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Transmission of cineraria isolate of tomato yellow ring virus by *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera, Thripidae)

N. MORTAZAVI, M. ALEOSFOOR & K. MINAEI

A b s t r a c t : The cineraria isolate of Tomato yellow ring virus (TYRV-CI) (Family Bunyaviridae, Genus Tospovirus) has been found for the first time in tomato fields, in Iran. The virus causes a serious disease in field crops and ornamental plants and like other tospoviruses, is transmitted by thrips. In this study we examined and compared the efficiency of two economically important thrips species: *T. tabaci* and *F. occidentalis* to transmit TYRV-CI to petunia (*Petunia hybrida*) and tomato (*Lycopersicon esculentum*) plants. Percentage transmission values of 68.57% and 34.28% for *T. tabaci* and *F. occidentalis*, respectively, were demonstrated by serological assay (DAS-ELISA) when petunia was used as the test plant. Both species of thrips were capable of transmitting TYRV-CI to petunia, how ever the efficiency of transmission was less for *F. occidentalis* than *T. tabaci*. A similar difference in efficiency of transmission between the two species of thrips was observed when the tomato was used as test plant. For both, however, efficiency of transmission of virus was greater in tomato plants.

K e y w o r d s : Tomato yellow ring virus, *T. tabaci*, *F. occidentalis*, DAS-ELISA.

Introduction

Members of the genus Tospovirus belong to the Bunyaviridae family, a large family of lipid-enveloped viruses containing a genome comprising three single strands of RNA (PARRELLA et al. 2003, PAPPU et al. 2009). The enormous impact of tospoviruses is due to their wide host range, at least 1090 host plant species are known, and their worldwide distribution (PARRELLA et al. 2003).

For a long time tomato spotted wilt virus (TSWV) was the only member of this genus but now, based on parameters including host range, vector species and modern serological and molecular data, 20 different species are recognized (PAPPU et al. 2009, RILEY et al. 2011). TSWV was the first tospovirus to be identified in Iran, in tomato fields (WINTER et al. 2002). More recently, however, a tospo-like virus has been isolated from tomato fields in the Varamin area, Iran (HASSANI-MEHRABAN et al. 2005). This species is distinct from all other tospoviruses both serologically and in terms of the nucleotide sequence of its SRNA. Also its N protein is closely related to that of Iris yellow spot virus (HASSANI-MEHRABAN et al. 2005, RASOULPOUR & IZADPANAH 2007). The name Tomato yellow ring virus (TYRV) was proposed for this virus due to its producing

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systemic chlorotic and necrotic spots on leaves and yellow rings on the fruit (HASSANI-MEHRABAN et al. 2005). This virus, which has been found infecting ornamental plants in greenhouses and some field crops, is considered to be a serious cause of disease in Iran (BEIKZADEH et al. 2012, MASSUMI et al. 2009, RASOULPOUR & IZADPANAH 2007). Tospoviruses are transmitted from plant to plant by thrips (Thysanoptera, Thripidae) in a persistent and propagative manner (ULLMAN et al. 1997, WIJKAMP et al. 1995, WHITFIELD et al. 2005). Until now 14 species in the family Thripidae have been reported to be vectors of tospoviruses (ULLMAN 1997, JONES 2005, HASSANI-MEHRABAN 2008, OHNISHI et al. 2006). Early stage larvae, only, are capable of acquiring virus from infected plants and after undergoing a latent period, the viruses transmitted to healthy plants by the adult stage (ULLMAN et al. 1997, WIJKAMP et al. 1995, WHITFIELD et al. 2005). *Thrips tabaci* and *Frankliniella occidentalis* are the two economic and important pests that are able to transmit, most of the viruses in the Tospovirus genus with differing efficiencies (JENSER et al. 2002, PAPPU et al. 2009, ROSELLO et al. 1996,TAVELLA et al. 2002). They are the best known vectors of TSWV (CHATZIVASSILIOU et al. 1999, 2002, NAGATA et al. 2002) and are amongst the most important thrips species found in tomato fields and greenhouses but their ability to transmit TYRV as well as their efficiency, no known well. The objective of the present study was to investigate the role of *F. occidentalis* as a vector for the transmission of TYRV-CI and to compare it with that of *T. tabaci*. The relative merits of *Petunia hybrida* and *Lycopersicon esculentum* as indicator plants were to be investigated also.

