Transmission of *Tomato spotted wilt virus* by the dark form of *Frankliniella schultzei* (Thysanoptera: Thripidae) originating in tomato fields in Paraguay

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(Received 28 August 2003; Accepted 11 November 2003)

**Abstract**

The efficiency of *Tomato spotted wilt virus* (TSWV) transmission by the dark form of *Frankliniella schultzei* was studied in Paraguay using the petunia leaf disk assay. Twenty percent of *F. schultzei* females collected in a field with TSWV-infected tomato plants were found to be transmitters of the virus. When larvae, up to 8 h old, were given a virus acquisition access period of 24 h, many adults, 88.9% and 72.2% of all males and females, respectively, transmitted the virus. These results indicate that the dark form of *F. schultzei* originating in Paraguanan tomato fields can transmit TSWV efficiently and is an important vector of the virus.

**Key words:** Frankliniella schultzei; dark form; Tomato spotted wilt virus; transmission efficiency; Paraguay

**INTRODUCTION**

Tospoviruses cause severe economic losses in the cultivation of a wide range of vegetable and ornamental plants in fields and greenhouses worldwide (German et al., 1992; Goldbach and Peters, 1994; Daughtrey et al., 1997; Peters, 1998). These viruses are transmitted by thrips (Thysanoptera: Thripidae) in both a propagative and a circulative manner (Ullman et al., 1993; Wijkamp et al., 1993). Ten thrips species have thus far been reported as vectors of these viruses: *Frankliniella occidentalis* (Pergande), *F. schultzei* (Trybom), *F. intonsa* (Trybom), *F. fusca* (Hinds), *F. bispinosa* (Morgan), *F. zucchini* Nakahara and Monteiro, *T. setosus* Moulton, *T. palmi* Karny, *T. tabaci* Lindeman and *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) (Mound, 2002).

*F. schultzei* is distributed between the latitudes 40° north and 40° south (Vierbergen and Mantel, 1991) and manifests dark and pale forms of body color. The former is primarily found south of the Equator and the latter north of the Equator. The dark form is known as a vector of four tospoviruses: *Tomato spotted wilt virus* (TSWV) (Samuel et al., 1930; Sakimura, 1969; Wijkamp et al., 1995), *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV) (Wijkamp et al., 1995) and *Chrysanthemum stem necrosis virus* (CSNV) (Nagata and de Ávila, 2000). On the other hand, the pale form has been reported to be an inefficient vector of TSWV and TCSV and a non-vector of GRSV (Sakimura, 1969; Cho et al., 1988; Mau et al., 1991; Wijkamp et al. 1995). The dark form is widespread throughout South America and is indicated as an important vector of tospoviruses in both Brazil (de Ávila et al., 1998) and Argentina (Williams et al., 2001).

In Paraguay, a disease known by growers as “vira cabeza” is caused by TSWV and affects tomato crops (Ishijima, 2002). Although the dark form of *F. schultzei* is observed primarily in tomato fields (Ishijima, 2002), it has never been shown how efficient this form is as a vector of the virus. Wijkamp et al. (1995) showed that the efficiency of TSWV transmission by the dark form adults of *F. schultzei* originating in a neighboring country, Brazil, is low (13.7%) when they are given virus-infected plants as newly hatched larvae. If this is also true of *F. schultzei* in Paraguay, this thrips species is unlikely to be an important vector of TSWV in tomato fields. However, recent studies suggest that the transmission efficiency of TSWV varies among populations in both *F. occidentalis* (van de Wetering et al., 1999a; Sakurai et al., 2002) and *T. tabaci* (Wijkamp et al., 1995; Chatzivassil-
In the dark form of *F. schultzei* as well, inter-population variation may exist in vector competence of the virus. In order to develop effective management programs for viral diseases, we must first determine the primary vector species and its competence of viral transmission in the infected area. In the present study, therefore, the frequency of TSWV-transmitters was investigated for dark form adults of *F. schultzei* collected in both TSWV-infected and non-infected tomato fields in Paraguay, and subsequently their potential to transmit the virus was tested after feeding TSWV-infected plant materials as newly hatched larvae in the laboratory.

**MATERIALS AND METHODS**

**Collection and maintenance of thrips.** *F. schultzei* females were collected from tomato fields in Caacupé and Caraguatay, Paraguay in February of 2000. The distance between the two fields was approximately 45 km. In the former field, symptoms of TSWV infection, i.e., bronzing, curling, wilting, necrotic streaks and spots, were observed on leaves of many tomato plants, and TSWV-nucleocapsid (N) protein was detected in all plants tested (*n*/H11005/10) by triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA). Although leaves of several tomato plants showed bronzing and curling in the latter field, all of them tested (*n*/H11005/12) were ELISA-negative for TSWV-N protein. In the present study, therefore, the tomato fields in Caacupé and Caraguatay were defined as TSWV-infected and non-infected, respectively. Females collected in these fields were confined in a ring cage made of a polymethylacrylate cylinder (80 mm diameter, 50 mm depth) with tea pollen and water (Murai and Loomans, 2001). Hatched larvae were reared on germinated seeds of broad bean, *Vicia faba* L., in a plastic container. The cultures were maintained at 25±1°C with a 16-h light photoperiod. Voucher specimens of thrips adults were deposited in the Laboratory of Insect Resources, Faculty of Agriculture, Tokyo University of Agriculture.

