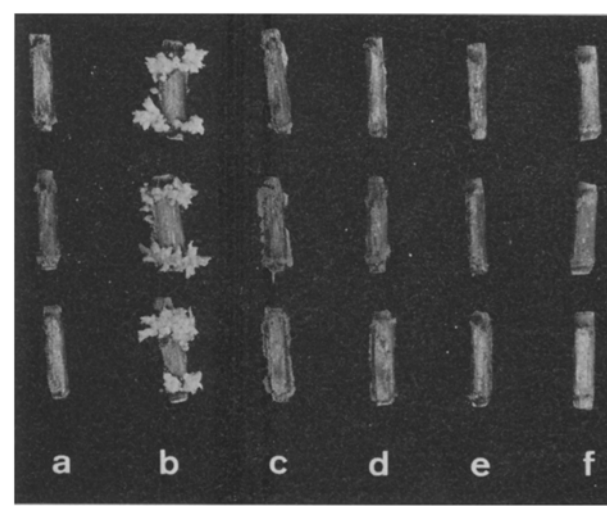

Fig. 2. Aspect of excised fragments taken from the first internode of partial florescences of *Begonia x richmondensis* cultured in vitro on the basal medium supplemented with IAA ($10^{-6}M$) (a); IAA ($10^{-6}M$) and BA ($5 \times 10^{-6}M$) (b); IAA ($10^{-6}M$) and 8-quinolinol sulphate at the concentration of 40 mg/l (c); 80 mg/l (d); 160 mg/l (e); and 320 mg/l (f). The picture is taken after 38 days of culture.

The outcome of our experiments confirms the results already stated by RAMESHWAR and STEPONKUS.

Zusammenfassung. Regenerationsexperimente an Segmenten aus Teilblütenstandsachsen von *Begonia x richmondensis* haben eine weitere Bestätigung dafür erbracht, dass 8-Hydroxy-chinolin bzw. 8-Hydroxy-chinolin-sulfat unter sterilen Bedingungen in vitro keine Cytokinin-Wirksamkeit besitzt.

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Effect of Toxic and Non-Toxic Sugars on Motility of Honey Bee (*Apis mellifera* L.) Spermatozoa

We recently reported[1] that motility can be induced in honey bee spermatozoa by suspending the semen in solutions containing sucrose, glucose, and fructose, or any combination of two thereof, and that the presence of sugar, rather than physical dilution, was the more important evocator of motility. Subsequently, we have conducted similar individual tests with sucrose, glucose, fructose, trehalose, maltose, melezitose (all either 'sweet to', tolerated by, or metabolized by bees)[2–6]; and mannose, galactose, melibiose, raffinose, rhamnose (all supposedly toxic to adult bees)[2–6].

The concentrations of the sugar solutions tested are recorded in the Table (those for sucrose, glucose, and fructose were the same as those used earlier[1] and were derived from Grace)[7]. The saline solvent for each was 0.85% NaCl in triple glass distilled water. 4 to 6 replicates (< 30 min elapsed time per replicate) of the following stepwise procedure were run for each of the sugars tested. 4 test tubes were used in each replicate. Tubes 1 and 2 each received 1 ml of saline; tube 3 received 1 ml of a sugar-saline control solution containing sucrose, glucose, and fructose[1], and tube 4 received 1 ml of the single sugar-saline test solution.

Step A: 1 µl of freshly collected honey bee semen introduced into each tube and gently mixed with the diluent. Step B: 1 drop of the mixture from each tube placed on a separate microscope slide and spermatozoal morphology and motility appraised. Step C: Tube 1 rechecked as a saline control. Step D: Tube 2 rechecked, 3 drops of the single-sugar-saline test solution added to it, and motility checked again. Step E: Tubes 3 and 4 rechecked.

The responses of spermatozoa to each diluent fell within one of three categories, each clearly distinct: 1. When 70% or more of the spermatozoa exhibited vigorous undulating movement, the diluent was considered to have induced motility. 2. When only 20–50% of the spermatozoa were motile, and then only feebly, the diluent was considered capable of inducing only incomplete motility. 3. When less than 1–2% of the spermatozoa were moving, the diluent was considered unable to induce motility; this latter result is expected for the saline controls[1].

Our findings are summarized in the Table. In all cases, the motility conditions observed at Steps B and D were in agreement. Further, motility was consistently lacking in the semen-saline control mixtures. Glucose, fructose, and sucrose induced motility as expected from earlier work[1,8]. Trehalose, a normal constituent of honey bee seminal plasma[9], was not as effective at inducing motility as we expected. Maltose and melezitose also induced motility, perhaps because they, as well as sucrose and trehalose, can be hydrolyzed to glucose and fructose by bees[6]. Galactose and melibiose, reputedly toxic to bees, were not toxic to spermatozoa but instead induced motility. These two sugars were shown toxic in earlier feeding tests[5,6], but the present findings hint they lack parenteral toxicity. Mannose, raffinose, and rhamnose, toxic to bees[2–6], did not induce motility.

Effects of added sugars on spermatozoal motility in saline solutions of honey bee spermatozoa: motility (+); partial motility (±); no motility (−).

Sugar	Concentration (g/l)	No. Observations	Effect on motility		
Glucose	0.7	4	4+		
Fructose	0.4	4	4+		
Sucrose	26.7	4	3+	1±	
Trehalose	0.5	4		3±	1−
Maltose	0.5	4	4+		
Melezitose	0.5	4	4+		
Mannose	0.5	6			6−
Galactose	0.5	6	4+	2±	
Melibiose	0.5	5		2±	3−
Raffinose	0.5	4			4−
Rhamnose	0.5	5			5−

These results confirm our earlier conclusions that motility of honey bee spermatozoa in vitro is greatly affected by the presence of sugar in the semen diluent and is affected little, if any, by dilution[1]. Likewise, as before, those sugars which induce motility are able to do so in very low concentrations (approximately 0.0363 mg/ml fructose at Step D for instance).

Zusammenfassung. Die Motilität der Spermien bei der Honigbiene *Apis mellifera* L. wird in vitro furch die Monosaccharide Glukose, Fruktose und Galaktose, die Dissacharide Maltose, Melibiose und Trehalose, sowie das Trisaccharid Melezitose ausgelöst. Mamose Rhamnose und Raffinose hingegen sind unwirksam. Von den wirksamen Zuckern sind Galaktose und Melibiose für Bienen giftig.

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20 August 1971.

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