

**PAPAYA MELEIRA VIRUS IS NEITHER TRANSMITTED
BY INFECTION AT WOUND SITES NOR BY THE WHITEFLY
*TRIALEURODES VARIABILIS***

S.P. Rodrigues¹, J.S. Andrade², J.A. Ventura^{1,2}, G.G. Lindsey³ and P.M.B. Fernandes¹

¹ Núcleo de Biotecnologia, Universidade Federal do Espírito Santo, UFES, 29040-090 Vitória, Espírito Santo, Brazil

² Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, INCAPER, 29010-901 Vitória, Espírito Santo, Brazil

³ Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch, South Africa

SUMMARY

Papaya meleira virus (PMeV), a double-stranded RNA (dsRNA) virus present in the latex of papaya (*Carica papaya* L.), is the causal agent of 'meleira' or 'sticky disease', which prevails in eastern Brazil. Disease management strategies in the orchards are impaired by the lack of knowledge on PMeV transmission. We have therefore evaluated inoculation methods (five mechanical and one biological) for virus transmission to different papaya cultivars using crude latex collected from symptomatic plants. Inoculated plants were kept under observation for symptom development and checked for the presence of viral dsRNA for over three months. Test plants wounded by cutting or abrasion, which resulted in latex exudation, were not infected, whereas PMeV dsRNA was detected 15 days after inoculation by latex injection into the stem apex. The whitefly *Trialeurodes variabilis* was unable to transmit PMeV from diseased to healthy papayas, even though the presence of the virus was ascertained in adults and nymphs. These data confirm previous field observations that failed to associate sticky disease with this whitefly species.

Key words: *Carica papaya*, dsRNA, mechanical inoculation, papaya sticky disease, virus inoculation, epidemiology, whiteflies.

INTRODUCTION

"Sticky disease" or "meleira" is an important disease of papaya (*Carica papaya* L.) in Brazil, especially in northern Espírito Santo and southern Bahia States, the main areas of papaya cultivation. The disease is characterized by intense and spontaneous exudation of latex that oxidizes and darkens. The fruits are visually imperfect and their flavour is compromised, making them unmarketable (Ventura *et al.*, 2001, 2003, 2004). Detection by Kitajima *et al.* (1993) of a single double-stranded

RNA (dsRNA) molecule in extracts from infected plants, suggested that the disease had a viral aetiology. Subsequently, latex from affected papaya was consistently found to contain a large number of spherical particles 50 nm in diameter, which were also present within the laticifer cells of leaf and fruit tissues. Inoculation of healthy plants with purified Papaya meleira virus (PMeV) particles resulted in the development of disease symptoms (Zambolim *et al.*, 2003). PMeV is widely distributed in infected papaya plants, and is present in high concentration in flowers and fruits (Kitajima *et al.*, 1993; Barbosa *et al.*, 1999; Ventura *et al.*, 2003; Rodrigues *et al.*, 2005).

Observations on PMeV infection in papaya orchards revealed a distribution of infected plants suggesting the involvement of a vector in virus transmission, although the distribution pattern of diseased plants along the rows suggested that PMeV might also be disseminated during pruning and harvesting (Ventura *et al.*, 2003). Laticifers, which extend throughout papaya plants (Esau, 1976), exude abundant latex after incision (Moutim, 1999). The exudation commonly occurs from wounds caused either by harvest tools (e.g. ladders and knives) and tractor movements during field work, possibly causing mechanical inoculation of healthy plants.

The whitefly *Bemisia tabaci* is a pest and possible disease vector to papaya in Brazil (Vieira and Correa, 2001; Culik *et al.*, 2003). In March to July there is the highest incidence of sticky disease, a period that follows directly a peak in whitefly population density, which occurs between February and April (Ventura *et al.*, 2003). Moreover, it was shown that the presence of dsRNA and sticky disease symptoms occurred six and eight months, respectively, after healthy papaya plants were exposed to PMeV-infected *B. tabaci* (Vidal *et al.*, 2000).

