

## Papaya (*Carica papaya*, Brassicales: Caricaceae) is not a Host Plant of Tomato Yellow Leaf Curl Virus (Tylcv; Family *Geminiviridae*, Genus *Begomovirus*)

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## PAPAYA (CARICA PAPAYA, BRASSICALES: CARICACEAE) IS NOT A HOST PLANT OF TOMATO YELLOW LEAF CURL VIRUS (TYLCV; FAMILY GEMINIVIRIDAE, GENUS BEGOMOVIRUS)

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Tomato is an important crop in Florida with a 2009 farm value of \$520,000,000 (USDA-Agricultural Statistics Service, 2010). Biotype B of the sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a principal pest of tomato both in Florida and in tropical/subtropical regions globally (Oliveira et al., 2001). This invasive insect vectors many plant viruses, among of the most detrimental of which are a group of begomoviruses collectively referred to as tomato yellow leaf curl virus (TYLCV) (Moriones & Navas-Castillo 2000; Glick et al., 2009). Typical symptoms of tomato infected with TYLCV include chlorosis and leaf curling.

A banker plant system has been developed for management of B. tabaci in greenhouses and is presently under evaluation (Xiao et al. 2011). The banker plant system relies on papaya (Carica papaya L.) and its host-specific whitefly, Trialeurodes variabilis Quaintance to support populations of Encarsia sophia (Girault & Dodd) parasitoid wasps. The parasitoids, in turn, search greenhouse crops such as poinsettia and tomato where they control B. tabaci. However, papaya is a documented host of B. tabaci (Costa et al. 1993; Anderson et al. 2005), so it is crucial to establish that papaya is not also a host plant for TYLCV. If papaya harbors TYLCV, this could be perceived as an unacceptable risk, impeding adoption by tomato growers of the papaya banker plant strategy.

TYLCV infectivity of papaya plants was tested in 60 cm  $\times$  60 cm  $\times$  60 cm screened laboratory cages. Whitefly adults used in the experiment originated from non-viruliferous and viruliferous B. tabaci biotype B colonies maintained on tomato at the USDA-Horticultural Research Laboratory in Fort Pierce, Florida since 1996 and 2001, respectively (McKenzie 2002; Sinisterra et al. 2005). The adult infection rate in the viruliferous whitefly colony is 100% (McKenzie unpublished data). Two tomato (Solanum lycopersicum cv. 'Florida Lanai') and 5 papaya (Carica papaya cv. 'Maradol') potted seedlings (30-35 cm in height with 6-8 leaves) were infested twice at the rate of 50 whitefly adults per plant by releasing adults at the base of each plant. Experimental plants were first exposed to whiteflies in the presence of the alternative host plant (tomato or papaya) for 72 h (choice conditions). Due to the unexpected strong host preference of B. tabaci for tomato compared to papaya during the first whitefly exposure, and to ensure papaya plants received an adequate number of whiteflies to transmit TYL-CV, plants were re-infested in the absence of the alternative host for 1 wk (no-choice conditions). Plants were treated with insecticidal soap to kill all whiteflies between infestations, after whitefly exposure, and at regular intervals while monitoring for onset of virus symptoms. The 2 "control" plants exposed to non-viruliferous whitefly adults (one papaya and one tomato) were caged together under choice conditions and caged separately under no-choice conditions. The 5 "TYLCV-exposed" plants exposed to viruliferous whitefly adults (4 papaya and 1 tomato) were caged together under choice conditions. The 4 "TYLCV-exposed" papaya plants remained together and the "TYLCV-exposed" tomato plant was caged separately under no-choice conditions.

The third fully expanded leaf from the top of each plant was sampled prior to whitefly exposure and again on d 32, 47, 61, 91 and 121 after initial whitefly exposure. To extract crude DNA, leaf tissue samples were frozen in liquid nitrogen and pulverized in 200uL Cartigen lysis buffer. Samples were then boiled for 5 min, diluted 1:20 in Cartigen dilution buffer, and stored at -20 °C until molecular analysis.

Samples were used in PCR assays to test for the presence of target regions of the following genes: a) tomato actin, b) papaya actin, and c) TYLCV C3. The actin assays were used to ensure that host-plant DNA was present in samples and that no contamination occurred between samples. The TYLCV C3 assay was used to check samples for the presence of TYLCV. The papaya actin primers and thermocycler conditions were identical to McCafferty et al. (2006) with the exception that the denaturing temperature was lowered to 94 °C. The tomato actin and TYLCV C3 primers used were described in Sinisterra et al. (2005). Thermocycler conditions were modified from Sinisterra et al. (2005) for amplifying genomic DNA. Thermocycler conditions for tomato actin were 35 cycles of 30 s each at 94 °C, 30 s at 58 °C, and 30 s at 72 °C. The thermocycler conditions for TY-LCV C3 were 35 cycles of 15 s each at 94 °C, 40 s at 46.5 °C, and 60 s at 72 °C. Each PCR was preceded by a denaturing step at 94 °C for 2 min and ended with a final elongation at 72 °C for 10 min. The PCR products were analyzed on a 1% agarose gel.

