PRODUCTION OF ENTOMOPATHOGENIC VIRUSES IN INSECT CELL CULTURES

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Insect viruses provide an effective control method against certain pests of oil palm plantations in the tropical countries.

The viruses used are that found on *Coelaenomenodera minuta* (Coleoptera, Chrysomelidae), the main pest of such plantations, and two isolated from defoliatry caterpillars, a Densovirus from *Casphalia extranea* and a Picornavirus from *Latoia viridissima* (Lepidoptera, Limacodidae).

Existing laboratory insect cell lines stocks do not permit the multiplication of these viruses because of their host specificities. We have therefore tried to establish new cell lines from host material.

Ovarioles from pupae of these insects were cultured on GRACE medium containing 20 % of foetal calf serum at 28°c.

After 48 hours, cells began to develop by budding from the explants. By the end of a week, cell multiplication had produced a cell mass. At this stage of the primoculture, an infection may be attempted either with the whole virus or by transfection.

The importance of this work lies on the development of specific cell lines with which to multiply these viruses. Production on an industrial scale for biological control is thus made possible. These cultures also enable the viruses to be cloned, so that the genetic variability of different strains can be studied and the production of metabolites by transfection of a mixture of both DNA from vector plasmide and virus made possible.

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Insect viruses provide an effective control method against certain pests of oil palm plantations in the tropical countries.
The importance of this work lies on the development of specific cell lines with which to multiply insect viruses.

Two types of viruses were isolated from insects in our laboratory, small non-enveloped viruses with DNA (Densoviruses) or RNA genome (Picornaviruses) an enveloped viruses with DNA genome (Polyhedrosis viruses). These viruses, isolated from several insect families, were identified and characterized. A Densovirus was isolated from the defoliatry caterpillar *Casphalia extranea* (Lepidoptera, Limacodidae). A Picornavirus and a Baculovirus from nuclear polyhedrosis were isolated from *Latoia viridissima* (Lepidoptera, Limacodidae).

We tried to multiply these viruses on existing laboratory insect cell lines stocks (*Spodoptera littoralis, Spodoptera frugiperda, Choristoneura fumiferana and Bombyx mori*). The Densovirus from *Casphalia extranea* was able to multiply on the *Bombyx mori* cell line.

After a cell culture of 3 hours, cells were infected with 0.8 OD purified virus suspension in GRACE medium during one hour at 28°c with a gently agitation. After removing the virus, new GRACE medium containing 10 % foetal calf serum was added, and cells were cultured during 7 days at 28°c. As no cytopathic effect (with the exception of a little cell deformation) could be observed, the cells were scrapped from the surface of culture flask and the virus was systematically extracted and purified.

The multiplication of the other viruses was, up to now, only possible on insect hosts (depending on proliferation in plantations). We have therefore tried to establish new cell lines from host material.

Ovarioles from pupae of these insects aseptically cut off, were cultured on GRACE medium containing 20 % of foetal calf serum at 28°c. After 48 hours, cells began to develop by budding from the explants. A week after, ilots are visible and after 2 weeks, cell multiplication had produced a cell mass. At this stage of the primoculture, an infection may be attempted either with the whole virus or by transfection.

Some difficulties appeared using insects collected during pests in plantation. The collect depend on proliferation of the insects. The life cycle of some of them give only one pupae per year. They were not necesseraly free from latent infection. For example, the primoculture of the *Coelaenomenodera minuta* (Coleoptera, Chrysomelidae) cells, isolated from the most important insect pest of oil palm plantations, died after 3 weeks for this reason. the culture showed some aspects of an in vitro infection with cytopathic effects.

The primoculture of *Casphalia extranea* was carried on during one month, then subcultured. A week later, cells released in the medium and died after 2 weeks without multiplication.

Only the primoculture of *Latoia viridissima* cells could allow the multiplication of the Baculovirus isolated from the host after a culture of 2 weeks. This was confirmed by the analysis on electron microscope of cells fixed with glutaraldehyde.

The results obtained with viruses isolated from *Latoia viridissima* and *Casphalia extranea* in primoculture and in cell line could allowed the initiation of different studies. The multiplication of these viruses is thus made possible. These cultures enable the viruses to be cloned, so that the genetic variability of different strains can be studied and the production of metabolites by transfection of a mixture of both DNA from vector plasmide and virus made possible.
Owing to the development of a new serum-free medium, lowering significantly the production coast, the culture on an industrial scale for biological control is now carrying out.

