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BIOLOGICAL CONTROL OF THE OIL PALM PEST *LATOIA VIRIDISSIMA* [*LEPIDOPTERA, LIMACODIDAE*], IN COTE D'IVOIRE, BY A NEW PICORNAVIRUS

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Among the major oil palm pest insects in the Côte d'Ivoire, *Latoia viridissima* Holland [*Lepidoptera, Limacodidae*] is the most frequently observed defoliator. During a pullulation of this species, a natural epizootic permitted us to demonstrate the occurrence of a small isometric RNA virus of 30 nm in diameter. The buoyant density of the virus particles was 1.34. The virus capsid contained 2 major proteins with molecular weights of 30,000 (55 %) and 31,000 (20 %) and 3 minor proteins. One genome component was detected with molecular weight $2{,}9 \times 10^6$. Agarose gel diffusion tests showed this virus was distinct from any other described insect Picornavirus.

Trials with different doses of viral suspensions were tested on industrial oil palm plantation, allocated by *L. viridissima,* from ground level, using an automatic air carried sprayer. One week after the treatment, a mortality gradient, increasing from 11 to 61 % according to the dose applied, was obtained. Two weeks after the treatment the mortality reached 92 % of the larvae in the treated parcels. During the next generation, the number of caterpillars on the same parcel was very low.

KEY-WORDS : *Latoia viridissima, Limacodidae,* Biological Control, insect Picornavirus, RNA virus.

Three larvae of *Lepidoptera Limacodidae* are known as defoliators of *Palmacae* in Côte d'Ivoire : *Latoia viridissima* Holland, *Latoia pallida* Möschl (**Mariau** *et al.*, 1981) and *Casphalia extranea* Walker (**Fedière,** 1983). During outbreaks of *L. viridissima,* the first in June 1979, in coconut plantation of I.R.H.O., Port-Bouet, situated near the coast to the south of Abidjan, and the second in September 1983 on oil palm at the Palmindustries plantation at Eloka, east of Abidjan near Bingerville, natural epizootics were observed and dead infected larvae were collected. Extracts of these caterpillars were examined by electron microscope and virus-like particles of 30 nm in diameter were found.

Isolates of 6 families of viruses pathogenic to insects have biological properties which shoud lead to their successful use as microbial control agents (**Payne,** 1982 ; **Entwistle,** 1983).

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Within the *Limacodidae,* several viruses have been used as biological control agents ; for example the Densovirus from *Sibine fusca* Stoll in Colombia (**Genty & Mariau,** 1975) and the virus β Nudaurelia from *Parasa lepida* Cramer in Indonisia (**Ginting & Desmier de Chenon,** 1987).

In this paper we report some properties of the virus which seems to be distinct from any other previously described insect picornarivus, and the results of field trials with a viral suspension tested in oil palm plantation.

MATERIALS AND METHODS

PURIFICATION AND CHARACTERIZATION OF THE VIRION

VIRUS STRAIN :

Dead infected larvae of *L. viridissima* were collected from coconuts plantation at Port Bouët in June 1979 and from oil palm plantation at Eloka, in September 1983.

Purification of the virus :

Extracts of insects either for infectivity assays or for virus characterization were prepared by homogenizing the infected larvae in 0.05 M Tris-Buffer (T.B.), pH 7.8, containing 0,5 % Sodium Dodecyl Sulfate. The extract was squeezed through Cheesecloth and the emulsion was centrifuged at 8,000 g for 10 min, the supernatant fluid was kept and the pellet re-extracted twice by sonication in T.B. The resulting supernatants were mixed and the virus was pelleted by centrifuging at 145,000 g for 1 h 30 at 4 °C. The pellet was allowed to resuspend overnight in small volumes of TB in the ratio of 1 ml for 5 g of infected larvae. This viral suspension was used for the pathogenicity tests and for all the field trials with different doses.

The partially purified suspension was then deposited on a 15 to 45 % (W/W) sucrose gradient in 0.05 M phosphate-Buffer (P.B.) pH 7.4, and centrifuged for 2 h at 200,000 g. The band containing the virus was collected, dialyzed, and the particles were further purified by 2 additional cycles of centrifugation in sucrose gradients. The virus particles were concentrated as above and stored at − 30°.

Electron microscopy :

Purified virus preparations were negatively stained with 2 % (W/W) uranyl acetate and the grids examined in a Siemens elmiskop 102 electron microscope.

