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LETTER TO THE EDITOR

Common weeds as alternate hosts of Mexican variant of Papaya meleira virus in papaya orchards in México

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Summary. – Presence of alternate hosts of plants is a great threat to the agriculture industry. Plants from several species growing in the papaya orchards affected by papaya sticky disease were examined for Papaya meleira virus (PMeV) infection causing this disease. The viral dsRNA was already detected in some plants from the family *Poaceae* or in watermelon. To identify new hosts of PMeV, we have collected 38 plant species belonging to 15 families of common weed species found in papaya-growing areas in México and used reverse-transcription PCR (RT-PCR) or quantitative real-time RT-PCR (RT-qPCR) for virus detection. We have detected the viral RNA in 11 species belonging to the families *Acanthaceae*, *Fabaceae* and *Poaceae*. Under experimental conditions, PMeV-Mx in *Panicum hirsutum* and *Ruellia nudiflora* inoculated weed species, showed that PMeV-Mx is able to replicate in plant cells of these species and spread in a systemic way. These results highlight the importance of weed species as potential virus reservoirs for PMeV-Mx.

Keywords: Papaya meleira virus; papaya sticky disease; Carica papaya; RT-PCR; TaqMan

One important aspect in plant disease epidemiology is the presence of alternate hosts of plant viruses. In a study conducted in Brazil, species belonging to several families of plants that grew in papaya orchards affected by papaya sticky disease (PSD), or "meleira", were assessed for papaya meleira virus (PMeV) infection (causal agent of PSD), and a viral dsRNA, with a molecular weight similar to that of PMeV, was detected in *Brachiaria decumbens* (the family *Poaceae*) (*3*). Later, molecular and experimental evidence demonstrated that watermelon (*Citrullus lanatus*) is an alternate host for PMeV-Mx. Quantification of PMeV-Mx RNA in non-inoculated leaves of watermelon seedlings showed that PMeV-Mx can replicate and move within this host (*1*). The identification of alternate hosts for the virus, which could be the source of infection, is important for determining factors that influence viral epidemiology and control strategies.

In this research, we evaluated the occurrence and infection capacity of PMeV-Mx for common weed species found in papaya-growing areas using RT-PCR and RT-qPCR to detect PMeV-Mx infection and determine the virus load, obtaining a more sensitive and thorough analysis of PMeV-Mx accumulation in these weed species. We collected common weed plant species, across a papaya-growing area, on the edges and between papaya

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Abbreviations: dpi = days post infection; PMeV = Papaya meleira virus; PSD = papaya sticky disease

Table 1. List of host and non-host weed species of Mexican variant Papaya meleira virus (PMeV-Mx) sampled in papaya crops

Plant species	Family	Acc. No.*	Life cycle	Natural infection with PMeV-Mx
Ruellia nudiflora (Engelm. and A. Gray) Urb	*Acanthaceae*	I. García 020	Perennial	Yes
Melampodium gracile Less.	*Asteraceae*	I. García 021	Annual	No
Tridax procumbens L.	*Asteraceae*	I. García 022	Annual	No
Viguiera dentata var. *helianthoides* (Kunth) S.F. Blake	*Asteraceae*	I. García 023	Annual	No
Commelina erecta L.	*Commelinaceae*	I. García 025	Perennial	No
Evolvulus alsinoides (L.) L.	*Convolvulaceae*	I. García 026	Annual	No
Ipomea triloba L.	*Convolvulaceae*	I. García 033	Annual	No
Merremia aegyptia	*Convolvulaceae*	I. García 024	Annual	No
Dioscorea sp.	*Dioscoreaceae*	I. García 038	Perennial	No
Acalypha alopecuroides Jacq.	*Euphorbiaceae*	I. García 028	Annual	No
Acalypha sp.	*Euphorbiaceae*	I. García 027	Annual	No
Euphorbia hypericifolia L.	*Euphorbiaceae*	I. García 031	Annual	No
Desmodium incanum DC.	*Fabaceae*	I. García 032	Perennial	Yes
Leucaena leucocephala (Lam.) de Wit	*Fabaceae*	I. García 029	Perennial	No
Piscidia piscipula (L.) Sarg.	*Fabaceae*	I. García 030	Perennial	No
Corchorus siliquosus L.	*Malvaceae*	I. García 037	Annual	No
Sida acuta Burm. f.	*Malvaceae*	I. García 035	Annual	No
Andropogon sp.	*Poaceae*	I. García 016	Annual	No
Bothriochloa pertusa (L.) A. Camus	*Poaceae*	I. García 011	Annual	No
Chloris ciliata Sw.	*Poaceae*	I. García 012	Annual	No
Dactyloctenium aegyptium (L.) Willd	*Poaceae*	I. García 003	Annual	Yes
Digitaria bicornis (Lam.) Roem. y Schult.	*Poaceae*	I. García 004	Annual	No
Echinochloa colona (L.) Link	*Poaceae*	I. García 001	Annual	Yes
Eleusine indica (L.) Gaertn.	*Poaceae*	I. García 002	Annual	Yes
Elytraria imbricata (Vahl) Pers.	*Poaceae*	I. García 019	Annual	Yes
Panicum hirsutum Sw.	*Poaceae*	I. García 005	Annual	Yes
Panicum hirticaule Presl.	*Poaceae*	I. García 006	Annual	Yes
Paspalum virgatum L.	*Poaceae*	I. García 007	Annual	Yes
Rhynchelytrum repens (Willd.) C.E. Hubb.	*Poaceae*	I. García 008	Annual	No
Setariopsis auriculata (Fourn.) Scribn.	*Poaceae*	I. García 009	Annual	No
Sporobolus buckleyi Vasey	*Poaceae*	I. García 010	Annual	No
Urochloa fusca (Sw.) B.F. Hansen y Wunderlin	*Poaceae*	I. García 015	Annual	Yes
Urochloa reptans L.	*Poaceae*	I. García 014	Annual	Yes
Gymnopodium floribundum Rolfe	*Polygonaceae*	I. García 036	Perennial	No
Neomillspaughia emarginata (Gross) Blake	*Polygonaceae*	I. García 039	Perennial	No
Borreria verticillata (L.) G. Meyer	*Rubiaceae*	I. García 034	Annual	No
Morinda yucatanensis Greenm.	*Rubiaceae*	I. García 041	Perennial	No
Capraria biflora L.	*Scrophulariaceae*	I. García 040	Biennial	No

