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Biological control of a Limacodid oil palm pest in Ivory Coast, by use of a small isometric virus

by

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INTRODUCTION

Three larvae of Lepidoptera Limacodidae are known as defoliators of Palmaceae in Ivory Coast : Latoia viridissima and Casphalia extranea on oil palm and Latoia pallida on coconuts. During an outbreak of the species L. viridissima, in march 1981, on the Palmindustries Eloka oil palm plantation, a natural epizootie was observed and dead infected larvae were collected. Extracts of these caterpillars were examined in electron microscope and virus-like particles of 30 nm in diameter were found.

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

Materials and methods

A - Purification and characterization of the virion

- Virus strain : Dead infected larvae of L. viridissima were collected from oil palm plantation at Eloka, in the South East of Ivory Coast, and these caterpillars were stored at - 30° C.

- Purification of the virus : Extracts of insect 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% S.D.S.. The extract was squeezed through cheese-cloth and the emulsion was centrifuged ant 8.000 g. for 10 min. the supernatant fluid was kept and the pellets re-extracted twice by sonication in the same volume of 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 to obtain a final concentration of 5 g. of frozen larvae per ml. This viral suspension was used for the pathogenicity tests and for all the field trials with different doses.

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The partially pure 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 light scattering band was collected, dialyzed and the particles were further purified by two additional cycles of sedimentation 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 elminskop 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 and Myers, 1565).

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 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 and 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 and 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 fragment were estimated by comparing its rate of electrophoretic migration in 2,5 % polyacrylamide gel (Peacock and Dingman, 1968) with these of the Drosophila C virus (Jousset et al. ,1977) andCricket paralysis virus (Eaton and Steacie, 1980) which are $3,0\ 10^6$ 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).

A - Pathogenicity tests and treatment trials

- Pathogenicity tests :

It is now well established that larval susceptibility to many virus decrease sharply as larvae get old (Evans, 1981), so, this laboratory test was effected with only older larval stages of L. viridissima.

Oral infection is by far the most common under natural conditions and the infection per os of larvae was carried out by painting oil palm leaflets with the viral suspension described above.

- Field trials :

A L. viridissima pest outbreak had occurred in the oil palm plantation of the I.R.H.O. La Mé Station on the plot F 52. In this place the palm-trees are 3 years old, 4-5 m high and do not yet have a large leaf volume. Two ground-level automatic sprayers were used : air-carrier sprayers (motorised appliance) and hand-operated compressed-air knapsack sprayers. In this paper we report the results obtained with the second equipment.

On the row n° 1, 5, 9, 13, 17 and 21 of the plot, we have look on the 5 trees at the north. 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 5, 13 and 21 were used as reference, without treatment. On the row 1, viral suspension, described above, was sprayed at a dose of 0,017 ml/palm,, that is to say 425 g. of dead larvae/ ha. on the row 9, 0,076 ml/palm i.e. 1902 g./ha. on the row 17, 0,148 ml/palm i.e. 3704 g./ha.

Results and discussion

A - Characterization of the virion

Examination of virion suspension under the electron microscope showed large numbers of isometric particles of 30 nm in diameter.
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 mμm and a minimum at 240 mμm. The average ratio of extinction at 260 mμm to that at 280 mμm was 1,72.

The purified virus preparation gave positive orcinol but negative diphenylamine reactions showing that the virus contains only RNA, protein and no detectable DNA.
The buoyant density of the virus was 1,34 g/ml after centrifugation at 200.000 g for 16 h at 20° C in pre-formed Caesium Chloride gradients.

Two major proteins (V P 1, V P 2) were found in purified virus particles which were analyzed by S.D.S - polyacrylamide gels electrophoresis. The average mol. wt values obtained from 6 determinations were 30. 000 (V.P 1) and 31.000 (V.P.2) accounting for 55 % (V P 1) and 20 % (V P 2) of the total virion protein. These polypeptides corresponded in their mol wt to the V P 2 and V P 3 reported for most of the insect picornaviruses but instead of the V P 1, three minor band with mol wt of 39.000 (8%), 44.000 (8%) and 51.000 (9%) were also detected in the gels.

The molecular weight of the RNA extracted from virus particles was estimated electrophoretically in 2,5 % polyacrylamide gel to be $2{,}9.\ 10^6$ daltons. This electrophoresis show that the virus genome is constituted by one RNA segment.

Serological studies with the antiserum of purified virus revealed that the virus is serologically quite different from Drosophila C virus as well as cricket paralysis virus.

From the physical and chemical properties mentioned above, this small isometric RNA virus of L. viridissima have provisionally been set aside from the Picornaviridae family, principally on account of its polypeptide composition.

B - Pathogenicity tests and field trials

- Pathogenicity tests in laboratory

These investigations have used 2559 infected larvae and 1072 reference larvae of L. viridissima.

Table I : Efficiency of L. viridissima virus in laboratory

Days after infection	3	5	7
infected larvae Population reduction(P.100)	73	89	90
reference larvae	12	28	34

Now, in order to show the effectiveness of the virus, we must determine the L D 50 for the host insect and establish the host range of the virus.

- Field trials

The results are collected in the table II.

Table II : Treatments trials with the viral suspension of L. viridissima.

	Row 1 dose : 0,017ml/palm i.e. 425 g/ha	Row 5 reference	Row 9 0,076ml/palm i.e. 1902g/ha	Row 13 reference	Row17 0,148 ml/palm i.e.3704g/ha	Row 25 reference
one week after the treatment. Population reduction (p.100)	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, one week after the treatment, a mortality gradient increasing from 11 to 61 % depending on dosage rates used.

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

The mortality observed in the reference rows proceed from the rapid spatial spread 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 which may persist in the soil. There is no doubt that soil constitutes an important long term reservoir.

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

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