Another whitefly species, *Trialeurodes variabilis*, has recently been shown to be a pest to Brazilian papaya and other fruit trees (Picaço *et al.*, 2003). This was first documented in papaya orchards of Espírito Santo in 2004, where the proliferation of saprophytic fungi on papaya fruit and leaves was associated with whitefly presence (Culik *et al.*, 2004).

In this study, we have investigated the relationship between mechanical damage to papaya plants and

PMeV infection, using different methods to simulate the damage observed *in vivo* and have also assessed, under controlled conditions, the ability of *T. variabilis* to act as a vector of PMeV.

MATERIALS AND METHODS

Plants and inoculum samples. Inoculation experiments were carried out at the Incaper Experimental Field, Linhares, Espírito Santo, using either three-month-old papaya plants (cv. Sunrise Solo) in 8 kg containers in an aphid-protected greenhouse, or stems of 18-month-old plants in the field. Crude latex containing virus particles (Kitajima *et al.*, 1993; Zambolim *et al.*, 2003; Rodrigues *et al.*, 2005) was collected by wounding the surface of green fruits from plants showing typical sticky disease symptoms. Presence of the virus in the latex was confirmed through nucleic acid extraction and analysis (see below). This crude latex was used as inoculum.

Mechanical inoculation. Five three-month-old papaya plants (cv. Sunrise Solo) were used for each inoculation. Healthy control plants were kept in a nearby greenhouse. Latex from plants was collected before and after inoculation and every 15 days over 90 days to be analyzed for the presence of dsRNA. Five mechanical inoculation methods, designed to simulate injuries caused by work tools and the movement of tractors, were tested. The first three methods all resulted in latex exudation; this was removed prior to abundantly brush painting the site of injury with inoculum. These methods were: cutting the leaf (method 1) or the leaf stalk (method 2) to a depth of 0.5 mm using a sterile blade, or manually scraping the leaf surface with washed sand (method 3). Alternatively, 500 μ l of inoculum was injected into the stem apex at irregular depths of less than 10 mm using a sterile syringe (method 4). Additionally, five 18-month-old papaya trees in the field had their stems abraded with a harvest ladder under the fruit column (method 5) prior to inoculation.

Whitefly transmission. To investigate whether *T. variabilis* whiteflies could transmit PMeV from diseased to healthy plants (method 6), 13-month-old papaya plants were divided in two groups in the greenhouse. A total of 24 plants was inoculated by stem injection with infected latex, with 8 plants being inoculated with phosphate buffer pH 6.0 as a control. One month post-inoculation, a large number of *T. variabilis* (>1000 units), collected from a papaya field showing no sticky disease, were introduced into the greenhouse. The virus acquisition access and/or virus inoculation access periods were optimal (Vidal *et al.*, 2000; Ventura *et al.*, 2003). A sample of these insects was analyzed for the presence of dsRNA prior to their introduction into the greenhouse.

Thirty days after insect addition, healthy papaya plants of different cultivars (cvs Golden, Sunrise Solo and Taiwan), were also placed in the greenhouse. Three plants of each cultivar were placed approximately 1.5 meters from the inoculated plants. Twenty days after addition of the plants of different cultivars, latex was withdrawn and adult whiteflies as well as nymphs were collected for dsRNA analysis.

Nucleic acid extraction and analysis. Viral dsRNA was extracted from papaya latex and from entire whiteflies as described previously (Rodrigues *et al.*, 2005). Briefly, latex was diluted with 0.1 M citrate buffer, pH 5.0 before being extracted twice with 1:1 (v/v) phenol:chloroform and once with chloroform before being precipitated with ethanol. The pelleted viral nucleic acid was dissolved in ultra-pure water, digested with DNase 1, and analyzed by electrophoresis using 1.0% (w/v) agarose gels, which were stained with ethidium bromide and photographed. Diagnosis by dsRNA extraction from plant latex was validated by RT-PCR analysis as described (Araújo *et al.*, 2007).

