

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<sup>1</sup> 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)<sup>2-6</sup>; and mannose, galactose, melibiose, raffinose, rhamnose (all supposedly toxic to adult bees)<sup>2-6</sup>.

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<sup>1</sup> and were derived from Grace)<sup>7</sup>. 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 step-wise 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<sup>1</sup>, 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<sup>1</sup>.

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<sup>1,8</sup>. Trehalose, a normal constituent of honey bee seminal plasma<sup>9</sup>, 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<sup>6</sup>. 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<sup>5,6</sup>, but the present findings hint they lack parenteral toxicity. Mannose, raffinose, and rhamnose, toxic to bees<sup>2-6</sup>, 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+
Trehalose	0.5	4	3±
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±
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<sup>1</sup>. 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 durch die Monosaccharide Glukose, Fruktose und Galaktose, die Disaccharide Maltose, Melibiose und Trehalose, sowie das Trisaccharid Melezitose ausgelöst. Mammose Rhamnose und Raffinose hingegen sind unwirksam. Von den wirklichen Zuckern sind Galaktose und Melibiose für Bienen giftig.

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<sup>1</sup> H. K. POOLE and J. F. EDWARDS, *Experientia* 26, 859 (1970).

<sup>2</sup> A. SOLS, E. CADENAS, and F. ALVARADO, *Science* 131, 297 (1960).

<sup>3</sup> K. VON FRISCH, *Naturwissenschaften* 14, 307 (1928).

<sup>4</sup> T. STAUDENMAYER, *Z. vergl. Physiol.* 26, 644 (1939).

<sup>5</sup> G. GEISSLER and W. STECHE, *Z. Bienenforsch.* 6, 77 (1962).

<sup>6</sup> A. MAURIZIO, *J. Insect Physiol.* 11, 745 (1965).

<sup>7</sup> T. D. C. GRACE, *Nature* 195, 788 (1962).

<sup>8</sup> T. MANN, *Biochemistry of Semen and of the Male Reproductive Tract* (John Wiley and Sons, Inc., New York 1964).

<sup>9</sup> M. S. BLUM, Z. GLOWSKA and S. TABER III, *Ann. ent. Soc. Am.* 55, 135 (1962).