VECTOR-VIRUS RELATIONSHIP FOR *MELANAPHIS SACCHARI* (ZEHNT.) (HEMIPTERA: APHIDIDAE) TRANSMITTING SUGARCANE YELLOW LEAF LUTEOVIRUS IN MAURITIUS

By

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Abstract

SUGARCANE yellow leaf virus (ScYLV) was reported in Mauritius in 1995. It spreads by vegetative propagation and is transmitted to healthy plants by the aphid, *Melanaphis sacchari* (Zehnt.) that prevails at a very low density in commercial plantations. The relationships pertaining to the vector and the luteovirus were investigated under laboratory conditions, as an understanding of the acquisition and transmission of the virus by the vector is a prerequisite for further studies on the epidemiology, economic importance and management of the disease at field level. An appropriate aphid rearing technique was developed so as to establish cultures of infected and healthy aphids for the relevant studies. To determine the acquisition time of the virus by aphids, infected plants of the sugarcane variety M 695/69 were infested with non-viruliferous aphids which were sampled daily over 4 consecutive days and tested by RT-PCR. *M. sacchari* acquired the virus within 24 h and more precise tests in the insectary, whereby aphids were sampled every 30 min over 6 h, indicated that the virus was acquired after 4.5 h of feeding on infected plants. The time taken for the viruliferous aphids to transmit the virus to disease-free tissue cultured plantlets was determined by allowing infected aphids from the breeding cages to feed on the plantlets for periods of 2, 8, 17 and 28 days. The virus was transmitted to healthy plants within 2 days. This is the first record of *M. sacchari* being a vector of ScYLV in Mauritius and the present laboratory data provide basic information for future field experimentation and research work on vector-virus interactions.

Introduction

Yellow leaf (previously referred to as yellow leaf syndrome) was first reported in Hawaii in 1988. The disease, which is now widespread across the world, is caused by Sugarcane yellow leaf virus (ScYLV), a Polerovirus of the Luteoviridae family (D'Arcy and Domier, 2005) which also contains barley dwarf virus, soybean dwarf virus, potato leaf roll virus and pea mosaic virus (Comstock and Gilbert, 2008). ScYLV is limited to the phloem tissue and is spread mainly by vegetative propagation through infected cuttings and transmitted to healthy plants by aphids (Rassaby *et al.*, 2004; Comstock and Gilbert, 2008). The aphid species proven to transmit the virus are the sugarcane aphid, *Melanaphis sacchari* (Zehntner), the corn leaf aphid *Rhopalosiphum maidis* (Fitch) and the rice root aphid, R. *rufiabdominalis* (Sasaki). The former species is a vector of three persistent viruses, namely millet red leaf, sugarcane yellow leaf and sugarcane mosaic viruses (Singh *et al.*, 2004). Not all aphid species infesting sugarcane are vectors of ScYLV (Schenck and Lehrer, 2000).

The disease was reported in Mauritius in 1994 but its epidemiology and the effect on yield were not clear. Symptoms were subsequently observed in several commercial varieties (Saumtally and Moutia, 1997), although the aphid population in commercial plantations is low. This paper

describes the incidence of the vector in the fields and studies in the insectary relating to the establishment of cultures of healthy and infected aphids, the determination of the acquisition time of the virus by aphids and the transmission of the disease to healthy plants. These studies will give a better understanding of the relationships pertaining to the vector and the virus. Data from these investigations may help to implement appropriate measures for timely provision of infected aphids for future studies on yield assessments of different varieties and the rate of transmission and ScYLV dissemination in the field with respect to the different genotypes detected locally.

Materials and methods

Incidence of aphids

To assess the potential of early infection in the epidemiology of the disease, surveys were initially conducted in transplanted seedling plantations set on four experimental stations located in the subhumid, humid and superhumid zones of Mauritius. Potted seedlings in the shadehouses at Réduit (humid region) were also surveyed.

