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Rayado fino virus: detection in salivary glands and evidence of increase in virus titer in the leafhopper vector *Dalbulus maidis*

Sumario. El virus del rayado fino del maiz se detectó en las glándulas salivales de insectos transmisores (*Dalbulus maidis*) examinadas al microscopio electrónico. Por otra parte, utilizando la técnica serológica de ELISA (enzyme-linked immunosorbent assay) se evidenció un incremento gradual del virus en el cuerpo de los insectos después de su adquisición. Estos resultados unidos a la información existente sobre el comportamiento biológico del virus en el vector, tipico de virus propagativos, son congruentes con la hipótesis de la multiplicación del virus del rayado fino en *D. maidis*.

Maize rayado fino virus (RFV) (4, 5) is transmitted by *Dalbulus maidis* DeLong & Wolcott in a persistent manner typical of viruses that multiply in their leafhopper vector. The existence of a long incubation period of 8-37 days, the retention of the virus through the moults, the lack of effect of abdominal punctures or remission by high temperature of transmission, have been considered as strong indirect evidence of a propagative cycle of the virus in *D. maidis* (3, 4, 5, 7, 9, 14). These characteristics are usually associated with propagative viruses (1, 13). Direct evidence of multiplication of RFV in *D. maidis* was still lacking.

The present paper reports the detection of the virus in the salivary glands of *D. maidis* by electron microscopy, and presents evidence of increase in virus titer in the insect's body. These results are compatible with the hypothesis that the virus multiplies in its insect vector.

Materials and Methods

The type strain of the virus (4) was used in this study and the transmission tests by *D. maidis* were performed as previously described (3). Antisera were produced by injecting rabbits with purified virus preparations (4, 5). The specific IgG fraction was purified by column chromatography through DEAE Sephadex A50, and used for the ELISA assay, which was performed with slight modifications as described by Clark and Adams (2). To determine the presence of virus and the increase in virus titer by ELISA, groups of 22 insects fed on infected plants for 24 hr were tested individually 2, 5, 10, 15, 20, 25 and 30 days after the acquisition feed. Single insects were macerated in 0.2 ml phosphate buffer saline, containing 0.5% Tween 20 and 4% bovine serum albumin. Viral protein concentration in an standard solution was determined as described by Lowry *et al.* (8), and a direct correlation was established between a serial dilutions of the standard and optical density at 405 nm. Foe electron microscopy young nymphs were exposed to infected plants for 24 hr and after an incubation period of 24 days at 22°C on healthy plants, the insects were dissected out and the salivary glands excised. The organs were inmediately fixed, dehydrated and embedded in Spurr resin essencially as previously described (12). Sections were cut using glass knives with a Porter-Blum MT-2B Ultratome, double stained with uranyl acetate and lead citrate, and examined in a HU-12A Hitachi Electron Microscope.

Results and Discussion

The results of the ELISA test are summarized in Table 1. RFV was detected in leafhoppers on day 2 but not on day 5 and again on the subsequent days. This suggests that the viral antigens detected on day 2 represent virus accumulated in the insects during their acquisition feeding period, but the concentration of such ingested virus dropped to undetectable levels by day 5. The number of positive insects increased in the subsequent days, and appears to reach a peak on day 25 decreasing by day 30. A similar behaviour in the number of infective insects has been observed in previous studies with RFV (3). The number of viruliferous insects detected by ELISA is higher than the number of transmitters determined by infectivity tests (3, 7, 9). This suggests that some insects may acquire but never transmit RFV. After day 5, absorbance at 405 nm, and protein concentration, rise with increasing time after virus acquisition, reaching a peak by day 20 and 25, and dropping afterwards. The standard deviation of the absorbance, which may be considered relatively high on days 20 and 25, is due to values much higher than the average.

Table 1. Serological detection of maize rayado fino virus in *Dalbulus maidis*.

