Serological detection of Fiji disease virus antigens in the planthopper *Perkinsiella saccharicida* and its inefficient ability to transmit the virus

R. I. B. FRANCKI*, C. C. RYAN†, T. HATTA*, J. ROHOZINSKI* and C. J. GRIVELL*

*Department of Plant Pathology, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia
†Bureau of Sugar Experiment Stations, Indooroopilly, Queensland, Australia

Individual adult *Perkinsiella saccharicida* reared on sugarcane infected with Fiji disease virus (FDV) were confined on healthy plants for 7 days to test for virus transmission and were then sacrificed and assayed for the presence of FDV-specific structures by immunoelectron microscopy. Only 15% of the insects contained FDV antigens and 6% transmitted the virus. FDV-specific antigens were detected in individual planthoppers by immuno-osmophoretic and enzyme-linked immunosorbent assays about as efficiently as by immunoelectron microscopy. The possible reasons for the inefficient acquisition and transmission of FDV by *P. saccharicida* are discussed.

**INTRODUCTION**

Sugarcane Fiji disease virus (FDV) is a member of the genus Fijivirus in the family Reoviridae (Matthews, 1982). The virus is transmitted by the planthopper *Perkinsiella saccharicida* Kirk, causing a serious disease of sugarcane (Hutchinson & Francki, 1973; Egan *et al.*, 1986). The first, second, and possibly third instars, but apparently not adult *P. saccharicida*, can acquire FDV from infected sugarcane and transmit it after a latent period of 12–14 days after which they remain infective for at least 16 days (Egan *et al.*, 1986). However, the proportion of insects transmitting the virus appears to be low (Baber & Robinson, 1951). Transovarial transmission of FDV has also been reported (Chang, 1977). These observations together with evidence from electron microscopy of thin sections of viruliferous insects (Francki & Grivell, 1972), indicate that FDV replicates both in sugarcane and the vector. In this paper we report experiments in which we tested individual insects reared on FDV-infected sugarcane for their ability to transmit the virus and for the presence of FDV antigens in extracts from them by three different methods.

**MATERIALS AND METHODS**

**Virus, plants and insects**

FDV was maintained in infected sugarcane (*Saccharum officinarum* L.) cv. NCo 310 colonized by *P. saccharicida* in an insectary for 4 months prior to the commencement of the experiments. Healthy test plants (cv. Q70) were raised from sets obtained from Tully (North Queensland) where FDV does not occur.

**Virus transmission tests**

Young adult *P. saccharicida* reared on the FDV-infected sugarcane were caged individually on virus-free sugarcane test plants raised from sets planted 3 weeks previously. After 7 days, the insects were recovered and frozen at -20°C until needed for serological tests. The test plants were watered with Metasystox and maintained free of insects in terylene cages for 9 weeks after which the cages were removed and, 1 week later, the plants were inspected for the presence of galls characteristic of FDV infection (Egan *et al.*, 1986). Fifteen test plants were caged without any
insects as controls but none developed disease symptoms.

Serological detection of FDV in insects

Antiserum prepared against FDV core particles, purified from gall tissue of virus-infected sugar-cane (Van der Lubbe et al., 1979), was used in all the serological tests. The antiserum had a titre of 1/64 to FDV core particles and 1/8 to double-stranded RNA in immunodiffusion tests but did not produce any precipitin lines when tested undiluted against extracts from healthy sugar-cane leaves.

To detect FDV antigens by immunoelectron microscopy, each frozen insect was ground in 30 μl of 20 mM phosphate buffer, pH 7.4, unless otherwise stated. A drop of the extract was placed on a piece of parafilm on which was floated a specimen grid with adsorbed antibodies prepared as described by Ofori & Francki (1983). After 30 min, the grids were rinsed twice with distilled water, stained with uranyl acetate and examined in a JEM 100 CX electron microscope.

Imuno-osmophoretic and enzyme-linked immunosorbent assay (ELISA) tests were done as previously described (Rohozinski et al., 1981; Chu & Francki, 1982).

RESULTS

Transmission of FDV by individual P. saccharicida and detection of virus-specific structures by immunoelectron microscopy

Data from three experiments (Table 1) show that 82 out of the 537 insects tested (15%) contained structures associated with FDV infection (Fig. 1): cores (indicated by arrows) which are degradation products of FDV particles (Hatta & Francki, 1977) and helical strands (indicated by arrowheads) which are thought to be constituents of viroplasm from infected cells (Hatta & Francki, 1981). Intact FDV particles were not detected and it is known that they are very unstable (Hatta & Francki, 1977). In other experiments, no FDV-specific structures were detected in P. saccharicida reared on healthy sugar cane.

All the planthoppers reared on FDV-infected sugarcane and tested for the presence of virus-specific structures were kept for 7 days on healthy plants before being sacrificed for immunoelectron microscopy. This precludes the possibility that the FDV-specific structures detected were from the insect gut, but indicates that the planthoppers were infected with the virus. Of the 82 infected insects, only 34 (41%) transmitted FDV.

