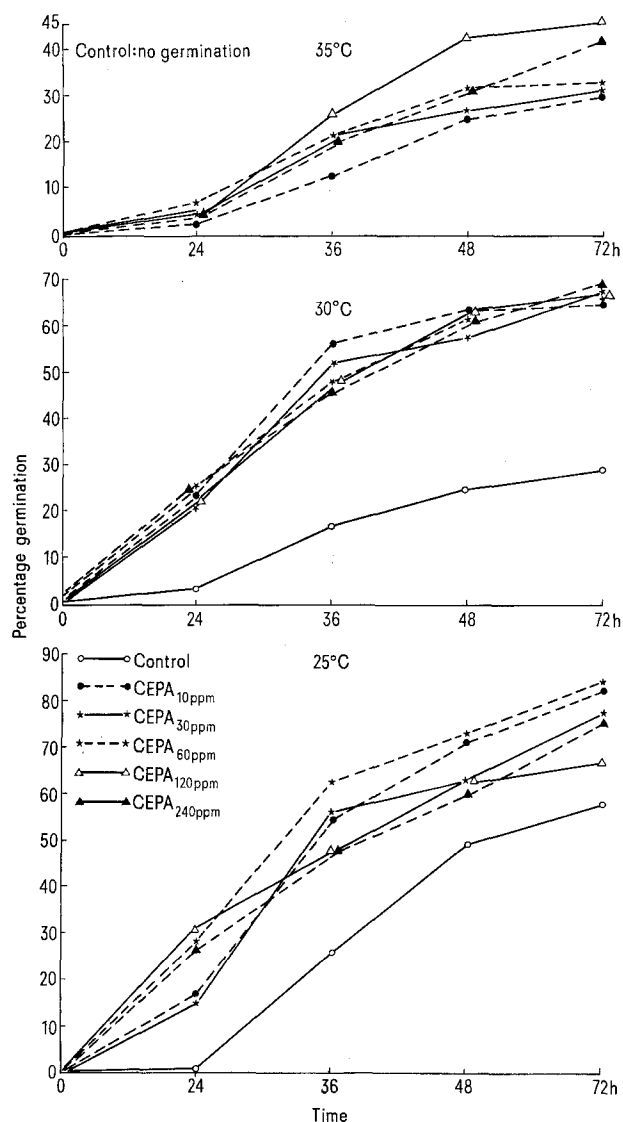


tion at 25°C and 30°C, but also prevents the dormancy caused by high temperatures. Thus, for instance, at a temperature of 35°C in control the seeds did not indicate any germination at all. However, in presence of various concentrations of ethrel, even at 35°C as much as 40%



Effect of ethrel on seed germination of lettuce at different temperatures.

germination was always observed. Therefore, a definite role of ethrel in prevention of thermodormancy is clearly perceptible. It might not be out of place to mention here that ethrel is also able to reverse the inhibition of lettuce seed germination caused by abscisic acid and other seed germination inhibitors<sup>2,5</sup>.

As early as 1965 HABER<sup>6</sup> had shown that gibberellic acid treatment of lettuce seeds caused a reversal of the effects of many of the known seed germination inhibitors. Recently, by using a procedure that enables a separation of the production of ethylene from the effect of ethylene, STEWART and FREEBAIRN<sup>7</sup> have shown that in lettuce seed germination gibberellin primarily induced its response by stimulating ethylene production. According to these authors heat treatment inactivates the ethylene synthesis without affecting ethylene action. Such a treatment, however, did not inhibit the activity of exogenously applied ethylene but did prevent the activity of gibberellic acid which presumably depended on ethylene synthesis. It is, therefore, logical to postulate that thermodormancy in lettuce seeds might be a result of the failure of endogenous synthesis of ethylene which is required for activation and synthesis of enzymes or hormones<sup>8</sup> in the early stages of seed germination<sup>9</sup>.

**Zusammenfassung.** Ethrel verhindert den Ruhezustand von «Cabbage»-Salatsamen, der durch hohe Temperaturen verursacht wird. Es wird angenommen, dass die Hemmung der Salatsamenkeimung durch hohe Temperaturen vermittelt wird, besonders durch ihre Wirkung auf die endogene Bildung des Äthylens, welches eine Schlüsselrolle bei der Samenkeimung spielt.

G. D. HARSH, O. P. VYAS, S. P. BOHRA and N. SANKHLA<sup>10</sup>

Botany Department, University of Jodhpur, New-Campus, Jodhpur (India), 20 October 1972.