Days after* acquisition	Number of viruliferous** insects/insects tested	Absorbance at 405 nm ($\bar{X}$)		Estimated protein **** concentration (ng.)
		Viruliferous	Healthy***	
Control	0/22		0.02 ± 0.02	0.0
2	8/22	0.15 ± 0.06		1.65
5	0/22	0.0		0.0
10	2/22	0.12 ± 0.02		1.32
15	2/22	0.12 ± 0.02		1.32
20	2/22	0.23 ± 0.13		2.46
25	5/22	0.17 ± 0.07		1.94
30	2/22	0.11 ± 0.02		1.19

* Healthy nymphs were given at 24 hr acquisition feed on infected plants and were tested individually by ELISA after the indicated incubation period.

** Insects positive by ELISA/insects tested.

*** Average absorbance of 15 healthy insects, not exposed to infected plants.

**** Viral protein concentration estimated as described by Lowry *et al.* (8).

The increase in absorbance could be explained by increase in virus titer due to replication in the leafhoppers. A similar virus growth pattern has been described for wound tumor virus, a prototype of viruses that multiply in their leafhopper vector (6, 11).

Virus particles were detected by electron microscopy in ascinar cells of the salivary glands of *D. maidis* (Fig. 1) exposed to infected plants but not in healthy insects. Virus aggregates were found in the cytoplasm of infected cells and correspond in size and shape to those observed in diseased leaves and purified preparations (4). Similar virus aggregates have been detected in parallel studies in the intestines and salivary glands of viruliferous *D. maidis* (E. W. Kitajima and R. Gámez, unpublished data). The presence of viral particles in cells of the internal organs may well be considered additional evidence of multiplication of RFV in its insect vector.

Based on the existence of a long incubation period and other characteristics of the virus-vector relationship, Gámez (3, 4) and others (5, 7, 9, 14) had suggested that RFV probably multiplied in *D. maidis* as it was transmitted in a manner typical of propagative viruses. The detection of increase in virus titer and the visualization of virus particles in the internal organs of *D. maidis* may be considered the first direct evidence of the existence of a propagative cycle of RFV in this insect.

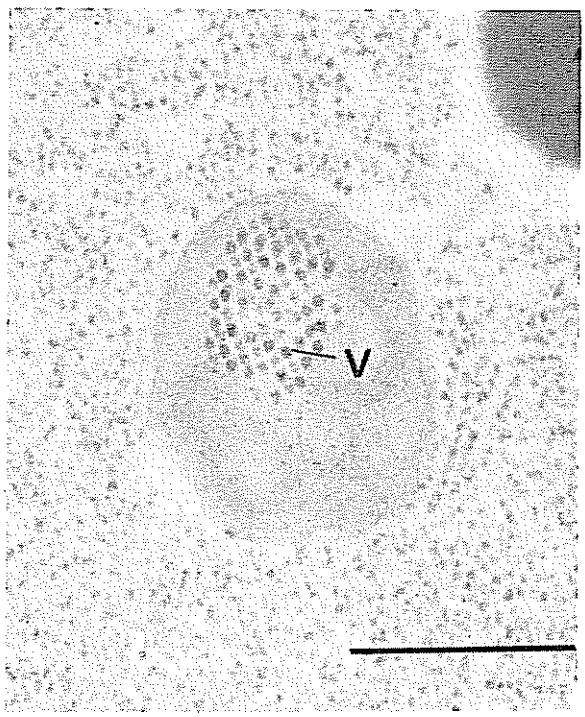

Fig. 1. RFV in the salivary glands of viruliferous *D. maidis*. Virus (V) aggregates were located in ascinar cells of the salivary glands within moderately dense membrane bound matrix. Bar is 0.5 μ.

The detailed description of the application of the ELISA test to RFV, and the results of further studies on the multiplication of RFV in *D. maidis* will be published elsewhere.

The results presented in this paper provide additional evidence in support of the hipothesis (4) that RFV and oat blue dwarf virus appear to represent a new group of small RNA viruses that propagate in their leafhopper vectors.

Note added in proof. In parallel and independent studies, the multiplication of RFV in its vector has been also demonstrated for an U. S. isolate of the virus (Gingery, R. E., D. T. Gordon and L. R. Nault. 1981. Purification and properties of a United States isolate of maize rayado fino virus. Unpublished).

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