Vector Relations in the Transmission and Epidemiology of Tospoviruses

D. Peters, I. Wijkamp, F. van de Wetering, and R. Goldbach

Department of Virology, Wageningen Agricultural University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

Tospoviruses are now recognized worldwide as limiting factors in the production of a large number of horticultural crops. The economic impact of the type species of these viruses, the tomato spotted wilt virus (TSWV), seems to be the highest because of its very broad host range. The current worldwide spread one of its most common vectors, the western flower thrips (Frankliniella occidentalis) and the abundance of Thrips tabaci in some solanaceous crops. The economic impact of recently described tospovirus species must still be assessed using reliable diagnostic tools. The spread of the tospoviruses and infection of the plant results from interactions between the plant, the vector and the virus. To better understand the epidemiology of tospoviruses, their interactions with various thrips species are reported.

Emergence of Tospoviruses

Occurrence of diseases caused by tospoviruses
Except for the countries or regions directly bordering the Sahara, and the Arabic Peninsula, TSWV occurs worldwide in all (sub)tropical and temperate climate zones. Although outbreaks of TSWV may be sporadic in some areas, they appear consistently in others and are a threat to some crops, e.g., tomato and other crops in Brazil (Resende et al. 1996) and tobacco in southern Europe (Ivantcheva-Gabrovskas 1959; Gajos 1972).

TSWV has repeatedly caused minor problems in western Europe and North America. The disease faded away in western Europe in the 1950s because of insecticide control of insect pests in greenhouses. However, a revival of tospovirus infections occurred in Europe during the late 1980s and early 1990s (Marchoux et al. 1991; Vaira et al. 1993) and became an important problem in the floral and vegetable industry of France, Italy, Portugal, and Spain. This revival of tospovirus infections in western Europe was preceded by an invasion of western flower thrips (WFT), Frankliniella occidentalis, into Europe (Zur Strassen 1986; Mantel and Van de Vrie 1988; Brødsgaard 1989). The majority of the infections occurred in greenhouses in northwestern Europe; whereas, in south and south-western Europe, high incidences of the disease were observed both in greenhouses and in the field. A further expansion of F. occidentalis over Europe in the late 1980s resulted in the emergence of infections in greenhouses in Poland (Kaminska and Korbin 1991). The recent discovery of F. occidentalis in Bulgaria (Jankulova, pers. comm.) and
Greece (Chatzivassiliou et al. 1996) was accompanied by severe outbreaks of tospovirus infections in crops that had not previously been affected. The outbreaks in Europe were preceded by severe outbreaks in the United States. These tospovirus epidemics were encountered in the groundnut industry of several southern and southeastern states, and in ornamental crops on the west and east coast as well as in Canada. The epidemics on the east coast and Canada were attributed to the expansion of *F. occidentalis*. The epidemics in the groundnut industry were attributed to *F. occidentalis* and to two other vectors, *F. schultzei* and *F. fusca*.

*F. occidentalis* also plays a role in the spread of tospoviruses in other areas of the world. This thrips species has been discovered in South Africa (Thompson and van Zijl 1996) and Australia (Latham and Jones, pers. comm.). Wongkaew (pers. comm.) considers *F. occidentalis* to be the most important vector for the spread of tospoviruses in groundnut crops in Thailand. Another tospovirus species, groundnut bud necrosis virus (GBNV), causes severe damage in the groundnut industry of India. This virus is endemic in the states of Maharashtra, Gujarat, Rajasthan, and western Uttar Pradesh. Increased incidence of this virus has been related to the continuous cropping of groundnut (Reddy et al. 1983). Originally, the spread of this virus was attributed to *F. schultzei*. However, the population development of this thrips species did not coincide with the development of the disease (Reddy et al. 1983). Later, Ranga Rao and Vijaya Lakshmi (1993) showed that this species was a poor vector of GBNV. The high incidence of GBNV in India is now attributed to *Thrips palmi* (Vijaya Lakshmi et al. 1995). This thrips species seems to be expanding its territory. Specimens are occasionally encountered on imported *Ficus benjamina* in the Netherlands. Although treated as a quarantine pest, it is doubtful that the importation of this thrips species can be stopped before it settles on other plant species. This species is also a vector of watermelon silver mottle virus (WSMV) and has been reported as a vector of TSWV (Fujisawa et al. 1988), an observation that must still be confirmed.

**Emergence of new tospovirus species**

TSWV was long believed to be the only member of the tomato spotted wilt virus group of plant viruses. Early failures to detect new species can be attributed partly to the use of inadequate detection and identification techniques. However, the expansion of WFT in the United States and Europe was followed by the discovery of a second tospovirus species, impatiens necrotic spot virus (INSV), in the United States (Law and Moyer 1990) and in Europe (Marchoux et al. 1991, de Ávila et al. 1992, Vaira et al. 1993). The host range of this virus is mainly ornamental plants, and it is rare in solanaceous crops. The origin of the reservoirs of this virus are still obscure. Plausible explanations are that either this virus was endemic in the west coast states of the United States and travelled along with the WFT, or that WFT was introduced into regions where INSV was endemic. Because WFT is the only vector found so far to transmit this virus (Wijkamp et al. 1995) endemicity of INSV in
Table 1. Members of the genus *Tospovirus* with serologically distinct N proteins.