<sup>5</sup> U. N. CHATTERJI, G. D. HARSH, D. SANKHLA and N. SANKHLA, *Biochem. Physiol. Pflanzen (Flora, Jena, Ser. A)* **161**, 572 (1971).

<sup>6</sup> A. H. HABER, *Pl. Cell Physiol.*, Tokyo **6**, 565 (1965).

<sup>7</sup> E. R. STEWART and H. T. FREEBAIRN, *Pl. Physiol. Lancaster* **44**, 955 (1969).

<sup>8</sup> K. TAKAYANAGI and J. F. HARRINGTON, *Pl. Physiol. Lancaster* **47**, 521 (1971).

<sup>9</sup> This work was carried out partly with a grant from U.G.C., New-Dehli. Grateful thanks are due to M/s Agromore (India) Ltd., for supply of ethrel.

<sup>10</sup> Present address: Technische Hochschule, Institut für Botanik, Arcisstrasse 21, D-8000 München 2, Germany.

## Raffinose in *Stomoxys calcitrans* Linn. (Diptera, Cyclorrhapha: Muscidae)

Carbohydrates, being utilized in various ways, play an important role in the metabolism of animals. During the course of study of sugars in the stable fly, *Stomoxys calcitrans* L., raffinose, a trisaccharide detected for the first time in an insect, is reported here.

**Materials and methods.** About 200 freshly laid eggs, 30 full-grown third-instar larvae, and an equal number of newly-formed white-pupae, and newly-emerged and adult flies were taken out from a culture of *S. calcitrans*, maintained in the laboratory. The newly-emerged flies were anaesthetized with carbon dioxide and fixed in cold

absolute ethanol, while the other stages were fixed directly. Sugars were extracted with warm 70% ethanol<sup>1</sup> and the extracts were evaporated to dryness in vacuo. Haemolymph and anal fluid collected from adult flies were used directly.

Sugars were separated by one-dimensional ascending paper chromatography. Each dried extract was dissolved in distilled water. Known volumes of each extract, the haemolymph and the anal fluid were chromatographed on

<sup>1</sup> D. FAIRBAIRN, *Can. J. Zool.* **36**, 787 (1958).

Whatmann No. 1 filter paper, without any treatment. The paper was developed in the upper layer of n-Butanol: Acetic acid: Water (4:1:5)<sup>2</sup> for 96 h without prior saturation of the chromatographic chamber. Pure solutions of known sugars were run parallel to the unknown ones for proper identification. Sugar spots were revealed by staining the dried developed chromatogram with silver nitrate reagent<sup>3</sup>.

**Results.** A comparison of the unknown spots with that of the known ones revealed the unusual presence of the trisaccharide, raffinose, in the extracts of eggs, larvae, pupae and newly-emerged flies, and in the haemolymph of adult *S. calcitrans*. However, this sugar could not be detected in the anal fluid.

In comparison to the other sugars found in the various stages of this fly (unpublished), raffinose was in moderate concentration in the larvae and newly-emerged flies, in low concentration in the eggs and white pupae, and only in traces in the haemolymph.

**Discussion.** This study on the stable fly *S. calcitrans* has shown, for the first time, the presence of raffinose in an insect. Recent studies of McLEOD<sup>4</sup> and PALMER<sup>5</sup> have shown that the trisaccharide, raffinose forms a substantial part of carbohydrate reserve of the embryo of cereal grains and that it is utilized during the early stages of germination and growth. Their findings lead us to presume that raffinose present in the freshly-laid eggs of *S. calcitrans*, is probably utilized during its embryogenesis.

The presence of raffinose in the larvae of *S. calcitrans*, in higher concentration than that in its eggs, leads us to believe that this sugar is procured afresh from the food, probably because of its nutritional value. This finds support from the recent studies of PRADHAN et al.<sup>6</sup> who have reported that raffinose is significantly superior to several other carbohydrates, for the growth and development of the larvae of *Chilo zonellus*.

**Résumé.** La raffinose a été découverte pour la première fois dans l'insecte *S. calcitrans*, une mouche suceuse de sang. Le sucre est présent dans les œufs fraîchement pondus, dans les larves au troisième stage, dans les pupes blanches et dans les toutes jeunes mouches. On a aussi discuté le rôle probable de ce sucre.

P. SINGH and P. D. GUPTA

Department of Zoology, University of Lucknow,  
Lucknow (India), 14 April 1972.

<sup>2</sup> S. R. PARRIDGE, *Biochem. J.* 42, 238 (1948).