Determination of the chemical composition of the virion :

Virus samples were tested for nucleic acid by the orcinol method (**Mejbaum,** 1939) and the diphenylamine reaction (**Giles & Myers,** 1965).

Determination of buoyant density of virus particles :

Buoyant density of the virus was determined in CsCl gradients. Virus samples were deposited on pre-formed 15 to 45 % (W/W) CsCl gradients and centrifuged at 200,000 g

for 16 h at 20 °C. Fractions of 0.3 ml were collected using an Isco gradient fractionator and their density was determined from measurements of their refractive index at 20 °C, according to **Rowlands, Sangar & Brown** (1971).

Spectrophotometric measurements :

U.V. absorption of purified virus was examined using a Beckman UV 5230 spectrophotometer.

Electrophoresis of virus polypeptides in S.D.S. polyacrylamide gels :

The size and number of proteins in the virus particles were assessed by comparing their electrophoretic mobilities with those of various standard marker proteins in 7.9 and 11 % polyacrylamide gels (**Weber & Osborn,** 1969).

Gel electrophoresis of virus RNA :

The virus genome was extracted from particles by proteinase K and sarkosyl treatment (**Hilz** *et al.,* 1975). The size and the number of RNA fragments were estimated by comparing their rate of electrophoretic migration in 2.5 % polyacrylamide gel (**Peacock & Dingman,** 1968) with those of the *Drosophila* C Virus (**Jousset** *et al.,* 1977) and Cricket Paralysis Virus (**Eaton & Steacie,** 1980) with molecular weights $3.0 \cdot 10^6$ and $2.8.10^6$ daltons respectively.

Antisera and serological tests :

Antisera were prepared in rabbits by intraveinal injection of 1 ml antigen (500 µg/ml) and intramuscular injection twice at weekly intervals with virus preparation emulsified in Freund's complete adjuvant. Gel immunodiffusion tests were done in 1 % agarose in P.B. (**Ouchterlony,** 1948).

PATHOGENICITY TESTS AND TREATMENT TRIALS

Pathogenicity tests :

This laboratory test was performed with only older larval stages of *L. viridissima* (fig. 1). Oral infection is by far the most common way of infection under natural conditions and per os infection of larvae was carried out by spreading oil palm leaflets with the viral suspension described above. The insects were kept in finemesh cages (30 × 20 × 20 cm), in groups of 100. Temperature and humidity conditions were identical with those in the plantation. 15 palm folioles were placed in each cage and changed every 2 days.

Field trials :

A *L. viridissima* pest outbreak had occurred in the oil palm plantation of the I.R.H.O. La Mé Station. In this place the palm-trees are 3 years old, 4-5 m high and do not have yet a large leaf volume. The palm trees were planted in an equilateral triangular pattern, 9 m per side, with an interline distance of 7-8 m. We report the results obtained with a hand-operated compressed-air knapsack sprayer.

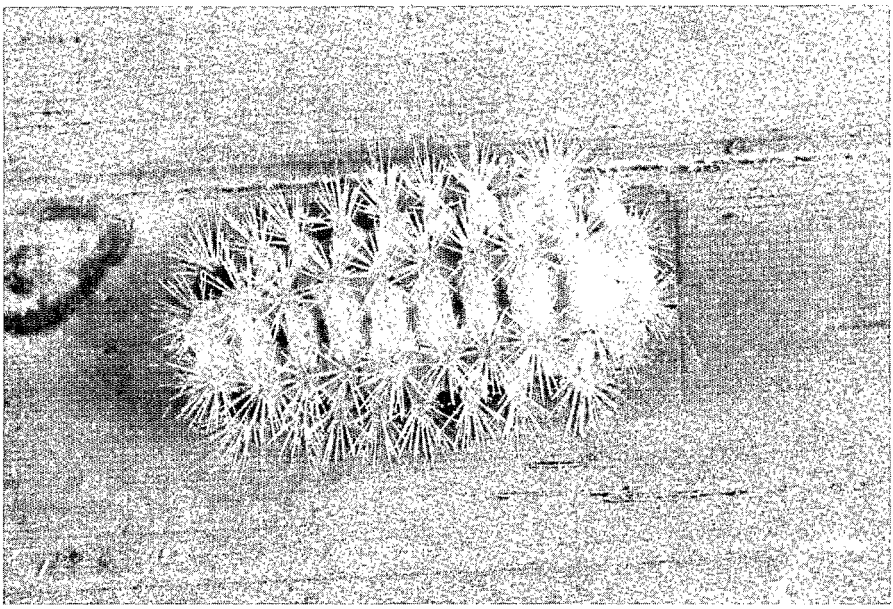

Fig. 1. Older larval stage of *L. viridissima* (2 cm length).