Material and methods

Thrips stock colonies

Non-viruliferous stocks of *T. tabaci* and *F. occidentalis* were collected from a Tomato field in Marvdasht (45 km north of Shiraz). Plants were shaken over a white tray and adult thrips were collected by brush and confined in a plastic box. They were immobilized by placing them in a refrigerator for a few minutes before confirming their identity. The specimens were allowed to oviposit on fresh Persian cucumbers in plastic boxes (20×7.5 cm size) located in a greenhouse maintained at 27±1ºC, 65±5 % R.H and 16 h light per 24 h. Progeny thrips were reared for two generations after which larvae from the confined adults were picked up and used in transmission tests.

TYRV isolate, source and plant material

Cineraria plants (*Senecio cruentus*), collected from a commercial greenhouse in Shiraz in 2011 and exhibiting symptoms of infection with TYRV were used as the source of virus. The identity of the virus, designated Tomato yellow ring virus-cineraria isolate (TYRV-CI), was confirmed by double antibody sandwich enzyme-linked immuno sorbent assay (DAS-ELISA) (CLARK & ADAMS 1977). Primary antibody (anti-TYRV IgG) was used at a dilution ratio of 1:600 and secondary antibody (anti-TYRV IgG conjugated with alkaline phosphatase) at a dilution ratio of 1:900. Infected plants were kept under greenhouse conditions at 27±2 C° with 65±5 % R.H and 16 h light per 24 h.

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The virus was transmitted to petunia plants (*Petunia hybrida*) by mechanical inoculation of an extract of infected cineraria leaf material suspended in 0.01 M potassium phosphate buffer containing 0.01 % M sodium sulphite, pH 7. Test plants were raised from seeds in sterile soil and were inoculated at the four to five leaf stage. Plants were kept in the dark for 24 hours immediately before inoculation to enhance their susceptibility to infection (AGRIOS 2005). Inoculated plants were maintained in a greenhouse under the same conditions as before. Uninoculated plants grown in sterile soil were placed under the cages as negative controls. Tomato (*Lycopersicon esculentum*) and Petunia (*Petunia hybrida*) plants to be used for comparing the efficiency of transmission of virus by *T. tabaci* and *F. occidentalis* were raised from seed and grown in 20 × 16 cm (width × height) pots in thrips-proof cages in the same greenhouse conditions as mentioned above.

Transmission test

The capacity of thrips to transmit virus was assessed by use of the petunia leaf disk technique (WIJKAMP & PETERS 1993). Leaf disks, 5 cm in diameter, were cut from plants showing signs of infection 3 weeks after inoculation. Each was placed in a separate Petri dish (5 cm in diameter) containing an agar medium, together with a larva of either *F. occidentalis* or *T. tabaci*, less than 4 hours old, to allow for acquisition of virus to occur. Petri dishes were sealed and incubated at 27±1ºC, 65±5 % R.H and 16 h light a day for 48 hours. Larvae were then transferred to two-week old healthy tomato plants in pots each covered by a plastic cylinder (7.5 × 15 cm). The plants were checked periodically for emergence of adults. When adults appeared they were transferred in groups of 10 to virus-free petunia plants at the four to five leaf stage, each contained within a plastic cylinder. The plants were kept in greenhouse conditions and were tested for the presence of virus.

In order to compare the efficiency of transmission of virus between tomato plants and petunia plants, infected larvae of *T. tabaci* and *F. occidentalis* were transferred to healthy petunia plants contained within plastic cylinders, and were allowed to develop to the adult stage. Groups of 10 adults of each species of thrips were transferred to both healthy petunia or tomato plants. In order to verify test plant preference, 10 tomato seedlings with 7 repetitions and 10 petunia seedlings with 7 repetitions were used for each species.

Detection of TYRV in plants

Petunia plants (*Petunia hybrida*) were sprayed with Metasistox insecticide two weeks after being presented with potentially infected thrips larvae and their infection status verified by DAS-ELISA. The absorbance values were read on a MIOS-Junior ELISA-reader at 405 nm. Samples with higher ELISA values than the average for healthy controls plus three times the standard deviation ($\bar{x}$+3SD) were considered to be infected.

Statistical analysis

In order to compare plant preference and efficiency of transmission of TYRV-CI by *T. tabaci* and *F. occidentalis* data from the transmission experiments were analyzed using the chi squared test (Minitab 16 software).