Tomato actin primers produced the expected ~240 base pair PCR product only in tomato for all sampling dates. Papaya actin primers produced the expected ~200 base pair PCR product only in papaya for all sampling dates. TYLCV C3 primers only produced the expected ~200 base pair PCR product in healthy tomato exposed to viruliferous whiteflies. None of the papaya plants showed evidence of infection with TYLCV. Data shown in Fig. 1 are for 121 d following virus exposure and

are identical to the results found at 32, 47, 61, and 91 d post-exposure.

Whiteflies preferred tomato to papaya under choice conditions. Papaya, however, is a known reproductive host of the sweetpotato whitefly (Costa et al. 1993; Anderson et al. 2005) and sweet potato whiteflies oviposit and remain viable after 96 h on papaya plants under no-choice conditions (Dickey personal observation). Thus both the primary literature and personal obser-

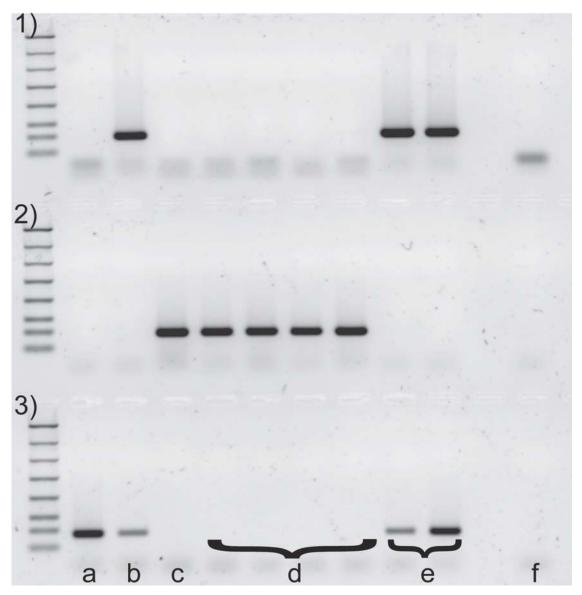


Fig. 1. Agarose gel showing presence/absence of the 200-240bp products from 1) TYLCV-C3, 2) Papaya actin, and 3) Tomato actin PCR assays 121 days after plant exposure to whitefly adults: a) Healthy tomato plant exposed to TYLCV negative whitefly adults, b) Healthy tomato exposed to TYLCV infected whitefly adults, c) Healthy papaya exposed to TYLCV negative whitefly adults, d) Healthy papaya exposed to TYLCV infected whitefly adults, e) TYLCV infected tomato positive control, f) water (negative control). Papaya plants exposed to TYLCV infected whitefly adults do not harbor the virus.

vations provide evidence that adult whiteflies fed on and exposed papaya plants to TYLCV under the no-choice conditions described herein. Only one variety of papaya, 'Maradol', was exposed to TYLCV and other varieties of papaya should also be screened prior to their use as banker plants in tomato production.

Tomato yellow leaf curl virus is among the most damaging disease causing agents in tropical and subtropical cultivated tomato worldwide, rendering its Bemisia tabaci biotype B whitefly vector a principal target of insecticides. The papaya banker plant system holds great potential as a non-chemical alternative by supporting a self-sustaining refuge for whitefly parasitoids (Xiao et al. 2011). Employing such strategies will reduce insecticide applications and the associated risk of insecticide resistance developing in whitefly populations. However, it is critical that a refuge for natural enemies of whitefly, not simultaneously harbor TYLCV. The demonstration that papaya is not a host of TYLCV is an important step toward integration of the papaya banker plant system in greenhouse cultivation of tomato. Furthermore, the observation of strong preference for tomato when given a choice suggests that if tomato is available, B. tabaci may largely avoid papaya banker plants. This report lends further support for the adoption of the papaya banker plant system in reduced-chemical management of sweetpotato whitefly in tomato greenhouses.

## Summary

Tomato yellow leaf curl virus and its vector, the sweetpotato whitefly threatens the economic value of tomato production. Use of papaya as a banker plant for whitefly biological control agents shows promise as a whitefly management strategy. This report shows that 'Maradol' papaya is not a host for tomato yellow leaf-curl virus, thus strengthening the case for adopting the papaya banker plant system in greenhouse tomato production.

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