<sup>3</sup> W. E. TREVELYAN, D. P. PROCTOR and J. S. HARRISON, *Nature, Lond.* 166, 444 (1950).

<sup>4</sup> A. M. MACLEOD, *Sci. Prog., Lond.* 57, 99 (1969).

<sup>5</sup> G. H. PALMER, *J. Inst. Brew.* 75, 505 (1969).

<sup>6</sup> S. PRADHAN, Investigations on insect pests of sorghum and millets, Final technical report P.L. 480 Project Grant No. FG-IN-227 Project No. A7-ENT-31 (1971).

## 5-Iodo-2-Deoxyuridine Resistance of Vaccinia Viruses in Cells Endowed with Thymidine Kinase Activity

5-iodo-2-deoxyuridine (IUdR) and 5-bromo-2-deoxyuridine (BUdR) inhibit the growth of DNA viruses by being incorporated in the viral genome<sup>1</sup>. For this, IUdR and BUdR must be previously phosphorylated by thymidine- and thymidilate kinases<sup>2</sup>. This explains why variants of Herpes and Pox virus which do not induce thymidine kinase are not inhibited by IUdR and BUdR in cells lacking this enzyme<sup>3,4</sup>. However, strains of Herpes and Pox virus have been obtained which develop in the presence of IUdR and BUdR in cells endowed with thymidine<sup>5,6</sup> kinase activity. The research referred to here was carried out to clarify the mechanism of this resistance.

**Materials and methods.** 5 fluoro-2-deoxyuridine (FUdR) (Calbiochem); IUdR and BUdR (K and K Labs. Plainview, USA); thymidine (Merck); <sup>3</sup>H thymidine (methyl T,

26 Ci/mM) and <sup>3</sup>H IUdR (3,8 Ci/mM) (Amersham). The experiments were carried out by using Genetron-treated, sucrose gradient purified pools of a vaccinia virus strain (I.S.M., Milan) and one of its IUdR-resistant variants<sup>7</sup>.

<sup>1</sup> A. S. KAPLAN, T. BEN PORAT and T. KAMIYA, *Ann. N.Y. Acad. Sci.* 130, 226 (1965).

<sup>2</sup> W. H. PRUSOFF, *Pharmac. Rev.* 19, 209 (1967).

<sup>3</sup> D. R. DUBBS and S. KIT, *Virology* 22, 214 (1964).

<sup>4</sup> S. KIT and D. R. DUBBS, *Biochem. biophys. Res. Commun.* 13, 500 (1963).

<sup>5</sup> W. FERRARI, G. L. GESSA, B. LODDO and M. L. SCHIVO, *Virology* 26, 154 (1965).

<sup>6</sup> H. E. RENIS and D. A. BUTHALA, *Ann. N.Y. Acad. Sci.* 130, 343 (1965).

<sup>7</sup> B. LODDO, M. L. SCHIVO and W. FERRARI, *Lancet* 2, 914 (1963).

Table I. Effect of IUdR on viral growth

IUdR* in the medium (µg/ml)	Infectious units produced after 30 h at 37°C	
	Sensitive strain	Resistant strain
—	8.3 × 10 <sup>7</sup>	5.0 × 10 <sup>7</sup>
6.25	1.8 × 10 <sup>5</sup>	1.6 × 10 <sup>7</sup>
12.5	7.5 × 10 <sup>4</sup>	3.3 × 10 <sup>7</sup>
25	< 10 <sup>4</sup>	1.6 × 10 <sup>7</sup>
50	< 10 <sup>4</sup>	1.6 × 10 <sup>7</sup>
100	< 10 <sup>4</sup>	3.3 × 10 <sup>6</sup>
200	< 10 <sup>4</sup>	1.6 × 10 <sup>5</sup>

\* Added to the cell cultures after the infection period

Table II. Effect of virus infection on the incorporation of H<sup>3</sup> thymidine and H<sup>3</sup> IUdR in DNA

Labelled compound in the medium*	Counts per min incorporated under acid insoluble form after 30 h at 37°C in		
	Uninfected cells	Sensitive virus infected cells	Resistant virus infected cells
(µCi/ml)			
H <sup>3</sup> thymidine 0.3	8.5 × 10 <sup>4</sup>	2.0 × 10 <sup>5</sup>	2.8 × 10 <sup>4</sup>
H <sup>3</sup> IUdR 1.0	3.8 × 10 <sup>4</sup>	7.6 × 10 <sup>4</sup>	1.1 × 10 <sup>4</sup>

\* Added to the cells soon after the end of the infectious period.