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SHORT COMMUNICATION

Physalis ixocarpa and *P. peruviana*, new natural hosts of *Tomato chlorosis virus*

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Abstract *Tomato chlorosis virus* causes yellow leaf disorder epidemics in many countries worldwide. Plants of *Physalis ixocarpa* showing abnormal interveinal yellowing and plants of *Physalis peruviana* showing mild yellowing collected in the vicinity of tomato crops in Portugal were found naturally infected with ToCV. *Physalis ixocarpa* and *P. peruviana* were tested for susceptibility to ToCV by inoculation with *Bemisia tabaci*, Q biotype. Results confirmed that ToCV is readily transmissible to both species. The infection was expressed in *P. ixocarpa* by conspicuous interveinal yellow areas on leaves that developed into red or brown necrotic flecks, while *P. peruviana* test plants remained asymptomatic. Infected plants of both *P. ixocarpa* and *P. peruviana* served as ToCV sources for tomato infection via *B. tabaci* transmission. This is the first report of *P. ixocarpa* and *P. peruviana* as natural hosts of ToCV.

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Tomato chlorosis virus (ToCV) is a whitefly-transmitted and phloem-limited virus in the genus *Crinivirus*, family *Closteroviridae*, that causes the 'yellow leaf disorder' of tomato; this has been present in Florida at least since 1989 (Wisler et al. 1998). ToCV is an emerging virus which has been reported recently in many countries of the Caribbean, the Far East, southern Africa, and northern and southern sides of the Mediterranean Basin. Symptoms in tomato include interveinal chlorosis, leaf brittleness, and limited necrotic flecking or leaf bronzing. In addition, ToCV was found naturally infecting sweet pepper in Spain (Lozano et al. 2003), zinnia in Taiwan (Tsai et al. 2004), and wild hosts like *Solanum nigrum* and *Datura stramonium* in Portugal and Spain (Louro et al. 2000b; Font et al. 2004; our unpublished results). The experimental host range of ToCV comprises at least 25 plant species in eight families (Wintermantel and Wisler 2006; Morris et al. 2006), and includes some important crops, ornamentals, and weeds. ToCV can be transmitted in a semi-persistent manner by three species of whiteflies (Hemiptera: Aleyrodidae), *Bemisia tabaci, Trialeurodes vaporariorum*, and *T. abutilonea* (Wisler et al. 1998).

In Portugal, ToCV has been present in tomato since at least 1998 (Louro et al. 2000a) and also has been reported to infect the weeds *S. nigrum*,

and *D. stramonium* (Louro et al. 2000b). Over the last years, the virus has become well established in the south of the country (Algarve region), causing annual epidemics of yellowing in tomato. In this report we present field and laboratory data on two new natural hosts of ToCV belonging to the genus *Physalis*.

During the summer of 2005, symptoms of interveinal yellowing were observed in some *Physalis ixocarpa* plants growing as weeds associated with processing tomato crops in Campo Maior (Alentejo region, central Portugal). During the summers of 2004 and 2005, symptoms of mild yellowing were also observed in some *P. peruviana* (syn. *P. edulis*) plants present in a vegetable garden in Faro (Algarve region, southern Portugal). Seventeen plants of *P. ixocarpa* from Campo Maior, and eight plants of *P. peruviana* from Faro were collected and tested for the presence of ToCV by RT-PCR. ToCV-specific primers were designed to amplify a 436 bp DNA fragment of the coat protein gene, MA380 (+) (5′-GTGAGACCCCGATGACAGAT-3′), and MA381 (-) (5′-TACAGTTCCTTGCCCTCGTT-3′), based on the sequence of a Spanish isolate of ToCV (Lozano et al. 2006). RT-PCR was performed using the SuperScript One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen). Total RNAs extracted from 0.2 mg of leaf tissue where first heat-denatured at 65°C for 5 min and quickly chilled on ice, and the reaction mixture was then added. After reverse transcription at 50°C for 30 min, and a denaturation step at 94°C for 2 min, PCR was carried out with 35 cycles of 15 s at 94°C, 30 s at 55°C, and 30 s at 72°C, in a Perkin Elmer 2400 Thermal Cycler. A DNA fragment of the expected size was amplified from some field samples but not from healthy controls. The 436 bp DNA fragment amplified from one sample of *P. ixocarpa* (210705/16) and one sample from *P. peruviana* (051105/6) were purified using the High Pure PCR Product Purification Kit (Roche) and sequenced in both directions using the primers used for amplification. Both coat protein sequences (GenBank accesion number EF187609 for 210705/16, and EF187610 for 051105/6) showed a nucleotide identity of 100% to those of ToCV isolates from Portugal obtained from infected tomato plants collected at Campo Maior or Faro (DQ335133 and DQ335134, respectively; our unpublished results), 99% to that of a ToCV isolate from Spain (DQ136146; Lozano et al. 2006), and 98% to that of an isolate from Florida (AY903448; Wintermantel et al. 2005).

