

centrations of monosaccharides (including glucosamine and lipid A) were detected by means of thin layer chromatography and by gas chromatography after silylation. Thin layer electrophoresis was used to detect oligosaccharides. The fatty acid composition of the lipid A was determined quantitatively by gaschromatography. Reducing sugars and free fatty acids were determined colorimetrically by the method of DUBOIS et al.⁶ and the method of LAUWERYS⁷, respectively.

The growth of *Bacillus macerans* and the degradation of the LPS were followed for 17 days at 37°C. It was found that first of all the LPS was hydrolysed into lipid A and a polysaccharide fraction. The latter fraction was not further hydrolysed as was seen from column and electrophoresis experiments. From the lipid A fraction, however, free fatty acids (FFA) and phosphate were partially liberated. In this way it was possible to identify the free amino groups of the disaccharide units. However, at this stage, no free glucosamine could be detected. It was also found that the liberation of the FFA in the growth medium reached a maximum level after 7 days (Figure). At this point the composition of these liberated FFA was

Fatty acid composition of the intact LPS of *S. typhimurium* type W and of the LPS after 7 days of growth

Fatty acids*	Intact LPS of <i>S. typhimurium</i> type W (%)	LPS of <i>S. typhimurium</i> type W after 7 days of growth (%)
12:0	trace	4.44
14:ob	trace	trace
14:o	11.8	4.03
15:ob	trace	trace
15:o	trace	trace
15:1	0.5	trace
16:ob	trace	trace
16:o	40.8	48.93
16:1	1.7	2.94
14:ob	18.0	3.53
18:o	19.1	7.56
18:1	8.1	12.10
18:2	trace	4.87
20:o	trace	11.60

*The first number gives the chain length; the second the number of double bonds. b means branched fatty acid.

determined and compared with the FFA composition of intact LPS. The results are summarized in the Table. From these results it can be concluded that the micro-organism grows at the expense of the fatty acids, liberated from the lipid A fraction.

After 17 days of growth at 37°C, intact polysaccharide as well as intact residual lipid A were detected. At this moment, however, FFA could not be found anymore, but free glucosamine was still present in the growth medium.

These results suggest that this *Bacillus macerans* species displays lipolytic activity, as it grows only at the expense of the lipid fraction of the LPS.

In this way, a strong lipolytic *Micrococcus* sp. was tested for its ability to degrade the same LPS. Although not isolated on a LPS containing mineral medium, as the isolated *Bacillus macerans*, it was found that this strain exhibits the same degradation pattern on LPS.

Identical experiments were carried out with purified LPS from *Salmonella minnesota* R-2051 and *Escherichia coli*, added to the mineral medium as sole source of carbon. The degradation pattern of these LPS by *Bacillus macerans* and by the *Micrococcus* sp. was found to be analogous.

Résumé. La biodégradation par *Bacillus macerans* des lipopolysaccharides (LPS), extraites de *Salmonella typhimurium*, *Salmonella minnesota* et *Escherichia coli* fut étudiée dans un milieu liquide minéral, contenant uniquement ces LPS comme sources de carbone. Il fut observé qu'après avoir effectué une hydrolyse des LPS le micro-organisme se développe sur les acides gras, libérés de la fraction lipidique. Après 17 jours de croissance la fraction polysaccharide était encore intact. Le même phénomène de biodégradation fut observé avec une souche typiquement lipolytique (*Micrococcus* sp.).

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⁶ M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS and F. SMITH, *Analyt. Chem.* 28, 350 (1956).

⁷ R. R. LAUWERYS, *Analyt. Chem.* 32, 331 (1969).

Lettuce Seed Germination: Prevention of Thermodormancy by 2-Chloroethanephosphonic Acid (Ethrel)

Recent evidence indicates that besides auxins, gibberellins, cytokinins and abscisic acid, ethylene might also be a major controlling factor of plant growth and development¹. Many seeds are known to produce ethylene naturally during the germination process and seedling growth. This suggests that ethylene might be involved in the growth and development of the embryonic or seedling plants¹. The new experimental chemical 2-chloroethanephosphonic acid (ethrel, CEPA), when applied to plants, mimics the effect of ethylene application in several physiological processes^{2,3}. The present report concerns the ability of ethrel to prevent the dormancy of lettuce seeds caused by high temperature.

