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Host range studies for *Tomato chlorosis virus*, and *Cucumber vein yellowing virus* transmitted by *Bemisia tabaci* (Gennadius)

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Abstract

The *Bemisia tabaci* (Gennadius) biotype B transmitted host range of *Tomato chlorosis virus* (ToCV), genus *Crinivirus*, Family *Closteroviridae*, and *Cucumber vein yellowing virus* (CVYV), genus *Ipomovirus*, Family *Potyviridae*, was studied. New experimental hosts were identified for each of these viruses. Seventeen species in eight plant families were assessed as potential hosts for ToCV. Infection in asymptomatic *Anthriscus cereifolium* (chervil) test plants by ToCV was confirmed by using a Real-Time PCR assay designed for ToCV. The presence of readily transmissible, infectious ToCV virions in *A. cereifolium* was confirmed by re-isolation of the virus via whitefly-transmission from *A. cereifolium* to *Lycopersicon esculentum* and *A. cereifolium*. This is the first report of the experimental transmission of ToCV by *B. tabaci* to a species within the Umbelliferae. All other hosts assessed for the presence of ToCV were found to be uninfected. Ten species in five families were assessed as potential hosts for CVYV. The CVYV host range identified included some important crops and common weeds, such as *L. esculentum*, *Nicotiana tabacum*, *A. cereifolium*, *Datura stramonium*, *Nicotiana benthamiana*, *Nicotiana clevlandii* and *Cucumis sativus*. Symptoms were present on *D. stramonium*, *N. benthamiana* and *C. sativus* control plants. The presence of infectious whitefly transmitted CVYV virions was confirmed solely for *D. stramonium* and *N. tabacum*, following re-isolation of the virus via *B. tabaci* transmission from all infected species to *C. sativus*. This is the first report of experimental CVYV transmission by *B. tabaci* to non-cucurbitaceous crop and weed hosts belonging to the Solanaceae or Umbelliferae.

Introduction

Tomato chlorosis virus (ToCV) is a whitefly-transmitted, phloem-limited virus belonging to the *Crinivirus* genus within the *Closteroviridae* family. ToCV is transmitted in a semi-persistent manner, by a number of whitefly vectors belonging to the family Aleyrodidae, which comprise: *B. tabaci* (Gennadius) (*B. tabaci*), biotypes A and B (Wisler et al., 1998a), and biotype Q, (Navas-Castillo et al., 2000), *Trialeurodes vaporariorum* and *Trialeurodes abutilonea* (Wisler et al., 1998a). ToCV has been described and characterised (Wisler et al., 1996, 1997, 1998a) and found to be the causative agent of a 'yellow leaf disorder' of greenhouse-grown tomatoes in north-central Florida. Symptoms on tomato include irregular chlorotic mottling that develops first on lower leaves and gradually advances toward the growing point. Interveinal yellow areas on leaves develop red or brown necrotic flecks, with no obvious symptoms produced on fruit or flowers (Wisler et al., 1998a). Yield reductions occur primarily due to the loss of photosynthetic area (Wisler et al., 1998b). Natural hosts of ToCV comprise *L. esculentum* (tomato), *C. annuum* (sweet pepper)

(Lozano et al., 2004), zinnia (Tsai et al., 2004), and two common weed species, *D. stramonium* reported the EPPO-datasheet on quarantine pests, and *Solanum nigrum* (Font et al., 2004). To date ToCV has been reported outside the EU/EPPO region in Morocco, Puerto Rico, the USA (Jones, 2003) and Taiwan, (Tsai et al., 2004). Within the EU/EPPO region ToCV has been recorded in tomato crops in Malaga and Almeria provinces, (the first report of ToCV epidemics in Europe), (Navas-Castillo et al., 2000), and Tenerife and Gran Canaria reported by the EPPO-datasheet on quarantine pests, Portugal, (Louro et al., 2000), Sardinia, Sicily and Apulia (Accotto et al., 2001), the south of France, (Decoin, 2003), and Greece (Dovas et al., 2002). Occurrence of the virus is associated with the whitefly vectors *B. tabaci* and/or *T. vaporariorum*. ToCV is included on the EPPO A2 List which is an indication of the quarantine significance of the virus.