After confirmation of natural infections, *P. ixocarpa* and *P. peruviana* plants were tested for ToCV susceptibility under experimental conditions using *B. tabaci* Q biotype as the vector (see Table 1 for details). Transmission experiments showed that ToCV was readily transmitted from infected tomato to healthy *P. ixocarpa* and *P. peruviana* (three out of three inoculated plants of each species were infected). Infection was confirmed by RT-PCR and by molecular hybridisation of petiole cross-sections tissue printing using a digoxigenin-labelled RNA probe that covers the coat protein gene of the Spanish Pl1-2 ToCV isolate. The probe was prepared using the DIG RNA Labelling Kit (Roche) and hybridisation conditions were as recommended by the manufacturer (DIG Application Manual for Filter Hybridization, Roche). Three weeks after inoculation, *P. ixocarpa* plants exhibited symptoms similar to those observed in nature consisting of conspicuous interveinal yellowing that evolved into brown necrotic flecks at about 60 days after inoculation (Fig. 1b). *Physalis peruviana* showed no viral symptoms even at 90 days after inoculation (Fig. 1d). Therefore, mild yellowing symptoms observed in naturally infected plants of *P. peruviana* were either not caused by ToCV, or different environmental conditions from those used in our experiments are required for symptom expression. Alternatively, genetic diversity of *P. peruviana* could explain the observed differences.

As a first step to understand the possible epidemiological significance of *P. ixocarpa* or *P. peruviana* as reservoirs of ToCV for epidemics in tomato, we tested if these hosts could act as sources of infection for tomato. Thus, transmission experiments from *P. ixocarpa* and *P. peruviana* to tomato were performed using *B. tabaci* Q biotype as the vector. Results indicated that ToCV was readily transmitted to healthy tomato plants from either *P. ixocarpa* or *P. peruviana*-infected plants (Table 1). Transmission was also

Table 1 *Tomato chlorosis virus* transmission using the whitefly *Bemisia tabaci*, Q biotype, as a vector

Source plant	Test plant	Number of plants infected/ number of plants tested[1]
Tomato[2]	*P. ixocarpa*	3/3
Tomato	*P. peruviana*	3/3
Tomato	Tomato	3/3
P. ixocarpa	*P. ixocarpa*	5/5
P. peruviana	*P. peruviana*	4/5
P. ixocarpa	Tomato	10/10
P. peruviana	Tomato	8/10

[1] Transmission was carried out with adults of *B. tabaci*, biotype Q, given a 48 h acquisition access period on the source plants infected with the Spanish Pl1-2 ToCV isolate. Whiteflies were then collected and caged in groups of 35 on leaf 2 from the apex of each test plant (4-leaf growth stage) for a 48 h inoculation access period. After killing the insects by insecticide treatment, test plants were maintained in a growth chamber until analysed 45 days later for ToCV infection by RT-PCR and molecular hybridisation