RESULTS

Nucleic acid extracts from sticky-diseased papaya samples presented an intense 12 kbp band in agarose gel (Fig. 1a). The band was previously demonstrated to be composed of dsRNA (Kitajima *et al.*, 1993; Rodrigues *et al.*, 2005), associated with PMeV particles (Zambolim *et al.*, 2003). To validate this result, the samples were submitted to RT-PCR (Araújo *et al.*, 2007). Sticky-diseased samples presented a band of about 506 kb, equivalent to amplification of the PMeV RNA-dependent RNA polymerase gene (Fig. 1b) (Araújo *et al.*, 2007). These results indicated the equivalence between the two diagnosis methods. Thus, we decided to use the

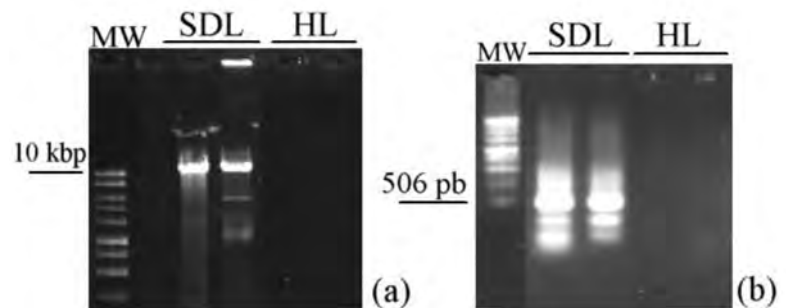


Fig. 1. Comparison of PMeV diagnostic methods. Sticky-diseased latex (SDL) was collected and submitted to PMeV diagnosis by (a) dsRNA extraction; (b) RT-PCR amplification. Healthy latex (HL) was used as negative control. The samples were separated on: (a) 1% agarose gel, where MW indicates a 1 kbp DNA molecular weight marker; (b) 1.5% agarose gel, where MW indicates a 100 bp DNA molecular weight marker.