Cucumber vein yellowing virus (CVYV) is a tentative member of the genus *Ipomovirus*, Family *Potyviridae* (Lecoq et al., 2000), and was first reported in *Cucumis* spp. in Israel (Cohen and Nitzany, 1960). CVYV causes severe disease of cucumbers and other cucurbits in the eastern Mediterranean basin (Lecoq et al., 2000), and considerable losses in cucumber infected with CVYV have been reported (Cuadrado et al., 2001). Symptoms reported on naturally infected cucurbits in southern Portugal (Louro et al., 2004) comprise vein yellowing, stunting and sudden plant death in protected melons (*Cucumis melo*), mild leaf chlorosis and split fruits with internal necrosis in watermelons (*Citrullus lanatus*) and vein clearing and mottling on leaves of cucumbers (*C. sativus*) and squashes (*Cucurbita pepo*). CVYV has been recorded in Spain, Israel, Jordan, Turkey (Jones, 2003), and southern Portugal (Louro et al., 2004) and included on the EPPO A2 list. CVYV-Isr and CVYV-Jor are two recognised strains of CVYV from Israel and Jordan respectively (Lecoq et al., 2000). These strains exhibit similar symptoms by mechanical transmission to cucumber and melon, but CVYV-Jor causes a more severe stunting in cucumber (Lecoq et al., 2000). Mechanical transmission of CVYV has been found to be an unreliable means of determining host range by some authors (Al-Musa et al., 1985; Yilmaz et al., 1989). Thus *B. tabaci* transmission was undertaken in this study to further examine the CVYV host range. *Bemisia tabaci* is an EPPO A2 listed pest, which is present in both protected and outdoor crops in Europe (Accotto et al., 2000; Jones, 2003).

Viruliferous whitefly associated with imported plants or plant produce, and the movement of infected plant material could introduce ToCV or CVYV to countries where they are not established in Europe. The identification of species which could potentially influence the epidemiology of ToCV and CVYV in Europe by serving as virus reservoirs and/or virus sources where traded, is important to develop an effective control strategy. The host range for these viruses transmitted by the B biotype of *B. tabaci* was investigated in this study to provide further information on the risks posed.

Materials and methods

Virus isolates and maintenance of the virus and the vector

An isolate of each of ToCV and CVYV were kindly provided by D. Janssen, (CIFA, Spain). A culture of *B. tabaci* biotype B was maintained on *Euphorbia pulcherrima* (Poinsettia) in a whitefly-proof perspex cage at 25 °C with a 16 h photoperiod. ToCV and CVYV were maintained via *B. tabaci* transmission on known susceptible hosts *L. esculentum* (tomato) cv. Moneymaker, and *C. sativus* (cucumber) cv. Telegraph Improved respectively. All whitefly transfers were carried out using an entomological pooter.

Whitefly transmission

Adult whiteflies from a nonviruliferous culture were given an acquisition-access period (AAP) of 48 h on viruliferous tomato cv. Moneymaker (ToCV) or cucumber cv. Telegraph improved (CVYV). An inoculation-access period (IAP) on two test plants (ToCV) or three test plants (CVYV) of each species at two true leaf stage of 48 h was given (Navas-Castillo et al., 2000; Cuadrado et al., 2001), using six adult *B. tabaci* per plant (ToCV) or ten adult *B. tabaci* (CVYV). ToCV test plants comprised *L. esculentum* cv. Moneymaker, *Phaseolus vulgaris* cv. The Prince, *Vicia faba* cv. Bunyard's Exhibition, *Daucus carota* cv. Nairobi, *Coriandrum sativum*,

Raphunus sativus cv. China Rose, *Brassica rapa* cv. Golden Ball, *Brassica pekinensis* cv. Tip Top, *Anthriscus cereifolium*, *Ocimum basilicum*, *Beta rubra*, *Pisum sativum* cv. Onward, *S. tuberosum* cv. Desiree, *Capsicum annuum* (Longum group) cv. chilli Apache, *Zea mays* cv. Prime Apache, *Fragaria ananassa* cv. Vesca, and *Solanum melongena* cv. Black Beauty. CVYV test plants comprised *C. sativus* cv. Telegraph Improved, *N. clevlandii*, *N. benthamiana*, *A. cereifolium*, *N. tabacum*, *D. stramonium*, *Petunia hybrida* cv. Crown, *L. esculentum* cv. Moneymaker, *Chenopodium quinoa* and *Allium cepa* cv. Red Barron. This procedure was repeated using healthy *B. tabaci* on two plants of the same species to provide healthy control material. After the IAP, test plants were fumigated with methyl bromide at a concentration of 15 g m^{-3} for 4 h, to give a concentration-time product of 60 g h m^{-3}. Fumigated plants were grown at 20 °C with a 16 h photoperiod for a period of 4 weeks, in a controlled environment cabinet within a quarantine facility for symptom development, then real-time PCR tests (ToCV) or RT-PCR (CVYV) were carried out for the detection of virus *in planta*. Re-isolation of virus was carried out for the PCR positive (ToCV) host species (chervil, to *L. esculentum*) to confirm the presence of infectious particles using the method described, except a PCR positive chervil plant was used for *B. tabaci* virus acquisition. Additionally, nine chervil test plants were assessed to determine whether transmission could occur from chervil to chervil. Re-isolation of CVYV was carried out for the RT-PCR positive host species to *C. sativus* to confirm the presence of infectious particles using the method described, except an RT-PCR positive plant of each species was used for *B. tabaci* virus acquisition.