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Results

Transmission efficiency of TYRV-CI by *T. tabaci* and *F. occidentalis*

Adults of both species transmitted TYRV-CI to petunia (Petunia hybrida) plants, but there were significant differences in their efficiencies (df=1, P= 0.004, χ^2= 3.88) (tab. 1). Comparison of transmission rates showed that T. tabaci transmitted TYRV-CI twice as efficiently as *F. occidentalis* (Fig. 1)

Tab. 1: Number of petunia plants infected with TYRV-CI by each species of thrips.

species	N
T. tabaci	20/35
F. occidentalis	12/35

N: number infected/number tested

Results by $\chi 2$ for comparing efficiency of transmission of virus in tomato plants and petunia by *T. tabaci* and *F. occidentalis* showed that there is a significant difference between the two host plants with values for *T. tabaci* (P=0.04, x^2 =4.08, df=1) and *F. occidentalis* (P=0.01, x^2 =6.49, df=1) being obtained. The transmission rate to tomato plants was significantly greater than to petunia plants for both species of thrips (Figs 2, 3).

Discussion

Approximately 5800 species are recognized in the order Thysanoptera (MOUND 2012) of which one hundred are considered to be crop pests causing damage by feeding or transmitting virus diseases to growing crops (INOUET et al. 2010, LEWIS 1997, TEULON & NIESEN 2005). Most pests in this order are members of the family Thripidae (MOUND 1997). *F. occidentalis* (PERGANDE) and *T. tabaci* (LINDEMANAND), known as Western Flower Thrips (WFT) and Onion thrips respectively, have been introduced throughout the world and are major pests of many crops (KIRK & TERRY 2003). Moreover, both species are known to be the best vectors of tospoviruses such as TSWV. TYRV-CI is a newly identified tospovirus that was detected in Iran in 2007 on cineraria plants in a greenhouse (RASOULPOUR & IZADPANAH 2007). This virus, like other tospoviruses, is transmitted by thrips. In the study reported here we first assessed the role of two species of *T. tabaci* and *F. occidentalis* in the transmission of TYRV-CI to an indicator plant (*Petunia hybrida*), then compared their relative efficiency. We showed by use of serological assays that both *T. tabaci* and *F. occidentalis* are able to transmit TYRV-CI to the indicator species. These results differ from those of HASSANI-MEHRABAN et al. (2005), who were unable to transmit TYRV-CI-T by thrips but agree with those of RASOULPOUR & IZADPANAH (2007) who demonstrated transmission of TYRV-CI to its hosts by *T. tabaci*. In our study we demonstrated that *F. occidentalis* also, was able to transmit TYRV-CI but less efficiently than *T. tabaci*. By comparison, *F. occidentalis* is the most efficient vector of TSWV for most of the country while failure to transmit this isolate by *T. tabaci* has been reported in some studies (NAGATA et al. 2002, 2004). On the other

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hand, *T. tabaci* has been observed to be the most effective vector species for transmission of iris yellow spot virus (SRINIVASAN et al. 2012). There is much evidence for significant differences in the ability of different species of thrips to transmit tospoviruses. Evidence for differences in competency and efficiency to transmit tospoviruses was first reported by WIJKAMP et al. (1995). NAGATA et al. (2004) showed that efficiency of transmission depended on both tospovirus isolate and species of thrips. They and others demonstrated that transmission efficiency, or vector competency, seems to be influenced by many factors including virus isolate, species of plant used for acquisition and inoculation of virus, species of thrips employed and mechanism of virus translocation (CHATZIVASSILIOU et al. 1999, NAGATA et al 2002, 2004). The thrips-virus relationship is complex. Virus acquisition takes place only in first and second instar larvae while transmission does not occur until the adult stage (RILEY et al. 2011). After acquisition, the virus binds to the mid-gut epithelium and replicates in the mid-gut epithelial and muscle cells. From there it migrates through the ligament to the salivary glands from where it is transmitted to healthy plants (WHITFIELD et al. 2005). In this way the quality of each step, the rate of virus multiplication and migration to the salivary glands and the different barriers to the progress of virus present in each species of thrips can affect the efficiency with which virus is transmitted (NAGATA et al. 2002). The lower transmission efficiency of *F. occidentalis* may be explained by one or all of the above mentioned factors. Indeed, of these factors, type of plant used for acquisition of virus and its subsequent passage by a particular vector species has an important effect on transmission efficiency (CHATZIVASSILIOU et al. 1999, NAGATA et al. 2002, 2004). It is known that viruliferous thrips transmit the virus to various hosts in a short time but at different rates (CHATZIVASSILIOU et al. 2002) and that suitability of plant host for vector feeding as well as its susceptibility to a specific isolate of virus are factors that may affect the outcome. Thrips have a shallow feeding habit on non-preferred hosts which will have an impact on the rate of the transmission and spread of virus in the field (CHATZIVASSILIOU et al. 2002, GERMAN et al.1992, VAN DE WETERING et al. 1998, ULLMAN et al. 1992). The importance of type of host plants on transmission rate demonstrated by some workers (TAVELLA et al. 2002) was reflected in our results, too.