**Detection of TSWV by ELISA.** In order to detect TSWV particles in infected tomato plants and individual viruliferous thrips, TAS-ELISA was carried out as previously described (Sakurai et al., 1998) using commercially available polyclonal antibody (PAb, American Type Culture Collection, Manassas, VA, USA) for coating the wells, and monoclonal antibody (MAb, Agdia Inc., Elkhart, IN, USA) for detecting TSWV-N protein. The concentration of PAb was 1 μg/ml in a carbonate buffer (0.05 M sodium carbonate, pH 9.6), and that of MAb was 0.25 μg/ml in a phosphate-buffered saline (PBS, 0.14 M NaCl, 1 mM KH₂PO₄, 8 mM Na₂HPO₄, 2.5 mM KCl, pH 7.4), including 0.05% Tween 20, 2% polyvinylpyrrolidone (PVP-40) and 0.2% bovine serum albumin. After reaction with an anti-mouse IgG-alkaline phosphatase conjugate (1 μg/ml), 100 μl of 1 μg/ml p-nitrophenyl phosphate substrate with a 0.01 M diethanolamine buffer (pH 9.8) was added to each well of the ELISA-microplate. After 1 h at room temperature, wells that turned obviously yellow were considered to be positive for TSWV; wells with no or barely perceptible color change were considered to be ELISA-negative.

**TSWV transmission by thrips collected in tomato fields.** Fewer than three females per plant were collected at random from the TSWV-infected tomato field in Caacupé (*n*/H11005/20) and the non-infected field in Caraguatay (*n*/H11005/12). The efficiency of TSWV transmission was tested using the petunia leaf disk assay (Wijkamp and Peters, 1993). Thrips were given an inoculation access period (IAP) of 24 h on a leaf disk (8 mm in diameter) of *Petunia hybrida* cv. Polo Blue in a 2.0-ml microtube. After the IAP, the leaf disks were floated on water for 2 days at 25±1°C with a 16-h light photoperiod and observed for symptom development. Transmission efficiency was calculated as the percentage of leaf disks that developed local lesions. TSWV infection of these disks with local lesions was confirmed by TAS-ELISA.

**TSWV transmission by thrips given an infected tomato leaf in the laboratory.** To elucidate the potential of *F. schultzei* adults to transmit TSWV in Paraguay, newly hatched larvae, up to 8 h old, were confined with a tomato leaf that had been confirmed positive for virus infection by TAS-ELISA. The larvae were given a virus acquisition access period (AAP) of 24 h in a Petri dish (90 mm diameter, 20 mm depth) covered with a stretched laboratory film. After the AAP, only larvae that remained on the leaf were transferred and reared on germinated broad bean seeds until adulthood. On the third to fourth day after adult emergence, males
(n=18) and females (n=18) were individually examined for transmission competence by the petunia leaf disk assay at 25±1°C with a 16-h light photoperiod. After this assay, they were stored at −30°C and then tested for infectivity by TAS-ELISA. Newly born larvae given an AAP with a virus-free tomato leaf instead of an infected one were used as controls (males: n=12; females: n=12).

RESULTS

TSWV transmission by thrips collected in tomato fields

Four of 20 females (20.0%) collected in the field with TSWV-infected tomato plants (TSWV-infected field) successfully transmitted the virus to petunia leaf disks in the laboratory, while no females (n=12) collected in the field in which TSWV-infected tomato plants were not found (non-infected field) transmitted the virus (Fig. 1).

TSWV transmission by thrips given an infected tomato leaf

The efficiency of TSWV transmission by *F. schultzei* adults was notably high when they were given an AAP of 24 h for the virus as newly hatched larvae. Local lesions were observed on leaf disks of 88.9% of males (n=18) and 72.2% of females (n=18), suggesting that they were able to transmit the virus (Fig. 2). All the disks with local lesions were ELISA-positive for TSWV-N protein. No males (n=12) or females (n=12) reared on non-infected plant materials during their larval stage became transmitters (Fig. 2). There was a significant difference in transmission efficiency between groups reared on infected or non-infected plants in each sex (Fisher's exact probability test, males: p<0.0001; females: p<0.0001), but no significant difference was found between males and females (Fisher's exact probability test, p=0.402). In the thrips that were provided an AAP for TSWV, the virus was clearly detected in 65.5% of the transmitters (n=29) and 14.3% of the non-transmitters (n=7) by TAS-ELISA. The rate of ELISA-positive subjects was significantly higher for transmitters than for non-transmitters (Fisher's exact probability test, p<0.05). Thus, the existence of TSWV-N protein was confirmed in many transmitting thrips. All thrips adults given healthy plants as newly hatched larvae were found to be ELISA-negative (males: n=12; females: n=12).