* Voucher specimens deposited in herbarium „U Najil Tikin Xiw" of Natural Resources Unit, Centro de Investigación Científica de Yucatán A.C.

plants, in an experimental papaya orchard in San José Kuché, municipality of Conkal, in the State of Yucatán, as well as in a commercial orchard in Alfredo Bonfil, in the State of Campeche, both in México. All plant samples were tested by RT-PCR amplification for the presence of PMeV-Mx, using specific primers based on genomic regions of the PMeV-Mx RNA-dependent RNA polymerase gene to amplify a 491-bp DNA fragment (*4*). In addition, PMeV-Mx viral load was determined in all positive weed samples by quantitative RT-PCR amplification (RT-qPCR) (*1*), in order to determine which species had the highest viral titers.

To understand the epidemiology of PSD in papaya fields in México, and identify the possible sources of initial virus inoculum, 38 plant species belonging to 15 families that grew on the edges and between papaya fields, were sampled in south-southeastern regions of México, during 2015 and 2016, and were assayed to detect a possible natural infection with PMeV-Mx. The presence of PMeV-Mx infection was detected in 11 species belonging to the families *Acanthaceae*, *Fabaceae* and *Poaceae*, pointing to the fact that PMeV-Mx could have a wide and diverse host range, including monocot and dicot plants (Table 1). In the two years of sampling, the species belonging to the family *Poaceae* that were positive for PMeV-Mx were the same. In all cases, PCR product of each sample was purified, sequenced, and confirmed as the expected viral sequence, showing 99% identity with the PMeV-Mx RdRp gene (KF214786.1).

Tests under experimental conditions, confirmed by RT-PCR and RT-qPCR, proved the presence and viral load of PMeV-Mx detected in non-inoculated leaves from inoculated plants *P. hirsutum* and *R. nudiflora* at 14 days post infection (dpi), producing the expected amplicon (491 bp) corresponding to the RdRp gene of PMeV-Mx. The sequencing of a single fragment of each sample confirmed that PCR products had the expected viral sequence, showing 99% identity with the PMeV-Mx RdRp gene (KF214786.1). Nevertheless, PMeV-Mx could not be detected in any of the inoculated *Dactyloctenium aegyptium* plants. None of the PMeV-infected latex inoculated plants showed PSD symptoms or any other symptom in the period from one to 90 dpi. Quantification of PMeV-Mx-RNA by RT-qPCR of non-inoculated leaves of inoculated plants showed that this virus accumulates and remains in both plants, but *P. hirsutum* had a higher viral load than *R. nudiflora*. PMeV-Mx was detected in *P. hirsutum* at 7 dpi, and CT values decreased in time (from 36 at 7 dpi to 33 at 28 dpi), indicating increased viral load (from 0.788 pg/µl at 7 dpi to 5.54 pg/µl at 28 dpi). On the other hand, it was found that the amount of PMeV-Mx-RNA in *R. nudiflora* showed a different dynamic. CT values, ranged from 37 at 7 dpi to 36 at 21 dpi, with estimated amounts of PMeV-Mx-RNA ranging from 0.560 pg/µl at 7 dpi to 1.140 pg/µl at 21 dpi. However, the CT values increased again to 37 at 28 dpi, with a decrease in the quantity of PMeV-Mx-RNA to 0.580 pg/µl.

PMeV-Mx in *P. hirsutum* and *R. nudiflora* inoculated weed species, showed that PMeV-Mx is able to replicate in plant cells of these species and spread in a systemic way. The viral load increased over time in *P. hirsutum*, but in case of *R. nudiflora*, it first increased over time and finally decreased, suggesting a reduction in the virus replication rate. Nevertheless, the present investigation demonstrated that *P. hirsutum* and *R. nudiflora* supports replication and systemic accumulation of PMeV-Mx after injection of infected latex. Therefore, these species can be considered as systemic hosts, because the virus spreads from the inoculated leaf to other, but not necessarily all, parts of the plant (*2*).

The findings, presented here, highlight the importance of weed species as potential virus reservoirs for PMeV-Mx and possibly other papaya viruses, implying the need for further investigations, in order to assess the impact of common weed hosts of PMeV-Mx upon the virus epidemiology.

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