The identification of *T. tabaci* and *F. occidentalis* as vectors of TYRV-CI, a new tospovirus isolate, is important to obtaining further data on TYRV-thrips interactions. These two species are important pests worldwide (FEKRAT et al. 2009, KIRK 2002) and it can be concluded from the work reported here have the potential to spread and cause TYRV epidemics in Iran and surrounding areas.

Although *F. occidentalis* may not transmit TYRV as efficiently as *T. tabaci*, but its powerful ability to survive and spread to most regions and crops (KIRK 2002), makes it likely to be an important vector for the spread of TYRV. Understanding TYRV vectors and the efficiency with which they spread virus is critical to managing this viral disease and is a strategy that has been used successfully for controlling virus epidemics in crops and weeds (WIJKAMP et al. 1995). Besides determining a difference in the transmission efficiency of *T. tabaci* and *F. occidentalis* we demonstrated that tomato is a better test plant than petunia for studying the transmission of TYRV-CI. This may be because the two species of thrips have a greater feeding preference for tomato. Furthermore, tomato is more susceptible than petunia to infection with TYRV-CI (TAVELLA et al. 2002). We conclude from the results of this study that host preference is a critical factor in determining vector capability and that further studies are essential to determining the role of host specificity in the transmission efficiency of *T. tabaci* and *F. occidentalis*.

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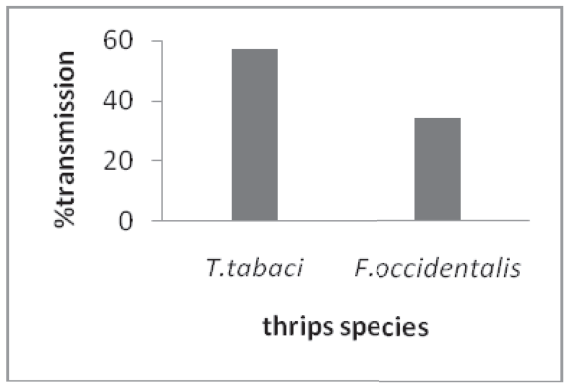

Fig. 1: Rate of transmission of TYRV-CI to petunia plants by *T. tabaci* and *F. occidentalis.*

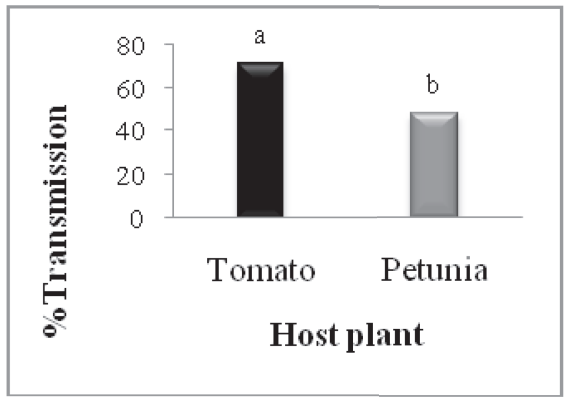

Fig 2: Comparison in TYRV-CI transmission efficiency between two host plants by *T. tabaci.*

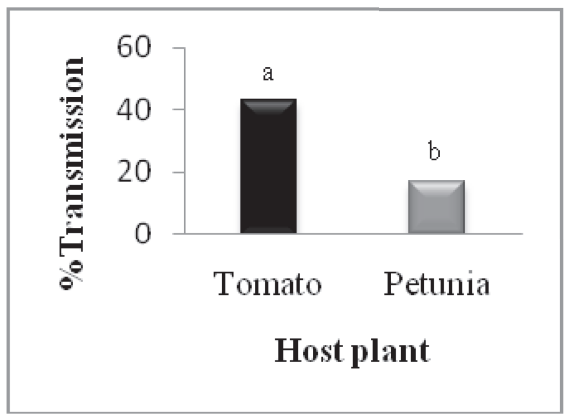

Fig 3: Comparison in TYRV transmission efficiency between two host plants by *F. occidentalis.*

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