Field surveys were then conducted to identify the aphid species, their distribution and their incidence in the various agroclimatic zones of the island. Surveys were carried out in commercial plantations and varietal preference was recorded. Leaf samples and aphids, if any, were collected at 25 points selected at random in each field. All aphids were kept in 70% alcohol and tested for whether they were viruliferous according to the method of Rassaby *et al.* (2004). To allow correlations to be made between the virus incidence and the vector population, the leaf samples were tested by tissue blot immunoassays (TBIA) using a cross-absorbed polyclonal antibody and the technique developed by Schenck *et al.* (1997). Confirmatory tests were effected with the leaf samples by reverse transcription polymerase chain reaction (RT-PCR) (Joomun and Dookun-Saumtally, 2008; Khoodoo *et al.*, 2008; MSIRI, 2008b).

Insect rearing

An investigation was carried out in the insectary to determine the most appropriate rearing method for *M. sacchari*. Young potted maize plants, potted sugarcane plants, cut leaves and cut shoots in water were infested with aphids in wooden cages (45 cm x 45 cm x 118 cm) and observations on insect development were recorded weekly over at least two generations.

Acquisition tests

Acquisition tests were conducted to determine whether *M. sacchari* is a potential vector of the ScYLV under controlled conditions in the insectary. Virus-free aphids, which were collected from healthy seedlings, were reared in three wooden insect cages (30 aphids/cage) each containing two cut shoots of infected plants of cane variety M 695/69 in water. After about two weeks, a random sample of five aphids was collected from each of these cages and tested for the presence of the ScYLV in single aphids by RT-PCR (MSIRI, 2008b). Positive results on the potential of *M. sacchari* as a vector of ScYLV then led to the establishment of additional tests to determine the time taken for the vector to acquire and transmit the virus.

About thirty virus-free aphids were collected from seedlings at Réduit and placed in contact with infected shoots of M 695/69 in wooden cages as described above. In addition to the three replicate cages with infected shoots, a control batch was also set with disease-free plantlets of variety R 570. Five aphids were removed from each of the four cages after 24, 48, 72 and 96 hours and kept in 70% alcohol for the detection of the virus.

Further tests were conducted to determine more precisely the acquisition time within 24 hours. One infected shoot of M 695/69 was placed in a conical flask containing water and was artificially infested with about eighty virus-free aphids collected from the seedlings. Three replicate flasks were set up and a control using disease-free plantlets of variety R 570 was also established. Five aphids were removed from each replicate flask every 30 minutes from 10 am for 5 ½ hours and kept in alcohol for virus detection.

Transmission tests

To determine whether infected aphids would transmit the virus to healthy plants, infected aphids from the breeding cages were placed in contact with three disease-free plantlets of R 570 in each wooden cage for different time periods of 2, 8, 17 and 28 days. All aphids from each of the cages were killed after the respective time periods by foliar application and soil drenching with thiamethoxam (Actara 25 WG) at 0.0625 g a.i./L. Samples of five aphids were taken at random from each plant in each cage to ascertain their infective nature. All the plants were subsequently transferred to insect-proof cages; after 45 days, leaves were sampled randomly from all the cages for the detection of the ScYLV by TBIA.

Results

Incidence of aphids

Results from the field surveys showed that the population of aphids in transplanted seedlings on three of the experimental stations was much lower than that at Réduit situated in the humid region. Surveys in the seed beds at Réduit indicated a high infestation and several colonies of about twenty aphids per leaf were sometimes encountered on the small plants despite the weekly cutting back prior to their transplantation to the commercial plantations. No ScYLV infection was detected in the leaves or aphids at the seedling stage on the four experimental stations.

Surveys in the commercial plantations showed that the only recorded aphid species was *M. sacchari* while the other potential vector, *R. maidis*, was not recorded. The population of *M. sacchari* was low island-wide, being prevalent in about 10% of the fields which were visited, except in one field at Savannah where hundreds of aphids comprising all stages were recorded on a few leaves in association with the soft scale, *Pulvinaria iceryi* Sign.

Insect rearing

M. sacchari could be successfully reared for 2–3 generations on young cut shoots of M 695/69 kept in water and this breeding method was adopted throughout the studies. Both apterous and alate forms of *M. sacchari* developed in large numbers (approximately 100–200 aphids per shoot) in the breeding cages. Young maize plants, cut sugarcane leaves and young potted plants were not appropriate for breeding aphids for several generations in the insectary.

