may be deduced that at low population density, under the climatic conditions of Trimmis, TTA traps attract a maximum number of males of *Z. diniana* for a whole season. Virgin females on the other hand attract a smaller number of males and have to be replaced each fortnight.

Tests with the *cis*-isomere (CTA) conducted at Trimmis in 1972 indicate that this substance is a strong antagonist of the natural pheromone and synthetic TTA. Male captures of traps furnished with 2 virgin females (controls) or 2 virgin females and a rubber stopper treated with 1 µl of paraffin oil and different concentrations of CTA are computed in Table III. These results indicate that 100 µg of CTA in paraffin oil completely or almost completely antagonize the action of the natural pheromone of 2 virgin females for about 4 weeks. The 9 males trapped during the period September 6–28 may have been caught accidentally, as indicated by the results of Table IV (last column). Antagonism of CTA and TTA is shown by the results of Table IV. The findings further strengthen the hypothesis that TTA is the natural pheromone of *Z. diniana*.

The results presented in this paper show that 1 µl of a 10% solution of TTA in paraffin oil applied to a rubber stopper presents a powerful lure for males of *Z. diniana*. Because of its long lasting superior effect it can more than only replace virgin females in monitoring traps. CTA at a relatively low concentration has proved to be such a powerful antagonist of the female pheromone of *Z. diniana* that it may be called a true synthetic antipheromone. It is hoped that the substance can be used to prevent pheromone guided meetings of the sexes of the larch bud moth when the population density of the species is low enough to prevent chance meeting[3]. It may thus become a tool for the control of *Z. diniana*. Theoretically much smaller quantities of CTA should be needed to reach this effect than if TTA was used for male confusion.

Zusammenfassung. Gummizapfen mit 1 µl 10%iger Lösung von *trans*-11-Tetradecenylacetat in Paraffinöl sind während 70–100 Tagen attraktiver für Männchen des Lärchenwicklers als Fallen mit je 2 virginen Weibchen der Art, die zudem alle 2 Wochen ersetzt werden müssen. Das *cis*-Isomer der Verbindung ist ein spezifischer Lockstoff für Männchen des Widderchens *Zygaena transalpina* und ein starker Antagonist sowohl des natürlichen Weibchenpheromons des Lärchenwicklers wie des synthetischen Lockstoffs.

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Biodegradation of Microbial Lipopolysaccharides

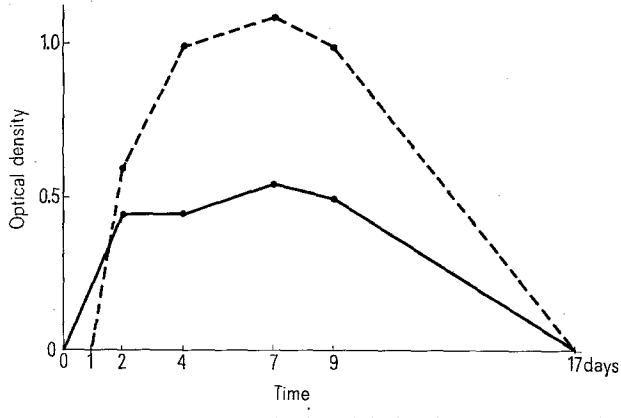
In a foregoing paper, VOETS and BEYAERT[1] described the isolation of a *Bacillus* species growing on a mineral medium with 0.2% lipopolysaccharides (LPS) extracted from a *Salmonella minnesota* R-250 strain.

In further investigations, the LPS fractions of the following bacterial strains were used: *Salmonella typhimurium* type W, *Salmonella minnesota* R-2051 and *Escherichia coli*. The impure LPS of *S. typhimurium* were obtained from Difco and purified by the hot phenol method of WESTPHAL et al.[2]. For the isolation of the LPS from *S. minnesota* R-2051, the strain was grown in batch cultures on the following medium: beef-extract 10 g; peptone 10 g; NaCl 5 g; distilled water 1 l (pH = 7.3). After 24 h of growth at 37 °C, the cells were centrifuged and acetone-dried. The LPS were extracted according to the method of GALANOS et al.[3]. Acetone-dried cells of *E. coli* were obtained from several batch cultures in nutrient broth and extracted according to the method of WESTPHAL et al.[2] as modified by O'NEILL and TODD[4].

Microbial strains growing on bacterial LPS were isolated on the following mineral medium, to which 0.2% LPS were added as sole source of carbon: $(NH_4)_2SO_4$ 1 g; K_2HPO_4 1 g; $NaH_2PO_4 \cdot 2\,H_2O$ 1.25 g; $MgSO_4 \cdot 7\,H_2O$ 0.5 g; $CaCl_2$ 0.01 g; $FeSO_4 \cdot 7\,H_2O$ 0.01 g; distilled water 1 l (pH = 7.0). On the purified LPS of *Salmonella typhimurium* type W, a few bacterial strains were isolated from a soil sample. Transfer of these strains to a liquid medium with the same composition resulted in weak growth. However, when glucose was supplied as sole source of carbon to the culture medium, abundant growth was obtained. According to Bergey's Manual of Determinative Bacteriology[5], a *Bacillus macerans* strain could be isolated in this way.

As a result of this phenomenon, further experiments were carried out with this species. The strain was inoculated into the mineral medium, containing LPS as sole source of carbon and in function of the incubation time – at 37 °C – the growth of the microorganism and the degradation pattern of the LPS were followed.

Intact LPS and its chemically degraded polysaccharide fraction (prepared by mild acid hydrolysis) could be separated easily on a Sephadex G 100 column. Small con-



---- = Liberation of free fatty acids (FFA) during the growth of *Bacillus macerans* on LPS from *Salmonella minnesota* R-2051.
—— = Liberation of free fatty acids (FFA) during the growth of *Micrococcus* sp. on purified LPS from *Salmonella typhimurium* type W.

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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 LAUWERYS[7], 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 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 microorganism 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 wel 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.

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

Fatty acids[a]	Intact LPS of *S. typhimurium* type W (%)	LPS of *S. typhimurium* 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

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

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 microorganisme 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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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 development[1]. 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 plants[1]. 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 substance[4]. 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-

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