RNA extraction

Total RNA extraction from all test plants was carried out according to the method of Korimbocus et al. (2002) adapted from Chang et al. (1993).

Reverse transcription-polymerase chain reaction (RT-PCR) for CVYV detection

CVYV specific primers and the RT-PCR method of Cuadrado et al. (2001) were used for the amplification of all CVYV total RNA extracts. RT-PCR was carried out using reverse transcriptase, *Taq* polymerase, and buffers obtained from Promega. The assay incorporated healthy and infected cucumber, and SDW controls, together with a healthy control for each species tested.

Real-time PCR probe and primer design for ToCV detection

The HSP70 sequences available on the NCBI database were used for primer and probe design, for real-time PCR detection (ie detection of PCR products during amplification) using TaqMan® chemistry. A forward primer, reverse primer and probe were designed within the conserved regions of the HSP70 gene without degeneracies so as to be specific for ToCV while including all known variants. The primer and probe design was carried out using Primer Express™ software (PE-Biosystems) as described in Mumford et al. (2000). Oligonucleotide primers and probe designed were designated as follows: Forward primer 258F: 5′ GTCTGTTCCGGCTGATTACAAGT 3′ reverse primer 331R: 5′ AATTGAAACCCAA AGAGGAACAAA 3′ and probe: 5′ TGGGCAG AGACTTTTCATGCAGGCA 3′ (FAM label). These sequences correspond to nucleotides 258–280, 331–308 and 282–306 respectively of ToCV NCBI Accession number AY048854. To determine the specificity of the assay the HSP70 sequences of all *Criniviruses* on the EMBL database were aligned using Meg Align™ software (DNASTAR INC., Madison, USA). A BLAST search of the designed primer and probe sequences obtained was carried out to compare all sequences with those present on the EMBL database. The oligonucleotide primer and probe sequences possessed a high degree of homology only to ToCV. The specificity of the assay was also assessed by comparison of healthy and ToCV infected *L. esculentum* total RNA extracts. The assay gave ct values <40 for ToCV infected plants but no amplification was observed in healthy plants.

Real-time RT-PCR assay for the detection of ToCV

Reactions for the detection of ToCV from total RNA extracts were carried out according to the method of Korimbocus et al. (2002) using 96-well plates and a 25 μl reaction volume containing 1 μl of RNA. Additionally, the cytochrome oxidase 1 primers and probe oligonucleotide sequences of

Weller et al. (2000) were utilised as an internal positive extraction control. Thermal cycling and fluorescence detection was carried out on an ABI Prism 7900 Sequence Detection System (PE-Biosystems) at generic cycling conditions (Mumford et al., 2000).

Sequence analysis of PCR products

PCR products from RT-PCR positive test plants were direct sequenced to provide additional confirmation of viral identity where re-isolation was either not achieved, or only one plant of three under test was found positive. A PCR product from each positive plant species was purified using a QIAquick PCR Purification Kit (Qiagen, Chatsorth, CA). Both strands were analysed using an ABI automated sequencer (Sequiserve, Germany), using the primers of Cuadrado et al. (2001).