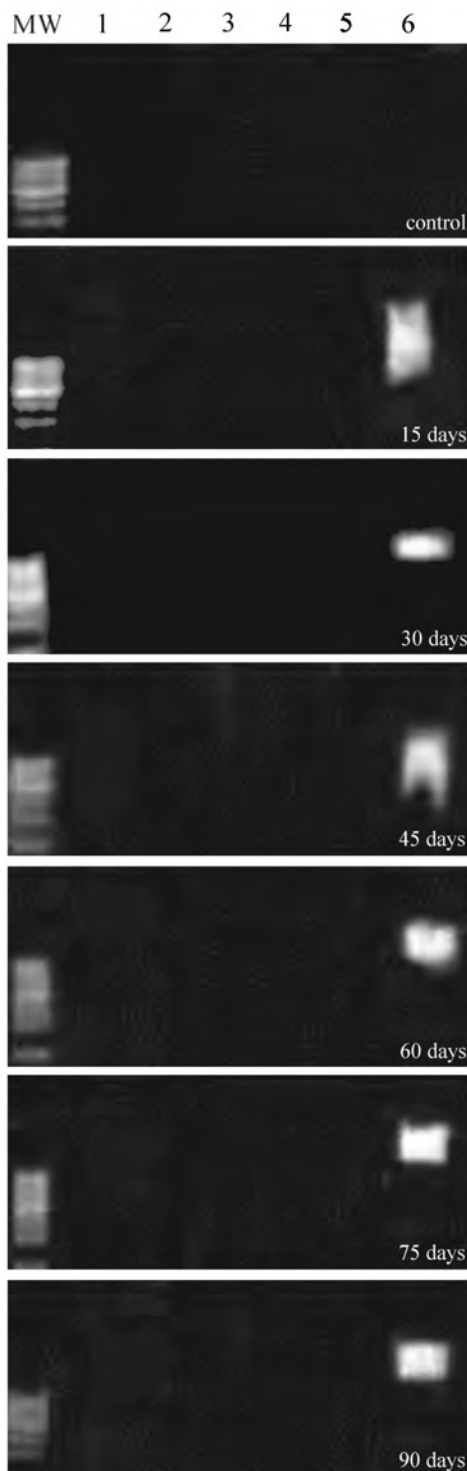


Fig. 2. Outcome of mechanical PMeV transmission trials to three-month-old cv. Sunrise Solo papaya seedlings inoculated by different methods. Latex was collected just before inoculation (control) and 15, 30, 45, 60, 75, and 90 days afterwards, and nucleic acids were extracted and digested with DNase I. Lane 1: negative control (non inoculated plants). Lanes 2 to 6 inoculated seedlings. Lane 2, inoculation with phosphate buffer, pH 6.0; lane 3, inoculation into razor blade cuts on the leaf; lane 4, inoculation into razor blade cuts on petioles; lane 5, inoculation after manually abrading the leaf surface with washed sand; lane 6, direct injection into the stem apex. The only successful inoculation method was by direct injection into the stem apex. MW, 1 kb DNA molecular weight marker.

dsRNA extraction method in the next steps of the work.

The inoculum used for all the mechanical inoculation tests was crude latex from diseased plants. Healthy papaya plants, which tested negative for the presence of PMeV prior to the start of the experiments (Fig. 1a, b), were inoculated with this crude PMeV-containing latex using different methods. Latex from all experimental plants was analyzed for the presence dsRNA at 15 day intervals post-inoculation. Experimental plants wounded by cutting or abrasion, with resulting latex exudation, remained free of PMeV symptoms and no dsRNA was found in their latex (Fig. 2). In contrast, when the infected latex was directly injected into tissues at the papaya stem apex, PMeV infection was obtained as indicated by the recovery of a dsRNA band after agarose electrophoresis (Fig. 2). The dsRNA was detected in two of the five inoculated plants after fifteen days and in a third plant after thirty days. Such plants had a more watery and translucent latex than the healthy ones and marginal scorching of the young leaves. These symptoms were similar to those of sticky-diseased papaya in the field (Ventura *et al.*, 2003).

The plants used in this work could not be monitored until their complete maturity as they grew under greenhouse conditions. However, we have already used PMeV-infected latex injection as an infection method in field experiments. In this case, mature plants showed

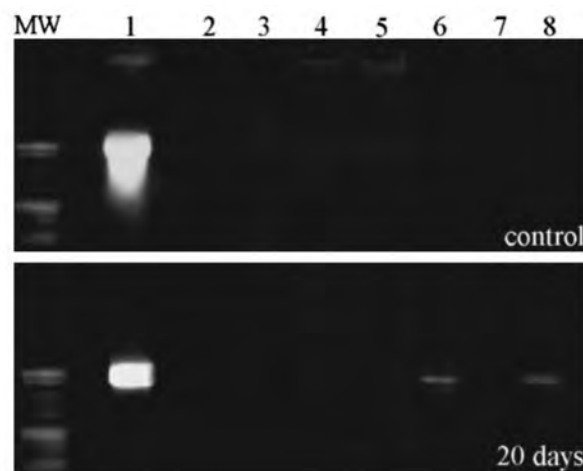


Fig. 3. Outcome of biological inoculation trials. Thirteen-month-old healthy papaya seedlings of cv. Sunrise Solo were divided in two groups which were inoculated via latex injection (lane 1) and phosphate buffer pH 6.0 (lane 2), respectively. One month later, both groups of seedlings were infested with *T. variabilis* adults. Thirty days after insect addition, healthy papaya of cvs Golden (lane 3), Sunrise Solo (lane 4) and Taiwan (lane 5), were placed in the greenhouse. Aliquots of latex from these plants and extracts from adult whiteflies (lane 6) and nymphs from healthy (lane 7) and infected plants (lane 8) were analyzed 20 days after exposure to whiteflies. dsRNAs were recovered only from plants inoculated by latex injection, from adult whiteflies, and nymphs from infected plants. MW, 1 kb DNA molecular weight marker.

the complete symptoms known for the naturally PMeV-infected papaya (unpublished information).