Virus strains :
FEDIERE, G., 1983
Recherches sur des viroses epizootiques de lépidoptères Limacodidae ravageurs de palmacées.
Thèse de doctorat de 3è cycle,USTL, Montpellier, 130 p.

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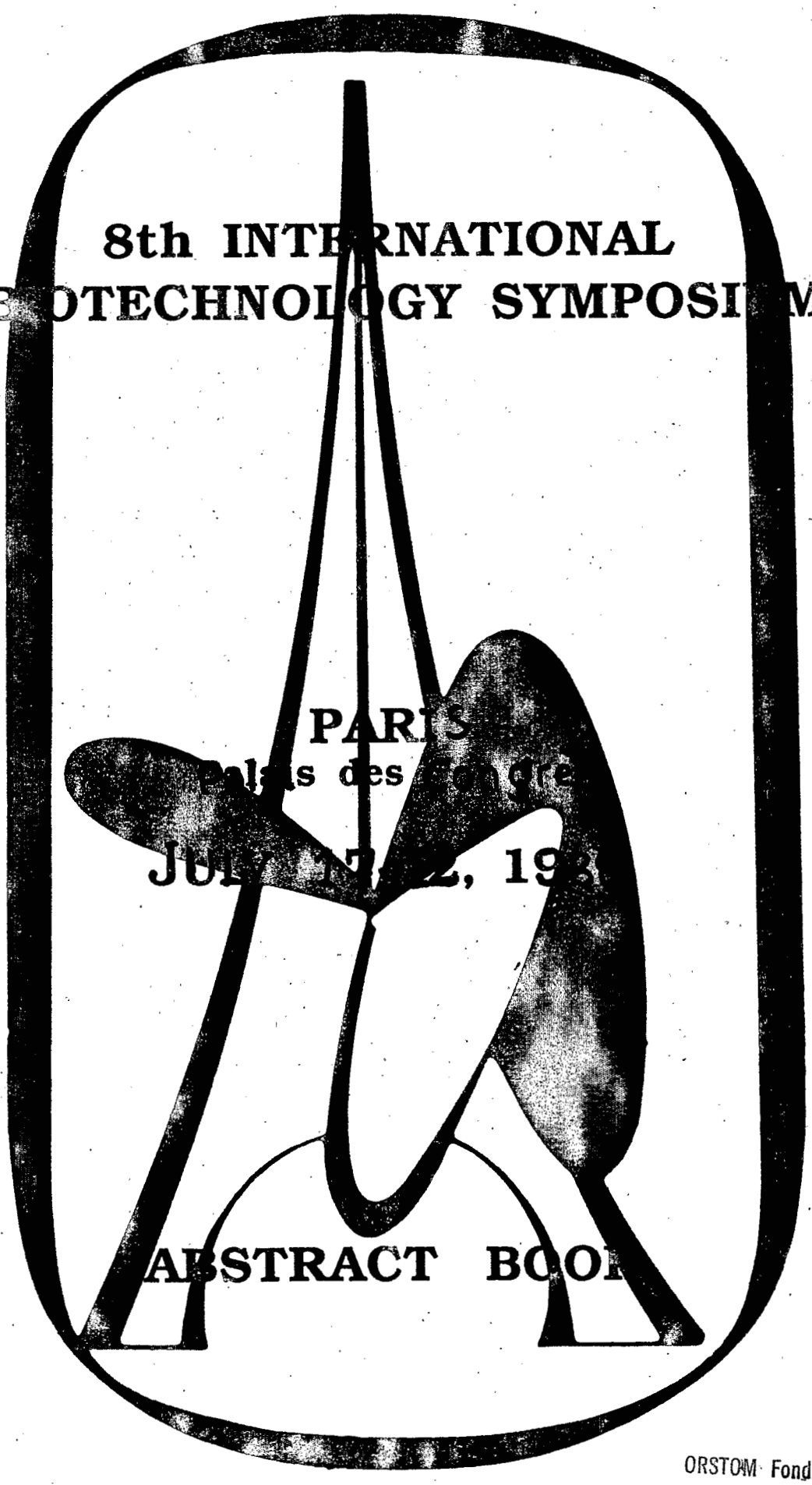

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ABSTRACT BOOK