DISCUSSION

*F. schultzei* has been reported to be a vector of TSWV (Samuel et al., 1930; Sakimura, 1962, 1969; Cho et al., 1988; Mau et al., 1991). Wijkamp et al. (1995) showed that the transmission effi-
ciency of the dark form of *F. schultzei* originating in Brazil (13.7%) is higher than that of the pale form originating in Northern Africa (2.3%). On the other hand, these rates are lower than those reported for *F. occidentalis* originating in the Netherlands (66.0%) and *F. intonsa* originating in Japan (31.8%), suggesting that the dark form of *F. schultzei* is not an efficient vector of TSWV when compared to *F. occidentalis* and *F. intonsa*. However, the present study demonstrates that dark form adults of *F. schultzei* originating in Paraguayan tomato fields accumulate and transmit TSWV at a high frequency when given infected plant materials as newly born larvae in the laboratory, and that transmitting adults exist in a field with infected tomato plants. In addition, *F. schultzei* is the only thrips species to be observed primarily in Paraguayan tomato fields (Ishijima, 2002). These findings indicate that this thrips species should be recognized as a highly efficient vector of TSWV and therefore as a serious threat to growers of tomato crops, particularly in Paraguay. Thus, in order to prevent economic damage to tomato production from viral disease, the prevalence of virus infection in *F. schultzei* adults should be monitored and their population density decreased appropriately in the fields.

Previous studies have suggested that the dark form of *F. schultzei* is widespread and is a significant vector of several tospovirus species in South America. *F. schultzei* adults originating in Brazilian populations efficiently transmit both TCSV (Wijkamp et al., 1995) and CSNV (Nagata and de Avila, 2000), and the wide dispersion of GRSV in Argentina may also be attributed to *F. schultzei* (Williams et al., 2001). The findings reported in the present study strongly suggest that TSWV should also be added to the list of tospoviruses efficiently vectored by the dark form of *F. schultzei* in South America.

Recently, intra-specific variation in the competence of some thrips species to transmit TSWV has been increasingly reported. In *F. occidentalis*, there exist differences in the ability of TSWV transmission among populations originating in various countries (van de Wetering et al., 1999a) and among different populations within Japan (Sakurai et al., 2002). In the same population, males have been found to transmit the virus more efficiently than females (Sakurai et al., 1998; van de Wetering et al., 1998, 1999b). In *T. tabaci*, adults of arrhenotokous populations, which consist of both males and females, transmit TSWV at a higher rate than those of thelytokous populations, which consist of only females (Wijkamp et al., 1995). In arrhenotokous populations, transmission efficiency is higher for males than for females (Chatzivassiliou et al., 1999) and depends on their host plant preference (Chatzivassiliou et al., 2002). As shown in the current study, inter-population variation is likely to exist both in the ability of the dark form adults of *F. schultzei* to transmit TSWV and in the relative occurrence of the two color forms (Sakimura, 1969; Wijkamp et al., 1995).

*F. occidentalis* was first recorded in 1990 in Japan (Fukuda et al., 1991; Hayase and Fukuda, 1991). As it spread quickly throughout the country (Saeki, 1998), Japanese growers have experienced economic losses to various crops and flowers due to direct feeding damage by this thrips species (Katayama, 1998) and diseases associated with TSWV (Kato and Katayama, 1998; Hanada, 1999). The occurrence of *F. schultzei* has never been reported in Japan. However, this thrips species is a common species in the international trade of plant materials and has been most frequently found on the list of thrips species intercepted at ports of entry in Japan (Hayase, 1991; Oda, 1993) and in the Netherlands (Vierbergen and Mantel, 1991; Vierbergen, 1995). Because of the small body size of *F. schultzei*, it is difficult to detect and identify. Therefore, this thrips species may easily invade Japan and spread in the near future. If *F. schultzei* populations are able to transmit TSWV efficiently as suggested in the present study, they can cause huge financial losses to Japanese growers in addition to the losses suffered due to *F. occidentalis*. It is thus crucially important to prevent the introduction of *F. schultzei* into Japan if possible, and if populations of this thrips species invade despite our efforts, we must take steps to eliminate them thoroughly before their expansion.

**ACKNOWLEDGEMENTS**

The author would like to thank Dr. S. Okajima (Tokyo University of Agriculture) for identifying the thrips and the deposit of voucher specimens, Dr. K. Natsuaki (Tokyo University of Agriculture) and the late Dr. C. Noda (Japan International Research Center for Agricultural Sciences) for supplying me with antiserum products, and Drs. T. Murai (National Institute of Fruit Tree Science, Japan) and Y. Narai (Shimane Agricul-
Plant Pathol. 48: 700–706.


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