Acquisition and transmission tests

Healthy aphids collected from seedlings multiplied rapidly on the infected leaves of cane variety M 695/69 in the insectary. After about four days, leaves showed pronounced yellowing and in about three weeks, a very high population of alate and apterous aphids was observed in all three cages. These aphids were found to be infected with ScYLV while aphids from the control cage tested negative. Laboratory tests clearly demonstrated that *M. sacchari* is a potential vector of ScYLV. Acquisition tests showed that the vector could acquire the virus within 24 hours as reported in the literature and more precise laboratory tests indicated that the virus was acquired after 4.5 hours in two replicate flasks (MSIRI, 2008a).

Tests on the aphids prior to the transmission studies confirmed their infective nature. Investigations on the transmission of the virus in the insectary at Réduit showed that the virus could be transmitted by viruliferous aphids to healthy plantlets of cane variety R 570 within two days (MSIRI, 2008a). All the leaf samples, except those sampled from plants that were exposed for a period of eight days, were found to be infected.

Discussions and conclusions

Field surveys in Mauritius have provided useful information on the epidemiology of ScYLV for the subsequent implementation of appropriate measures for its management and for future studies. The only aphid species that was found to be associated with the disease was *M. sacchari*. Similar investigation of infestation and transmission efficiency reported in the literature showed that *M. sacchari* was the only vector important for the field spread of the disease in Hawaii (Lehrer *et*

al., 2007), the two other *Rhopalosiphum* species being unimportant. In Mauritius, the distribution of *M. sacchari* was limited and its population in the commercial plantations was low as colonies were detected in very few localities.

This contrasts with the situation in Guadeloupe where *M. sacchari* is widespread in all areas and ScYLV appears to be spread by aphid vectors and infected cuttings (Edon-Jock *et al.*, 2007). However, in Louisiana, the incidence and rate of disease increase remained low despite the wide occurrence of the potential vectors (McAllister *et al.*, 2008) which have a low rate of dispersal (Lehrer *et al.*, 2007). In Mauritius, the widespread and high incidence of the virus in commercial varieties, the absence of infected aphids in transplanted seedlings and the low vector population in commercial fields indicate that the main source of infection is through the vegetative propagation of infected cane setts as reported by Khoodoo *et al.* (2008). This is despite the fact that our studies highlighted the short time needed for the vector to acquire and transmit the virus**.**

In this present study, the aphids were killed in alcohol right after removing them from the infected plants. The acquisition time might have been shorter if the aphids were left on healthy plants for some time to allow the virus to multiply in the aphids.

These observations in Mauritius correlate well with previous studies carried out by Edon-Jock *et al.* (2007) in Guadeloupe, where virus incidence was consistently high in R 579 even though colonisation by aphids was low. Similarly, no indication of long-distance transfer via aphids could be seen in Hawaii, indicating that it may be possible to produce and use virus-free seed cane for planting of high-yielding but susceptible cultivars (Lehrer *et al.*, 2007).

Aphid control using insecticides as proposed in the literature should be adopted judiciously. In Mauritius, this practice is not warranted due to the low vector population and because established biological control of major sugarcane pests through a complex of predators and parasitoids may be disrupted by insecticide use.

In countries where control of the vector population is necessary, biological control of the main vector species, *M. sacchari*, integrated within a varietal development program may be attempted as revealed by the high potential of the coccinellid predator, *Diomus terminatus* Say, in adult voracity tests in Louisiana (Akbar *et al.*,. 2009; Akbar and Reagan, 2006).

The present study highlighted the fact that initial contamination occurs in the field probably by aphid vectors and subsequently setts issuing from infected fields help in the transmission of the disease**.** The short acquisition and transmission time by the vector is definitely of concern, especially as four different genotypes have been detected so far as revealed by genetic diversity studies (Joomun and Dookun-Saumtally, 2008; MSIRI, 2008b).

As genotypes vary in their virulence, future studies can be carried out to determine whether there is a differential pattern in the transmission and acquisition of the different biotypes of the virus by *M. sacchari*. Additionally, the information obtained on transmission and acquisition will be useful to obtain infected plants for field experimentation, using *in vitro* plantlets as initial starting material.