Results

ToCV symptomatology and host range

A total of seventeen species belonging to fifteen genera in eight plant families were assessed as potential hosts for ToCV. Test plants from transmission experiments were all found asymptomatic, and were virus negative following real-time PCR testing, designed for the specific detection of ToCV except one of two *Anthriscus cereifolium* (cv. chervil) test plants (one *A. cereifolium* plant did not survive fumigation post-transmission), (Table 1). Healthy chervil and tomato controls were asymptomatic and found to be negative using the real-time PCR assay together with the water control (data not shown). Chlorotic symptoms indicative of ToCV were present on two of two PCR positive *L. esculentum* (tomato) transmission control plants. The presence of infectious particles was confirmed by re-isolation of the virus from the asymptomatic PCR positive host identified in this study- (chervil), via *B. tabaci* biotype-B transmission to tomato, and chervil to chervil transmission was investigated (Table 2). Post re-isolation from chervil, three of three tomato test plants were found positive by real-time PCR, and displayed symptoms indicative of ToCV infection, confirming the presence of infectious particles, and that ToCV was readily transmitted from chervil to tomato. All nine asymptomatic chervil test plants were found real-time PCR positive, confirming that ToCV was readily transmitted from chervil to chervil.

CVYV symptomatology and host range

A total of ten species belonging to eight genera in five plant families were assessed for the presence of CVYV. The host range was identified by RT-PCR assay and amplicons obtained were of the expected size 449 bp (Cuadrado et al., 2001) and viral identity was confirmed by sequencing. Six *B. tabaci* Gennadius transmitted CVYV hosts were identified belonging to four genera in two plant families by RT-PCR which comprised *L. esculentum*, *D. stramonium*, *N. benthamiana*, *N. tabacum*, *N. clevlandii*, *A. cereifolium* and *C. sativus* transmission control plants (Table 3). Healthy controls of each species under test were asymptomatic and RT-PCR negative. Water controls for the assay were found negative (data not shown). Vein yellowing symptoms characteristic of CVYV were observed on both *D. stramonium*, and *C. sativus* control plants. A single *N. benthamiana* test plant found infected, exhibited chlorosis and rugosity. All other CVYV infected species were asymptomatic. The number of infected test plants found was variable between species by comparison with susceptible *C. sativus* control plants (Table 3). The most susceptible hosts by this comparison were *L. esculentum* and *N. clevlandii*, where all three test plants of each host were found to be infected. CVYV was successfully re-isolated, via *B. tabaci* transmission to *C. sativus* test plants, solely from the solanaceous hosts *D. stramonium* and *N. tabacum*, and *C. sativus* control plants (Table 4) confirming the presence of infectious CVYV particles for these hosts. The number of infected test plants found was identical for *D. stramonium* and *N. tabacum*. CVYV was less readily transmitted to these hosts than *C. sativus*, by comparison with the number of infected *C. sativus* control plants. Vein yellowing symptoms were present on *D. stramonium* and *C. sativus* controls; however *N. tabacum* was asymptomatic. Therefore symptoms present were as recorded for these hosts prior to re-isolation.

Table 1. Experimental host range of ToCV when inoculated by *Bemisia tabaci*

Test plant[a]	Common name	Cultivar	Family	Taq-Man®[b]	Mean C_T[c]	Internal control – Mean C_T[d]	Symptoms[e]
Beta vulgaris	Beetroot	Mixed vars	Chenopodiaceae	–	40	21.20	NS
Raphanus sativus	Radish	China Rose	Cruciferae	–	40	21.42	NS
Brassica rapa	Turnip	Golden Ball	Cruciferae	–	40	22.21	NS
Brassica Chinensis	Chinese cabbage	Tip Top	Cruciferae	–	40	21.64	NS
Ocimum basilicum	Basil	NA	Larniaceae	–	40	23.56	NS
Pisum sativum	Pea	Onward	Leguminosae	–	40	20.45	NS
Phaseolus vulgaris	Dwarf French bean	The Prince	Leguminosae	–	40	21.47	NS
Vicia faba	Broad bean	Bunyard's Exhibition	Leguminosae	–	40	22.43	NS
Zea mays	Sweet corn	Prime Apache	Poaceae	–	40	34.65	NS
Fragaria vesca	Strawberry	NA	Rosaceae	–	40	24.85	NS
Solanum melongena	Aubergine	Black beauty	Solanaceae	–	40	21.15	NS
Solanum tuberosum	Potato	Desiree	Solanaceae	–	40	21.11	NS
Capsicum annuum, Longum Group	Cayenne Pepper	Chilli Apache	Solanaceae	–	40	21.57	NS
Anthriscus cereifolium	Chervil	NA	Umbelliferae	+*	31.10	19.90	NS
Coriandrum sativum	Coriander	NA	Umbelliferae	–	40	20.41	NS
Daucus carota	Carrot	Nairobi	Umbelliferae	–	40	18.80	NS
Lycopersicon esculentum	Tomato	Money-maker	Solanaceae	+**	21.07, 18.88	23.10, 21.68	CL

[a]Two test plants caged together and inoculated with ToCV by 30 *B. tabaci* given a 48 h acquisition access period on source plants (*L. esculentum*), and a 48 h inoculation access period on test plants.