Seeds of *Lactuca sativa* 'Cabbage' were allowed to germinate at 3 different temperatures (25°C, 30°C and

35°C), alone and in presence of ethrel (CEPA), in glass petri dishes lined with a single layer of filter paper moistened with 5 ml of the test solution. Ethrel (ACP-68-250) containing propylene glycol as carrier base was used as an ethylene-generating substance⁴. Germination percentage was scored commencing from 24 h till 72 h after incubation. The results of this study are incorporated in the Figure. It is clear that ethrel not only stimulates the rate as well as final percentage of germina-

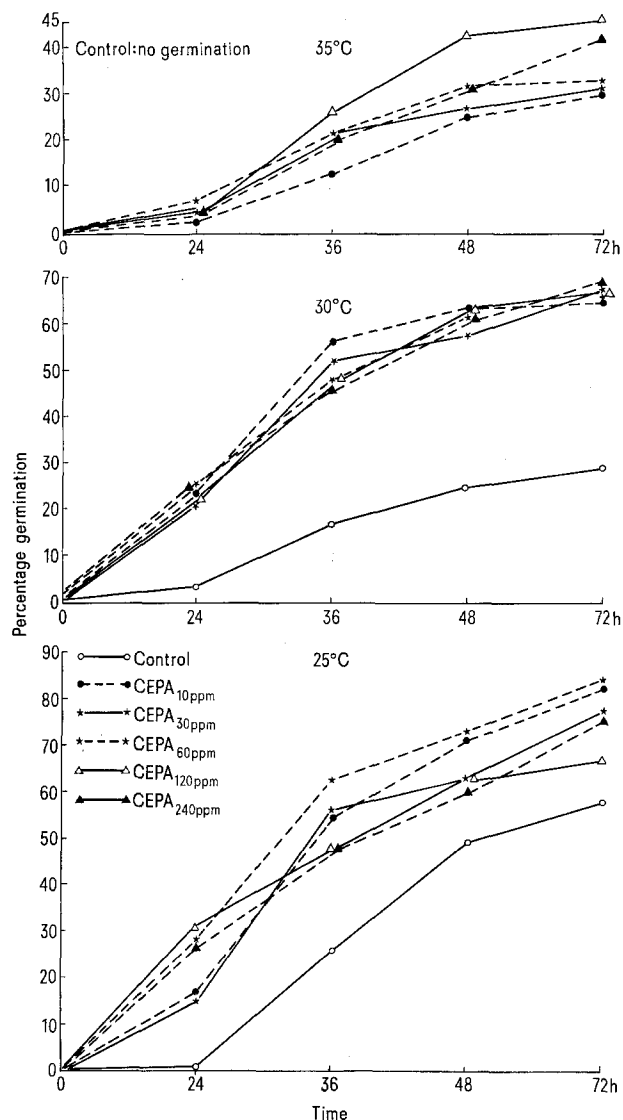
¹ H. K. PRATT and G. D. GOESCHL, *A. Rev. Pl. Physiol.* 20, 541 (1969).

² N. SANKHLA, Thesis, University Jodhpur (1971).

³ R. C. DE WILDE, *Hort. Science* 6, 364 (1971).

⁴ Technical Data Sheet-Ethrel. Amchem Products (1969).

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^{2,5}.

As early as 1965 HABER⁶ 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 FREEBAIRN⁷ 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 hormones⁸ in the early stages of seed germination⁹.

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.

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⁷ E. R. STEWART and H. T. FREEBAIRN, Pl. Physiol. Lancaster 44, 955 (1969).

⁸ K. TAKAYANAGI and J. F. HARRINGTON, Pl. Physiol. Lancaster 47, 521 (1971).

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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% ethanol¹ 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

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