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RELATION VECTEUR-VIRUS DE *MELANAPHIS SACCHARI* (ZEHNT.) (HOMOPTÈRE: COCCIDE), VECTEUR DU VIRUS DE LA FEUILLE JAUNE DE LA CANNE À SUCRE

Par

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MOTS-CLÉS: *Melanaphis sacchari,* Acquisition, Transmission, Feuille Jaune.

Résumé

LA MALADIE de la feuille jaune de la canne à sucre à été répertoriée à Maurice en 1995. Le virus (*Sugarcane yellow leaf virus* – ScYLV) est propagé par boutures infectées et est transmis par le puceron *Melanaphis sacchari* (Zehnt.) présent à faible densité dans les plantations commerciales. Des études ont été effectuées au laboratoire sur la relation virus-vecteur, car des données sur l'acquisition et la transmission du virus sont nécessaires pour déterminer l'épidémiologie, l'impact économique et la gestion de la maladie. Une méthode fut établie pour l'élevage en masse de pucerons infectés et sains. L'acquisition du virus fut déterminée en exposant les pucerons sains à des plantes infectées de la variété M 695/69. Des échantillons de pucerons ont été prélevés chaque jour sur une période de quatre jours et analysés par RT-PCR. Les résultats démontrent que le virus est acquis dans les 24 heures et des essais plus précis, en prélevant des pucerons chaque trente minutes sur une période de six heures indiquent que le vecteur peut acquérir le virus après avoir été exposé pendant 4.5 heures aux plantes infectées. Le temps requis pour la transmission du virus par le vecteur à été évalué en exposant les pucerons infectés aux plantules *in vitro* pour une période de 2, 8, 17 et 28 jours. Le virus était transmis aux plantules en 2 jours. L'étude démontre que *M. sacchari* est un vecteur du ScYLV et les données préliminaires obtenues seront importantes pour des études plus approfondies au champ.

RELACIONES VECTOR-VIRUS PARA *MELANAPHIS SACCHARI* (ZEHNT.) (HEMIPTERA: APHIDIDAE) TRANSMISOR DEL VIRUS AMARILLO DE LA HOJA LUTEOVIRUS EN MAURICIO

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Palabras clave**: *Melanaphis sacchari*, Adquisición, Transmisión, Hoja Amarilla.**

Resumen

EL VIRUS de la hoja amarilla de la caña de azúcar (ScYLV) se registró en Mauricio en 1995. El virus se dispersa por propagación vegetativa y se transmite a plantas sanas por el áfido, *Melanaphis sacchari* (Zehnt.) que prevalece en poblaciones muy bajas en plantaciones comerciales. Las relaciones que tienen que ver con el vector y el luteovirus se investigaron bajo condiciones de laboratorio, ya que entender el proceso de adquisición y transmisión del virus por el vector, es un prerrequisito para posteriores estudios sobre la epidemiologia, importancia económica y manejo de la enfermedad a nivel del campo. Se desarrolló una metodología apropiada de cría del áfido para establecer cultivos de áfidos infectados y sanos para los estudios pertinentes. Para determinar el tiempo de adquisición del virus por los áfidos, se infestaron con áfidos no virulentos, plantas infectadas con el virus de la variedad de caña de azúcar M 695/69. Los áfidos se muestrearon diariamente durante 4 días consecutivos y se probaron por RT-PCR. *M. sacchari* adquirió el virus en 24 h y ensayos más precisos en el insectario, donde los áfidos se muestrearon cada 30 min durante 6 h, indicaron que el virus se adquirió después de 4.5 h de alimentarse en plantas infectadas. El tiempo que toma a los áfidos viruliferos para transmitir el virus en tejido libre de virus de plántulas se determinó permitiendo a los áfidos infectados de las jaulas de cría alimentarse de las plántulas por periodos de 2, 8, 17 y 28 días. El virus se transmitió a plantas sanas en 2 días. Este es el primer registro de *M. sacchari* como vector de ScYLV in Mauricio y los datos de laboratorio de este estudio proveen información básica para futuras investigaciones de campo y trabajos sobre interacciones entre vector y virus.