[b]TaqMan® = Real-time fluorescent RT-PCR assay, + = positive result and – = negative result, +* = one plant positive of two tested , +** = two plants positive of two tested.

[c]Mean C_T-ToCV = Mean C_T value (per pair of microplate wells) – Threshold cycle-a value below 40 indicates a positive result.

[d]Internal control Mean C_T = Mean C_T value (per pair of microplate wells).

[e]Symptoms assessed one month post-transmission. NS = No symptoms, CL = chlorosis.

Table 2. ToCV re-isolation from Real-Time PCR positive *Anthriscus cereifolium*: confirmation by *Bemisia tabaci* transmission to *Lycopersicon esculentum* and *Anthriscus cereifolium*

Test plant[a]	TaqMan®[b]	Mean C_T –ToCV[c]	Internal control-Mean C_T[d]	Symptoms[e]
A. cereifolium 1	+	24.35	22.44	NS
A. cereifolium 2	+	24.01	23.13	NS
A. cereifolium 3	+	25.17	23.27	NS
A. cereifolium 4	+	20.29	17.74	NS
A. cereifolium 5	+	26.48	24.32	NS
A. cereifolium 6	+	24.19	22.77	NS
A. cereifolium 7	+	20.83	18.83	NS
A. cereifolium 8	+	20.11	18.23	NS
A. cereifolium 9	+	21.04	18.49	NS
L. esculentum 1	+	19.49	21.65	CL
L. esculentum 2	+	18.57	19.37	CL
L. esculentum 3	+	17.65	19.49	CL

[a]Test plants derived from TaqMan® positive source plant – *A. cereifolium* caged with *B. tabaci* and given a 48 h acquisition access period, then transferred to groups of two test plants caged together with 30 *B. tabaci* and given a 48 h inoculation access period.
[b]TaqMan® = Real-time fluorescent RT-PCR assay, + = positive result and – = negative result.
[c]Mean C_T-ToCV = Mean C_T value (per pair of microplate wells) – Threshold cycle-a value below 40 indicates a positive result.
[d]Internal control Mean C_T = Mean C_T value (per pair of microplate wells).
[e]Symptoms assessed one month post-transmission. NS = No symptoms, CL = chlorosis.

Table 3. Experimental host range of CVYV as determined by (*Bemisia tabaci*) transmission

Test plant[a]	Common name	Cultivar	Family	RT-PCR[b]	Number[c]	Symptoms[d]
Nicotiana clevlandii	NA	NA	Solanaceae	+	3	NS
Nicotiana benthamiana	NA	NA	Solanaceae	+	1	CL/R
Allium cepa	Onion	Red Barron	Liliaceae	–	0	NS
Petunia hybrida	Petunia	Crown (mixed)	Solanaceae	–	0	NS
Nicotiana tabacum	Tobacco	NA	Solanaceae	+	1	NS
Datura stramonium	Jimson weed	NA	Solanaceae	+	2	VY
Lycopersicon esculentum	Tomato	Moneymaker	Solanaceae	+	3	NS
Chenopodium quinoa	Quinoa	NA	Chenopodiaceae	–	0	NS
Anthriscus cereifolium	Chervil	NA	Umbelliferae	+	1	NS
Cucumis sativus	Cucumber	Telegraph Improved	Cucurbitaceae	+	3	VY

[a]Three test plants caged together and inoculated with CVYV by 30 *B. tabaci* given a 48 h acquisition access period on source plants (*C. sativus*), and a 48 h inoculation access period on test plants.
[b]RT-PCR = Reverse transcription polymerase chain reaction assay, + = positive result and – = negative result.
[c]Number = Number of test plants, of three, found positive by RT-PCR.
[d]Symptoms assessed one month post-transmission. NS = No symptoms, CL = chlorosis, R = Rugosity.