Each palm frond was treated for 30 s and all fronds per tree were sprayed. After each tree was treated the pressure within the knapsack sprayer was checked.

On the plot row n^{os} 1, 5, 9, 13, 17 and 21, we looked on the 5 trees at the northern end. On each tree, caterpillars were counted before and twice after treatment at weekly intervals on fronds at level 9, 17 and 25.

The row n^{os} 5, 13 and 21 were used as reference, without treatment. On the row 1, the viral suspension (5 g of infected larvae per ml) was sprayed, after dilution in water, at a dose of 0.017 ml/palm, i.e. 425 g of dead larvae/ha. On the row 9, 0.076 ml/palm i.e. 1,902 g/ha. On the row 17, 0.148 ml/palm i.e. 3,704 g/ha. The dose rate per hectare was estimated by multiplying the result obtained per palm frond, first by 35 to obtain the dosage per tree, and then by 143, the number of trees per hectare.

RESULTS AND DISCUSSION

CHARACTERIZATION OF THE VIRION

Examination of purified viral suspensions under the electron microscope showed large numbers of isometric particles 30 nm in diameter (fig. 2) similar to those found within the *Picornaviridae*.

The U.V. absorption spectra of virus preparations served as criterion of their purity. The spectra were typical of a nucleoprotein, with a maximum at 260 nm and a minimum at 240 nm. The average ratio of extinction at 260 nm to that at 280 nm was 1,72.

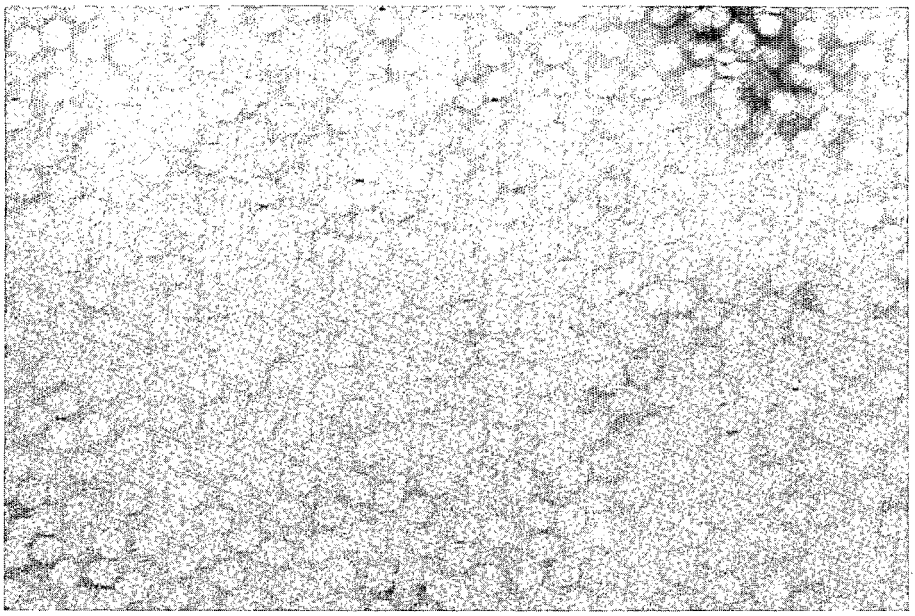

Fig. 2. Electron micrograph of the negatively stained virus particles 30 ± 1 nm in diameter isolated from *L. viridissima* (× 130 000).

The purified virus preparations gave positive orcinol but negative diphenylamine reactions indicating that the virus contains only RNA, protein and no detectable DNA.

The buoyant density of the virus was 1.34 determined after CsCl isopynic centrifugation which could be analogous to that of insect Picornaviruses.

Two major proteins (VP 1, VP 2) were found in purified virus particles. The average mol. wt. values obtained from 6 determinations were 30,000 (VP 1) and 31,000 (VP 2) accounting for 55 % (VP 1) and 20 % (VP 2) of the total virion protein. These polypeptides corresponded in their molecular weight to the VP 2 and VP 3 reported for most of the insect picornaviruses but instead of the VP 1, 3 minor bands were also detected in the gels.

