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Short communication

Mercurialis ambigua and *Solanum luteum*: two newly discovered natural hosts of tomato yellow leaf curl geminiviruses

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Abstract

The yellow leaf curl disease of tomato is caused by a complex of virus species, two of which, tomato yellow leaf curl virus (TYLCV)-Sar and TYLCV-Is, are involved in epidemics of southern Spain. Plants of *Mercurialis ambigua* and *Solanum luteum* showing abnormal upward leaf curling and leaf distortion collected in the vicinity of tomato crops were found to be naturally infected with TYLCV-Is and TYLCV-Sar, respectively. These weed species, as well as *Datura stramonium* and *S. nigrum*, which had also been found to be naturally infected by TYLCVs in the same region in previous studies, were tested for susceptibility to TYLCV-Sar or TYLCV-Is by *Agrobacterium tumefaciens*-mediated and by *Bemisia tabaci* inoculation. Results indicated that both TYLCV-Sar and TYLCV-Is were able to infect *D. stramonium* and *M. ambigua*, whereas only TYLCV-Sar infected *S. nigrum* and *S. luteum*. Implications for the epidemiology of TYLCV are discussed. This is the first report of *M.ambigua* and *S. luteum* as hosts of TYLCV.

The name tomato yellow leaf curl virus (TYLCV) refers to a group of virus species of the genus *Begomovirus* in the family Geminiviridae of plant viruses (Rybicki et al., in press) that cause devastating damage to tomato (*Lycopersicon esculentum* Mill.) crops worldwide (Czosnek and Laterrot, 1997). These viruses are transmitted by the whitefly *Bemisia tabaci* Gen. (Homoptera: Aleyrodidae) in a circulative manner (Cohen and Nitzany, 1966; Mehta et al., 1994; Caciagli et al., 1995). TYLCV infections have caused severe yield losses to tomato crops of south and southeastern Spain since they were first reported in 1992 (Moriones et al., 1993). Isolates belonging to the TYLCV-Sar species were associated with these early infections (Noris et al., 1994). In the same region, the presence of TYLCV-Is was reported a few years later to be associated with more severe epidemic outbreaks in tomato and with a new disease in common bean (*Phaseolus vulgaris* L.) (Navas-Castillo et al., 1997; 1999). TYLCV-Sar and TYLCV-Is are virus species taxonomically close to each other; isolates of each species reported from Spain (GenBank accession numbers Z25751 and AF071228) showed a nucleotide sequence similarity between their genomes of about 78%. After a few years, a progressive displacement of TYLCV-Sar by TYLCV-Is has been observed in tomato epidemics of southern Spain (Sánchez-Campos et al., 1999). Maintenance of virus inoculum in wild hosts in a certain area might provide a means for viruses to survive through the seasonal cycle and to explain their predominance during epidemics. Therefore, the knowledge on the weed reservoirs of TYLCV-Sar and TYLCV-Is may help to understand their epidemiology and could provide basic information for implementation of effective management strategies. In this work, data on weed species that are reservoirs of TYLCV-Sar or TYLCV-Is in southern Spain are presented.

Symptoms of abnormal upward leaf curling and leaf distortion were observed in plants of *Mercurialis ambigua* L. fil. and *Solanum luteum* Mill. growing in