Sequence analysis

Direct sequencing of RT-PCR products from putative CVYV infected hosts was carried out to confirm viral identity. The consensus sequences for all amplicons obtained were 445 bp in length, 4 bp smaller than that reported by Louro et al. (2004) due to the sequencing reaction terminating this number of bases shorter than that reported. 100% homology was found for all sequenced amplicons both with each other as expected since one CVYV isolate was employed, and with the CVYV capsid protein (CP) sequence recorded on the GenBank accession number AY424869, (Louro et al., 2004). Additionally all sequences shared 99% sequence identity with GenBank accession number AY290865-CP sequence, 99% identity with accession number AJ301640-CP sequence and 96% identity with accession number AF233429-CP sequence. A Spanish isolate of CVYV was used in this study and the sequence identity found was in agreement with that previously reported by Louro et al. (2004) for a CVYV isolate from cucumber in Portugal, which also utilised the

Table 4. CVYV re-isolation from RT-PCR positive plant species, by *Bemisia tabaci* transmission, to *Cucumis sativus*

Source plant[a]	Test plant[b]	RT-PCR[c]	Number[d]	Symptoms[e]
C. sativus	*C. sativus*	+	2	VY
L. esculentum	*C. sativus*	−		NS
D. stramonium	*C. sativus*	+	1	VY
N. benthamiana	*C. sativus*	−		NS
N. clevlandii	*C. sativus*	−		NS
A. cereifolium	*C. sativus*	−		NS
N. tabacum	*C. sativus*	+	1	NS

[a]RT-PCR positive source plant caged with *B. tabaci* given a 48 h acquisition access period, then transferred to groups of three *C. sativus* test plants caged with 30 *B. tabaci* and given a 48 h inoculation access period.
[b]Groups of three *C. sativus* test plants were caged with 30 *B. tabaci* and given a 48 h inoculation access period.
[c]RT-PCR = Reverse transcription-polymerase chain reaction assay, + = positive result and − = negative result
[d]Number = Number of test plants, of three, found positive by RT-PCR.
[e]Symptoms assessed 1 month post-transmission. NS = No symptoms, VY = vein yellowing.

primers of Cuadrado et al. (2001) and reported 95.6% identity to the sequence published for a CVYV isolate from Israel AF233429-CP sequence, and 99% identity with an isolate from Spain, AY290865.

Discussion

This study identified new host(s) for ToCV and CVYV. To date, a partial experimental host range has been previously reported for ToCV (Wisler et al., 1998a, b) which identified four members of the family Solanaceae as ToCV hosts comprising *Physalis wrightii*, *S. tuberosum*, *N. clevlandii* and *N. benthamiana*; in addition *Lactuca sativa*, family Compositae, was found to be a non-host. The present study has demonstrated that *B. tabaci* will transmit ToCV from the most economically important host, *L. esculentum*, to *A. cereifolium* (chervil), an annual weed which is common in southern Europe, naturalized in Britain, and also cultivated as a culinary herb (Clapham et al., 1962). In addition it was found that *B. tabaci* will efficiently transmit ToCV from *A. cereifolium* to tomato, and from *A. cereifolium* to *A. cereifolium*. Thus in nature *A. cereifolium* could serve as an alternate and readily transmissible ToCV weed reservoir, and an infectivity cycle could possibly occur between *L. esculentum* and *A. cereifolium*. There is no report to date of the presence of ToCV in *A. cereifolium* field sources in Europe, however, due to the polyphagous nature of the vector (Williams et al., 1996), and as an experimental asymptomatic weed reservoir, which is hardy to −10 °C (Huxley et al., 1999), this host potentially poses a phytosanitary risk. The only other known tomato infecting *Crinivirus*, *Tomato infectious chlorosis virus* (TICV), exhibits symptoms which are similar to ToCV (Wisler et al., 1996). The two viruses are readily distinguished, however, by differences in vector specificity. TICV is transmitted solely by the greenhouse whitefly *Trialeurodes vaporariorum* (Wisler et al., 1996). This is the first report of experimental transmission of ToCV by *B. tabaci* to a species within the Umbelliferae.