Analyses of the viral RNA by polyacrylamide gel electrophoresis revealed a unique band with a molecular weight estimated to be $2.9 \cdot 10^6$ daltons.

TABLE 1

Efficiency of L. viridissima *virus in laboratory*

Days after infection	3	5	7
Infected larvae Population reduction (%)	73	89	90
Control larvae	12	28	34

Serological studies using the antiserum prepared against purified virus revealed that the virus is serologically different from *Drosophila* C Virus as well as Cricket Paralysis Virus.

From the physical and chemical properties of this small isometric RNA virus of *L. viridissima* we propose to classify it provisionally as an atypical member of the *Picornaviridae* according to the ICTV classification (**Matthew,** 1982).

PATHOGENICITY TESTS AND FIELD TRIALS

Pathogenicity tests in laboratory :

This test was performed with 2,559 infected larvae and 1,072 control larvae of *L. viridissima.* Results recorded in table 1 reveal that in laboratory, the *L. viridissima* virus is very efficient. Five days after infection the mortality reached 89 % of the larvae. The mortality observed in the controls was attributable to a natural infection by the virus, latent within the population and increased by stress resulting from captivity.

Field trials :

These observations are based upon 500 to 1,500 specimens counted on between 35 to 52 palm fronds.

The results are summarized in table 2.

TABLE 2

Treatments trials with the viral suspension of L. viridissima

	Row 1 dose 425 g/ha	Row 5 control	Row 9 1,902 g/ha	Row 13 control	Row 17 3,704 g/ha	Row 21 control
One day before the treatment. Average number of caterpillars per palm frond	50	69	64	60	80	33
One week after the treatment. Population reduction	11 %	7.8 %	44.2 %	40.9 %	61.4 %	49.2 %
Two weeks after the treatment	83.5 %	85.4 %	96.6 %	99.5 %	97.2 %	97.1 %

It is interesting to note, that one week after the treatment, the mortality gradient increased from 11 to 61 % depending on dosage rates used.

Two weeks after the treatment, the epizootic caused the death of 92 % of the larvae on the treated field.

These trials included contaminated controls ; one week after spraying there was no significant differences (Student's test) between the treatments and their controls. The mortality observed in the reference rows proceeds from the rapid dispersion of the virus infection. Virus may be liberated by the breakdown of the host and may be spread very widely by the action of wind, rain and associated fauna. The number of caterpillars during the subsequent generations in the treated areas was nil to very low. Virus can remain

infectious for months in host corpses, which may adhere to plants for long periods and we know that the immature stages of this species develop exclusively on the palm fronds, from oviposition to pupation (**Fediere,** 1983).

Now, after these small scale field trials, it should be interesting for control to determine the effective minimum virus dosage required.

RÉSUMÉ

Lutte biologique contre *Latoia viridissima* [*Lepidoptera, Limacodidae*] ravageur du palmier à huile en Côte d'Ivoire, par un nouveau Picornavirus

Parmi les principaux insectes ravageurs du palmier à huile en Côte d'Ivoire, *Latoia viridissima* Holland [*Lepidoptera, Limacodidae*] est le défoliateur le plus couramment observé. Lors d'une pullulation de cette espèce une épizootie nous a permis de mettre en évidence un petit virus icosaédrique à ARN de 30 nm de diamètre. La densité apparente de la particule virale est de 1,34. La composition polypeptidique du virus consiste en 2 protéines majeures de faible poids moléculaire 30 000 (55 %) et 31 000 (20 %) et en 3 protéines mineures. Le génome viral est constitué d'un fragment d'ARN dont le poids moléculaire est estimé à $2{,}9 \cdot 10^6$. Des tests d'immunodiffusion en gel d'agarose montrent que ce virus est distinct des autres Picornavirus d'insectes connus.

Des essais de traitements terrestres ont été menés sur une plantation industrielle de palmiers à huile envahie par *L. viridissima,* avec différentes doses de suspension virale en utilisant un pulvérisateur automatique porté. Il est intéressant de noter une semaine après le traitement un gradient de mortalité augmentant de 11 à 61 % selon la dose utilisée. Deux semaines après le traitement l'épizootie causa la mort de 92 % des larves sur la parcelle traitée. Le nombre de chenilles durant la génération suivante sur cette parcelle fut presque nul.

MOTS CLÉS : *Latoia viridissima, Limacodidae,* lutte biologique, Picornavirus d'insectes, virus à ARN.

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