the vicinity of tomato fields in the Málaga province, southern Spain. One apical young leaf per plant was collected from several symptomatic plants. Total nucleic acids (TNA) extracts were obtained and dot blot and Southern blot hybridization analyses performed (Noris et al., 1994). For hybridization, digoxigenin (DIG)-labeled DNA probes that specifically recognize TYLCV-Sar or TYLCV-Is were prepared (Navas-Castillo et al., 1999). The detection of TYLCV viral DNA forms in Southern blot hybridizations of TNA extracts from samples that reacted positively in dot blot hybridizations was used to confirm the presence of TYLCV infections. Analysis of the symptomatic samples collected showed the presence of TYLCV-Is or TYLCV-Sar in naturally infected *M. ambigua* and *S. luteum* plants, respectively (Table 1). Previous surveys of spontaneous host species conducted in the same region (Sánchez-Campos et al., 1999) recorded TYLCV-Sar and TYLCV-Is naturally infecting *Datura stramonium* L. and TYLCV-Sar infecting *Solanum nigrum* L. plants showing symptoms of abnormal upward leaf curling and leaf distortion similar to those reported in this work for *M. ambigua* and *S. luteum*. TYLCV infections on *S. nigrum* plants have been reported from southeastern Spain (Bedford et al., 1998) and Italy (Davino et al., 1994), and on *M. annua* L. plants from Lebanon (Abou-Jawdah et al., 1998). However no details were given in these cases about which TYLCV species was involved in field infections. Our data indicated that only TYLCV-Sar was found in field-infected plants of *S. luteum*, and only TYLCV-Is was in *M. ambigua*. Since only a small number of field samples was analyzed, these plant species were experimentally inoculated with either TYLCV-Sar or TYLCV-Is to determine if any incompatibility exist in certain virus/plant species combination. *Agrobacterium tumefaciens*-mediated inoculations were performed (Noris et al., 1994) using the infectious clones obtained for Spanish isolates of TYLCV-Sar (Noris et al., 1994) or TYLCV-Is (Navas-Castillo et al., 1999). One month after inoculation, plants were analyzed for TYLCV-Sar or TYLCV-Is infection by dot blot and Southern blot hybridization of TNA extracted from young tissues. Results indicated that both TYLCV-Sar and TYLCV-Is were able to infect *M. ambigua*, whereas only TYLCV-Sar infected *S. luteum* plants (Table 2). However, for TYLCV-Sar infection of *M. ambigua*, only three plants out of 18 inoculated were found infected (Table 2), and all these showed a very low virus titre (data not shown). Therefore, a certain resistance to TYLCV-Sar infection seemed to exist in *M. ambigua*. By *A. tumefaciens*-mediated inoculation, most of the *D. stramonium* and tomato control plants were infected by either TYLCV-Sar or TYLCV-Is whereas, as for *S. luteum*, only TYLCV-Sar could infect *S. nigrum* control plants (Table 2). By *B. tabaci* inoculation, only TYLCV-Sar could be transmitted to *S. luteum* and TYLCV-Is to *M. ambigua* test plants although at a lower transmission efficiency than to the *S. nigrum* plants used as control (Table 3). In the same experimental conditions, 25% to 100% of the tomato control plants were infected by *B. tabaci* transmission (not shown).

The possible epidemiological significance of TYLCV-infected *S. luteum* or *M. ambigua* plants for epidemics in tomato was analyzed. Transmission experiments to tomato were performed using *B. tabaci* as vector and *S. luteum* and *M. ambigua* plants

Table 1. Identification of TYLCV-Sr and TYLCV-Is in samples from plants of weed species with symptoms of abnormal upward leaf curling and leaf distortion collected in the vicinity of tomato fields in the Malaga province, southern Spain

Weed species	No. of plants sampled	No. of plants positive for:[a]		
		Sr	Is	Sr + Is
Mercurialis ambigua	3	0	3	0
Solanum luteum	5	5	0	0

[a]TNA extracts of samples were analyzed by dot blot and Southern blot hybridization using DIG-labeled DNA probes specific to TYLCV-Sar or TYLCV-Is. Sr, infected by TYLCV-Sar; Is, infected by TYLCV-Is; Sr + Is, infected by TYLCV-Sar and TYLCV-Is.

Table 2. Infection with TYLCV-Sr or TYLCV-Is of test plants of weed species inoculated with TYLCV-Sar or TYLCV-Is by *Agrobacterium tumefaciens*-mediated inoculation

Species	No. of plants infected/no. of plants tested when inoculated with:[a]	
	TYLCV-Sar	TYLCV-Is
Mercurialis ambigua	3/18	16/18
Solanum luteum	14/14	0/14
S. nigrum	12/12	0/12
Datura stramonium	9/11	16/16
Lycopersicon esculentum	14/14	14/14

[a]TNA extracts obtained from young tissues of plants 60 days after inoculation were analyzed by dot blot and Southern blot hybridization using DIG-labeled DNA probes specific to TYLCV-Sar or TYLCV-Is. *S. nigrum*, *D. stramonium*, and tomato (*L. esculentum*) were used as controls.