To investigate whether PMeV could be transmitted by *T. variabilis*, 24 healthy papaya plants were directly injected with infected latex at the stem apex and left in the greenhouse for one month prior to the introduction of an massive population of whiteflies. Viral dsRNA was found in the latex of these plants (Fig. 3). In parallel, eight additional plants were injected with phosphate buffer as control. One month after the exposure to whiteflies, healthy papayas of cvs Golden, Sunrise Solo and Taiwan were introduced into the greenhouse. Three weeks later, latex, adult, whiteflies and nymphs were collected and analyzed for the presence of PMeV. All papayas remained uninfected throughout the experiment despite the presence of a large whitefly population (Fig. 3). Adult whiteflies and nymphs collected from the greenhouse showed the presence of viral dsRNA, indicating that they had acquired PMeV during the course of the experiment (Fig. 3). In contrast, nymphs collected from control plants as well as from introduced plants of the different cultivars failed to show the presence of dsRNA indicating that they did not carry PMeV (Fig. 3).

DISCUSSION

Normal papaya orchard management often results in mechanical injury to the lactifer-rich tissue, and it has been proposed that such wound sites might facilitate PMeV infection (Ventura *et al.*, 2003). Although some of these injuries are accidental, for example those caused by tractors and from ladders used for pruning and harvesting, others are deliberate, like those caused by pruning. Since knives are not disinfected between plants, we investigated whether such injuries allow the spread of PMeV infection.

We show that such injuries are not the reason for spread of PMeV infection throughout a plantation since none of the methods used to simulate possible mechanical injuries resulted in plant infection. We believe that this failure is due to the rapid coagulation of the latex exudate. The interconnected duct system (Esau, 1976) maintains papaya lactifers under high turgor pressure, which results in abundant latex exudation occurring immediately after damage to any tissue. The exudate polymerizes rapidly (Moutim *et al.*, 1999), forming a barrier between the lesion and the environment, which has been proposed to protect injured tissues in lactifer-rich plants (Mahlberg, 1993; Datta and Iqbal, 1994; Hunter, 1994). Infected latex had to be injected into stem apex tissues in order to overcome the laticifer-based defense system, presumably because this method only causes moderate tissue damage. It also places the injected PMeV particles adjacent to viable plant cells, a pre-requisite for the establishment of plant virus infections

(Carrington *et al.*, 1996). Using this method, PMeV dsRNA was detected 15 days post-inoculation, with the amount of the dsRNA present increasing over the 90 days of the experiment.

T. variabilis and other whiteflies are major agricultural pests, as they feed on plant sap, causing increased plant susceptibility to abiotic and biotic stress (Berlinger, 1986). Most plant virus arthropod vectors, such as *T. variabilis* and other whiteflies, have a common feeding mechanism, whereby their mouthparts pierce the plant cell wall by mechanical force, possibly with the help of salivary and gut enzymes (Gray and Banerjee, 1999). Since this method of feeding is similar to the artificial inoculation described, it was surprising that no whitefly transmission was observed, especially as papaya was their only source of food. Moreover, adult whiteflies tested positive for PMeV, as did nymphs collected from infected plants. We would postulate that *T. variabilis* inject little into plants when feeding as they may not interact efficiently with the virus (Gray and Banerjee, 1999). Low virus titres on insect mouthparts and/or the time of feeding have also been proposed to be factors influencing virus transmission (Grill and Holt, 2000). PMeV-containing material injected by whiteflies might be countered by localised latex polymerization, peptidases (Moutim *et al.*, 1999) or by polyphenols present in the tissues.

The present data confirm our two-year field observations where no correlation between *T. variabilis* population density and the occurrence of sticky disease was found (unpublished information). Other insects that occur in papaya orchards are currently being investigated as possible vectors.