<table>
<thead>
<tr>
<th>Status of insects</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survived after transmission test</td>
<td>209/277</td>
<td>167/216</td>
<td>161/201</td>
<td>537/694</td>
<td>77</td>
</tr>
<tr>
<td>Survivors which transmitted FDV and contained virus-specific structures</td>
<td>22/209</td>
<td>9/167</td>
<td>3/161</td>
<td>34/537</td>
<td>6-3</td>
</tr>
<tr>
<td>Survivors which failed to transmit FDV but contained virus-specific structures</td>
<td>18/209</td>
<td>22/167</td>
<td>8/161</td>
<td>48/537</td>
<td>8-9</td>
</tr>
<tr>
<td>Survivors which transmitted FDV but no virus-specific structures were detected</td>
<td>1/209</td>
<td>3/167</td>
<td>0/161</td>
<td>4/537</td>
<td>0-7</td>
</tr>
</tbody>
</table>

*aCaged individually on healthy sugarcane plants for 7 days.
*bNumber of planthoppers observed/total number of insects in treatment.
Fig. 1. Detection of FDV cores (arrows) and helical strands (arrowheads) by immunoelectron microscopy of individual Perkinsiella saccharicida. Both structures are virus-specific (see text for explanation). Bar represents 100 nm.

during feeding on test plants for 7 days (Table 1). Only 4 of the 455 (0.9%) insects in which no virus-specific structures were detected transmitted virus.

Detection of FDV-specific antigens in individual P. saccharicida by immuno-osmophoresis and ELISA

The suitability of immuno-osmophoretic and ELISA methods for detecting viral antigens in P. saccharicida was tested. Both methods have been shown to be satisfactory for detecting FDV in virus-induced galls on sugarcane, ELISA being the more sensitive (Rohozinski et al., 1981).

In a series of tests, each of 150 insects from the colony reared on FDV-infected sugarcane was ground in 100 µl of 0.14 M phosphate buffer, pH 7.4; 30-µl and 10-µl aliquots were used for ELISA and immuno-osmophoresis respectively. The remaining extracts were frozen and some used later for immunoelectron microscopic assay. Results (Fig. 2) showed that extracts from 116 of the insects (77%) produced colour reactions below OD$_{405}$ nm = 0.2 in ELISA; none of these produced positive reactions in the immuno-osmophoretic tests. When extracts from eight of these insects were taken at random and tested by immunoelectron microscopy, no FDV-specific

Fig. 2. Detection of FDV-specific antigens in individual Perkinsiella saccharicida by ELISA and immuno-osmophoretic tests. The shaded area indicates insects that assayed positive for the virus in immuno-osmophoretic tests.
structures were detected (insects 1–8, Table 2). It can thus be concluded that they were from uninfected insects.

Extracts which reacted strongly in ELISA (OD<sub>405 nm</sub> > 0-4) accounted for 16% (24 out of the 150 insects) of the samples, and all produced positive immuno-osmophoretic reactions (Fig. 2). When some of these extracts were checked by immunoelectron microscopy all were shown to contain virus particles (insects 14–20, Table 2) and thus all were infected with FDV. The variation in colour intensity among the samples in ELISA tests (Fig. 2) suggests that FDV antigen concentration in the insects varied considerably. From 11 of the extracts which reacted weakly in ELISA (OD<sub>405 nm</sub> between 0-2 and 0-4), virus was detected by immuno-osmophoretic tests in seven but not in the other four extracts (Fig. 2). Furthermore, there were discrepancies in the immuno-osmophoretic and immunoelectron microscopic assays on such samples (insects 9–13, Table 2). These extracts may have contained low concentrations of FDV which were near the limit of detection by both tests. Of the 150 insects used, a maximum of 35 (23%) appear to have been infected by FDV, a proportion greater than that in the experiments detailed in Table 1.

**DISCUSSION**

It appears that immunoelectron microscopy, ELISA and immuno-osmophoretic tests are all useful but not perfect methods of screening *P. saccharicida* for FDV infection. They are not completely reliable when testing insects containing low concentrations of virus (Table 2). Immunoelectron microscopy has the advantage that it is based on the visual detection of virus-specific structures (Fig. 1) but has the disadvantage of being time-consuming and hence presents logistic problems for mass sampling. Both ELISA and immuno-osmophoretic tests are more suitable for handling large numbers of samples. Of these, ELISA has already been shown to be more sensitive for detecting FDV antigens in sugarcane tissues (Rohozinski et al., 1981) and to be a sensitive and efficient method of detecting antigens of another Fijivirus, maize rough dwarf, both in plants and individual planthopper vectors (Caciagli et al., 1985).

There is little doubt that only a low proportion (less than one in four) of the insects were infected with the virus even though they were reared on infected sugarcane for at least three generations (4 months). Moreover, less than half of the infected insects transmitted FDV in spite of having access to the test plants for 7 days. These data confirm the conclusion reached by Baber & Robinson (1951) and our own casual observations (unpublished) that the planthopper is an inefficient FDV vector.

There are several possible reasons why such a
large proportion of the planthoppers escaped infection. Among these are: (1) the colony may have been genetically heterogeneous, some of the insects being immune to FDV; (2) the feeding habits of *P. saccharicida* and the distribution of virus in the plant tissues may have been such that the chance of an insect ingesting sufficient virus for infection was rare; (3) the virus taken up may have been insufficiently stable to have reached susceptible cells of the insect gut as infectious particles. There are also several factors which could be responsible for inability of some of the infected insects to transmit the virus to test plants. The feeding behaviour of *P. saccharicida* on sugarcane could account for the lack of transmission, resulting in introduction of virus particles into susceptible cells of the plant being a rare event. Instability of the virus particles *in vitro* (Hatta & Francki, 1977) could also be an important factor.

ACKNOWLEDGEMENTS

This work was supported by grants from the Rural Credits Development Fund of the Reserve Bank of Australia, the Australian Department of Primary Industry and the Utah Foundation.

REFERENCES


This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.