The six non-cucurbitaceous hosts for CVYV belonging to two families found in this study considerably broadens the host range identified by previous researchers. Previous experimental transmission experiments using the vector *B. tabaci*, have been carried out by Al-Musa et al. (1985), Yilmaz et al. (1989) and Mansour and Al-Musa (1993). These studies only identified hosts belonging to the *Cucurbitaceae*; however CVYV has been subsequently experimentally transmitted from the natural weed host *Sonchus oleraceus*, Family Asteraceae, via *B. tabaci* to cucumber (Janssen et al., 2002). Some species found CVYV positive in our study have previously been reported as non-CVYV hosts (Yilmaz et al., 1989; Mansour and Al-Musa, 1993). These are *N. clevelandii*, *N. benthamiana*, *N. tabacum*, *D. stramonium*, *L. esculentum* and *C. quinoa*. These host range studies were carried out without the benefit of increased sensitivity afforded by modern molecular methods. This may explain why our results for some of the asymptomatic hosts identified in this study using the RT-PCR assay of Cuadrado et al. (2001) do not concur with those previously reported. In addition vein

yellowing symptoms were present in our study for *D. stramonium*. This may be due to use of a different strain of CVYV, (CVYV-Jor) reported by Lecoq et al. (2000) to produce a more severe stunting in cucumber than CVYV-Is but for which sequence information is not yet available. Putative 'dead end' hosts were identified in this study for CVYV, where re-isolation of infectious particles to *C. sativus* test plants by *B. tabaci* transmission was not achieved. These comprised host species belonging to the Solanaceae notably including the host species *L. esculentum*, or Umbelliferae. The possible mechanism for this is unknown. CVYV has not been reported to date in field sources of *L. esculentum* in Europe. This is the first report of experimental transmission of CVYV by *B. tabaci* to non-cucurbitaceous crops comprising Solanaceous and Umbelliferous specie(s). Some crop and common weed hosts common to both CVYV and ToCV were identified in this study. Of these both *D. stramonium* and *N. tabacum* have been previously reported as hosts of the *Begomovirus Tomato yellow leaf curl virus* (Kegler, 1994). The epidemiological significance of this is that these hosts may potentially serve as sources of multiple *B. tabaci* transmitted viruses, in addition to possibly contributing to virus epidemics in the European-Mediterranean region. Natural infection of weed species belonging to the families, Convolvulaceae, Malvaceae, or Asteraceae has previously been reported to coincide with an outbreak of CVYV in cucurbitaceous crops in south-eastern Spain (Janssen et al., 2002).

For countries where ToCV and CVYV are of quarantine significance an understanding of all the hosts that may carry *B. tabaci* transmitted viruses, and the countries in which these occur is required to identify the most important risks and effectively identify and monitor trade pathways. ToCV and CVYV are not known to be seedborne and the major crops at risk are not propagated by tubers, corms or rhizomes. Therefore, introduction would most likely be from the importation and movement of infected seedlings or growing plants reported by the EPPO-datasheet on quarantine pests. In addition the global spread of viruliferous polyphagous *B. tabaci* biotype B as a 'hitch-hiker' on traded plant material is a major factor in the global increase in whitefly-transmitted diseases (Jones, 2003). The *Begomovirus Tomato yellow leaf curl virus* has previously been detected *in vector* (*B. tabaci*) on plant material imported to the UK (Morris et al., 2002). The risk of CVYV becoming established in the UK from introductions of viruliferous *B. tabaci* 'hitch-hiking' on non-host plants is unlikely as the ability of *B. tabaci* to transmit the virus is lost after a 4–6 h period (Harpaz and Cohen, 1965). ToCV is retained by *B. tabaci* for 2–3 days (Wisler et al., 1998a). The UK is part of an EU protected zone for *B. tabaci*, and in the UK the vector is unlikely to survive other than in protected environments; however it is regularly intercepted on imported plant material (Morgan and Macleod, 1996), and subjected to successful containment and eradication action (Bartlett, 1992; Cheek, 1999). Significantly, should ToCV be introduced to the UK or other European countries, an alternate ToCV vector, *T. vaporariorum*, is widespread within the European-Mediterranean region.

Our experimental transmission study has identified new hosts which may influence the epidemiology of ToCV and CVYV in Europe by serving as virus reservoirs, and/or virus sources where traded. The potential effect on yield for the new hosts identified which are of economic importance is currently undetermined. Notably, the majority of new hosts identified were asymptomatic and therefore pose a potential phytosanitary risk. The effect of potential mixed virus infections in the hosts identified in this study with regard to *B. tabaci* transmission is not currently known. Control of these hosts where they occur as weeds is recommended as part of an Integrated Crop Management strategy. This study will contribute to UK pest risk assessment studies.

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