[2] Solanum lycopersicum cv. Moneymaker

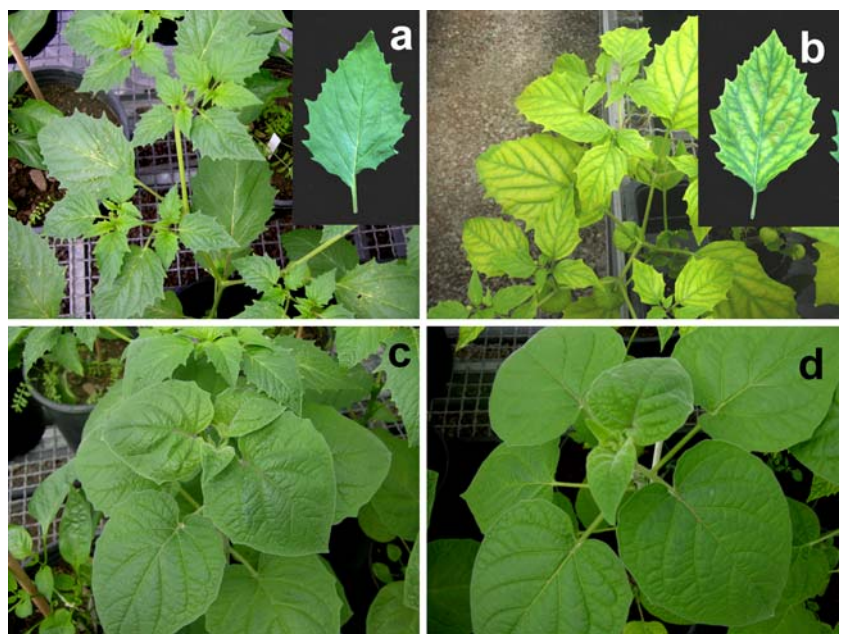

Fig. 1 Plants of *Physalis ixocarpa* (**a, b**) and *P. peruviana* (**c, d**) infected with *Tomato chlorosis virus* by *Bemisia tabaci* transmission (**b, d**) compared to plants given equal treatment but with non-viruliferous whiteflies (**a, c**). Symptoms consisting of conspicuous interveinal chlorosis that evolved into brown necrotic flecks were observed only on infected *P. ixocarpa* plants (**b**), while infected *P. peruviana* plants remained asymptomatic (**d**)

confirmed between plants of the same species, i.e. from infected *P. ixocarpa* to healthy *P. ixocarpa*, and from infected *P. peruviana* to healthy *P. peruviana* plants (Table 1). Therefore, *P. ixocarpa* and *P. peruviana* could be relevant as reservoirs for ToCV epidemics in tomato in Portugal, similar to that proposed for the weeds *S. nigrum* and *D. stramonium* (Louro et al. 2000b). The presence of ToCV in wild reservoirs could be of special relevance for ToCV epidemics in central Portugal. Thus, *P. ixocarpa* could maintain both ToCV and the vector *B. tabaci* in the Alentejo region where only one cycle of processing tomato is grown per season. In the absence of overlapping tomato crops, weeds could help to complete the annual infection cycle and to maintain virus inoculum between tomato epidemics.

This study provides relevant information about natural reservoirs of ToCV in Portugal that might contribute to the understanding of the epidemiology of this virus. However, additional information is needed for a more precise knowledge of the role of reservoirs in ToCV epidemics. Critical

aspects such as periods in which ToCV reservoirs are present in the field, their relative predominance among natural weed flora, the frequency of ToCV infections, or their behaviour as hosts for *B. tabaci* populations should be studied for a more comprehensive understanding of this pathosystem. In this sense, it is noteworthy that the high populations of *B. tabaci* found during our sampling were abnormal for the Alentejo region that was an EU protected zone for this insect until March 2006.

Although *P. ixocarpa*, and two other species in the same genus, *P. alkekengi*, and *P. wrightii*, have been described recently as experimental hosts for ToCV (Wintermantel and Wisler 2006), to our knowledge, this is the first report of *P. ixocarpa* and *P. peruviana* as natural hosts of this virus.

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