REFERENCES

- Araújo M.M.M., Tavares E.T., Silva F.R., Marinho V.L.A., Souza Júnior M.T., 2007. Molecular detection of Papaya meleira virus in the latex of *Carica papaya* by RT-PCR. *Journal of Virological Methods* **146**: 305-310.
- Berlinger M.J., 1986. Host plant resistance to *Bemisia tabaci*. *Agriculture, Ecosystems and Environment* **17**: 69-82.
- Carrington J.C., Kasschau K.D., Mahajan S.K., Schaad M.C., 1996. Cell-to-cell and long-distance transport of viruses in plants. *The Plant Cell* **8**: 1669-1681.
- Culik M.P., Martins D.S., 2004. First record of *Trialeurodes variabilis* (Quaintance) (Hemiptera: Aleyrodidae) on *Carica papaya* L. in the state of Espírito Santo, Brazil. *Neotropical Entomology* **33**: 659-660.
- Datta S.K., Iqbal M., 1994. The laticifer system in vascular plants. In: Iqbal M (ed.). *Growth Patterns in Vascular Plants*, pp. 137-161. Timber Press, Portland, OR, USA.
- Esau K., 1976. *Anatomía Vegetal*. Ediciones Omega S.A. Barcelona, Spain.
- Gray S.M., Banerjee N., 1999. Mechanisms of arthropod transmission of plant and animal viruses. *Microbiology and Molecular Biology Reviews* **63**: 128-148.

- Grill M.P., Holt J., 2000. Vector feeding period variability in epidemiological models of persistent plant viruses. *Ecological Modelling* **126**: 49-57.
- Kitajima E.W., Rodrigues C., Silveira J., Alves F., Ventura J.A., Aragão F.J.L., Oliveira L.H.R., 1993. Association of isometric virus-like particles, restricted to laticifers, with "meleira" (sticky disease) of papaya (*Carica papaya*). *Fitopatologia Brasileira* **8**: 118-122.
- Moutim V., Silva L.V., Lopes M.T.P., Wilsom Fernandes G., Salas C.E., 1999. Spontaneous processing of peptides during coagulation of latex from *Carica papaya*. *Plant Science* **142**: 115-121.
- Picaço M.C., Moura M.F., Moreira M.D., Antonio A.C., 2003. Biologia, identificação e manejo de moscas-brancas em fruteiras. In: Zambolim L. (ed.) Manejo Integrado, Produção integrada, Fruteiras Tropicais, Doenças e Pragas, pp. 243-284. UVS Publisher, Viçosa, Brazil.
- Rodrigues S.P., Galvão O.P., Andrade J., Ventura J.A., Fernandes P.M.B., 2005. Método de diagnóstico molecular simples para a detecção do vírus da meleira do mamoeiro em látex e tecidos de plantas infectadas. *Summa Phytopathologica* **31**: 273-275.
- Ventura J.A., Costa H., Tatagiba J.S., 2001. Sintomatologia da meleira do mamoeiro e sua importância para o "roguing". *Fitopatologia Brasileira* **26** (Suppl.): 536.
- Ventura J.A., Costa H., Tatagiba J.S., Andrade J.A., 2003. Meleira do mamoeiro: etiologia, sintomas e epidemiologia. In: Martins D. dos S. (ed). Papaya Brasil: Qualidade do Mamão para o Mercado Interno, pp. 267-276. INCAPER, Vitória, Brazil.
- Vidal C.A., Nascimento A.S., Barbosa C.J., Marques O.M., Habibe T.C., 2000. Experimental transmission of "sticky disease" of papaya by *Bemisia argentifolli* (Bellows & Per-ring). *21st International Congress of Entomology, Foz do Iguaçu 2000*: 819.
- Vieira M.R., Correa L.S., 2001. Ocorrência de moscas-brancas (Hemiptera: Aleyrodidae) e do predador *Delphastus pusillus* (Le Conte) (Coleoptera: Coccinellidae) em mamoeiros (*Carica papaya* L.) sob cultivo em ambiente protegido. *Neotropical Entomology* **30**: 171-173.
- Zambolim E.M., Alonso S.K., Matsoka K., Carvalho M.G., Zerbini F.M., 2003. Purification and some properties of Papaya meleira virus, a novel virus infecting papayas in Brazil. *Plant Pathology* **52**: 389-394.

Received April 23, 2008

Accepted September 5, 2008