Table 3. Bemisia tabaci transmission of TYLCV-Sar or TYLCV-Is from tomato to *Solanum luteum* or *Mercurialis ambigua*

Target plant species	Virus source	*Bemisia tabaci* biotype used for transmission	No. of plants infected/no. of plants tested in replica[1]		
			1	2	3
Solanum luteum	TYLCV-Sar	B	1/8	0/10	—[2]
S. luteum	TYLCV-Sar	Q	0/10	0/10	—
S. luteum	TYLCV-Is	B	0/10	0/10	—
S. luteum	TYLCV-Is	Q	0/10	0/10	—
Mercurialis ambigua	TYLCV-Sar	Q	0/10	0/10	0/10
M. ambigua	TYLCV-Is	Q	1/6	3/10	0/10
S. nigrum	TYLCV-Sar	Q	7/15	—	—
S. nigrum	TYLCV-Is	Q	0/10	—	—

[1]Transmission was done with adult *B. tabaci* whiteflies that were given a 24-h acquisition access period (AAP) on tomato (*Lycopersicon esculentum* cv. Moneymaker) plants infected with either TYLCV-Sar or TYLCV-Is. After the AAP, insects were collected and caged in groups of 10 on leaf number two from apex of each test plant (four leaves growth stage) for a 24-h inoculation access period (IAP). After the IAP insects were killed and plants were maintained in a growth chamber until analyzed 60 days later for TYLCV-Sar or TYLCV-Is infection as described by Sánchez-Campos et al. (1999). *S. nigrum* was used as control.
[2]—, replica not done.

TYLCV-infected by *B. tabaci* transmission (Table 3) as source plants. The results indicated that both plant species serve as a source for TYLCV transmission to tomato (Table 4). Therefore *S. luteum* and *M. ambigua* are reservoirs for epidemics of TYLCV in tomato as has also been shown for other weed species like *D. stramonium* or *S. nigrum* (Cohen and Nitzany, 1966; Bedford et al., 1998).

Moderate to low *B. tabaci* transmission rates of TYLCV were obtained in this work from tomato to *S. luteum* or *M. ambigua* or vice versa. However, this should not lead to an underestimation of the significance of these species as reservoirs of TYLCV-Sar or TYLCV-Is in the epidemiology of these viruses. *B. tabaci* pressure on host plants in southern Spain, especially during the warm season is much higher than that simulated in our experiments. Therefore, the probability of transmission would greatly increase. Moreover, the presence of TYLCV-Sar and/or TYLCV-Is in local reservoirs could be of special relevance for TYLCV epidemics in southern Spain because mild weather conditions allow many wild plant species to vegetate all year round. This is of particular concern for *M. ambigua* and *S. nigrum* because they are found frequently associated with tomato crops in southern Spain, whereas *S. luteum* is less frequent and, therefore, might have a more limited role in TYLCV epidemiology. *D. stramonium*, *M. ambigua*, *S. nigrum*, and *S. luteum* may all be involved in epidemics in other countries affected by TYLCV because they are weed species present in many temperate regions worldwide.

Table 4. Bemisia tabaci transmission of TYLCV-Sar or TYLCV-Is acquired from *Solanum luteum* or *Mercurialis ambigua* infected plants, to tomato test plants

Acquisition source species	Virus source	*Bemisia tabaci* biotype	No. of plants infected/no. of plants tested in replica[1]	
			1	2
Solanum luteum	TYLCV-Sar	B	1/10	—[2]
S. luteum	TYLCV-Sar	Q	6/10	—
Mercurialis ambigua	TYLCV-Is	Q	3/10	2/10

[1]Transmission was done with adult *B. tabaci* whiteflies that were given a 24-h AAP on *S. luteum* or *M. ambigua* plants infected with TYLCV-Sar or TYLCV-Is, respectively. After the AAP, insects were collected and caged in groups of 10 on leaf number two from apex of each tomato (*Lycopersicon esculentum* cv. Moneymaker) test plant (four leaves growth stage) for a 24-h IAP. After the IAP insects were killed and plants were maintained in a growth chamber until analyzed 60 days later for TYLCV-Sar or TYLCV-Is infection as described by Sánchez-Campos et al. (1999).
[2]—, replica not done.

This study provides information on weed species that are natural reservoirs of TYLCV-Sar and TYLCV-Is in southern Spain as well as on incompatibilities of certain TYLCV/weed species combinations that might

contribute to the understanding of the epidemiology of these viruses. Additional information is needed, however, for a more precise knowledge on the role of weed reservoirs in TYLCV epidemics in southern Spain. Critical aspects such as periods in which TYLCV weed reservoirs are present in the field, their relative predominance among natural weed flora, the frequency of TYLCV infections, or their behavior as hosts for *B. tabaci* populations should be studied for a more comprehensive understanding of the pathosystem. This appears to be the first report of *M. ambigua* and *S. luteum* as hosts of TYLCV species.

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