<table>
<thead>
<tr>
<th>Established species:</th>
<th>Homology to N protein of TSWV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato spotted wilt virus (TS WV)</td>
<td>100</td>
</tr>
<tr>
<td>Groundnut ring spot virus (GRSV)</td>
<td>78</td>
</tr>
<tr>
<td>Tomato chlorotic spot virus (TCSV)</td>
<td>76</td>
</tr>
<tr>
<td>Impatiens necrotic spot virus (INSV)</td>
<td>55</td>
</tr>
<tr>
<td>Watermelon silver mottle virus (WSMV)</td>
<td>29</td>
</tr>
<tr>
<td>Groundnut bud necrosis virus (GBNV)</td>
<td>29</td>
</tr>
<tr>
<td>Melon spotted wilt virus (MS WV)</td>
<td></td>
</tr>
<tr>
<td>Peanut chlorotic fanleaf virus (PCFV)</td>
<td></td>
</tr>
<tr>
<td>Peanut yellow spot virus (PYSV)</td>
<td></td>
</tr>
<tr>
<td>Other possible new members isolated from:</td>
<td></td>
</tr>
<tr>
<td>Chrysanthemum (Brazil)</td>
<td></td>
</tr>
<tr>
<td>Chrysanthemum (The Netherlands)</td>
<td></td>
</tr>
<tr>
<td>Iris (The Netherlands)</td>
<td></td>
</tr>
<tr>
<td>Onion (USA)</td>
<td></td>
</tr>
<tr>
<td>Onion (Brazil)</td>
<td></td>
</tr>
<tr>
<td>Tomato (Brazil)</td>
<td></td>
</tr>
<tr>
<td>Cucumber (Brazil)</td>
<td></td>
</tr>
</tbody>
</table>

Europe means that before the arrival of this thrips species, it was transmitted by an unknown thrips species in an unknown reservoir.

After the discovery of INSV, two additional isolates with a great overlap in host range with TSWV were recognized. Tomato chlorotic spot virus (TCSV) and groundnut ringspot virus (GRSV) were recognized only after serological and genome-sequence analysis of the nucleocapsid (N) protein. TCSV has been reported only from South America and GRSV only from South America and South Africa. A fifth virus WSMV, has been described in Japan and Taiwan (Yeh and Chang 1995). This virus has a host range almost identical to that of TSWV. However, in contrast to TSWV, WSMV infects cucurbits systematically. A virus from Taiwan that reacts with GBNV antiserum has an N-protein gene that is identical in base sequence to WSMV (Heinze et al. 1995). This virus and WSMV forms with GBNV a fourth serogroup. Besides these viruses, other viruses have been found that can be considered as new species and incorporated into new serogroups (Table 1).

**Thrips species and transmission of tospoviruses**

Vector competence, defined as the innate capacity to vector tospoviruses, is restricted to a number of thrips species in the genera *Thrips* and *Frankliniella.*
Table 2. Confirmed thrips vectors of tospoviruses.

<table>
<thead>
<tr>
<th>Latin Name</th>
<th>Common Name</th>
<th>Tospoviruses</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Frankliniella fusca</em></td>
<td>Tobacco thrips</td>
<td>TSWV, INSV</td>
</tr>
<tr>
<td><em>F. intonsa</em></td>
<td></td>
<td>TSWV</td>
</tr>
<tr>
<td><em>F. occidentalis</em></td>
<td>Western flower thrips</td>
<td>TSWV, TCSV, GRSV, INSV</td>
</tr>
<tr>
<td><em>F. schultzei</em></td>
<td>(dark form) Common blossom thrips</td>
<td>TSWV, TCSV, GRSV</td>
</tr>
<tr>
<td></td>
<td>(light form) Melon thrips</td>
<td>TSWV, TCSV</td>
</tr>
<tr>
<td><em>Thrips palmi</em></td>
<td></td>
<td>GBNV, WSMV</td>
</tr>
<tr>
<td><em>Thrips setosus</em></td>
<td></td>
<td>TSWV</td>
</tr>
<tr>
<td><em>Thrips tabaci</em></td>
<td>spp. <em>communis</em> Onion thrips</td>
<td>TSWV</td>
</tr>
<tr>
<td>spp. <em>tabaci</em></td>
<td></td>
<td>TSWV</td>
</tr>
</tbody>
</table>

*TSWV tomato spotted wilt virus; TCSV tomato chlorotic spot virus; GRSV groundnut ringspot virus; INSV impatiens necrotic spot virus; GBNV groundnut bud necrosis virus; WSMV watermelon silver mottle virus.

Seven thrips species are competent to transmit tospoviruses (Table 2). The last one added to this list, *F. intonsa*, was first reported as a vector by Umeya et al. (1988). It is an efficient vector of TSWV (Wijkamp et al. 1995). This species is also involved in vectoring TSWV in dahlia in the Netherlands (Blom and Asjes, pers. comm.). Its role as a potential vector in the spread of TSWV deserves attention because it has been recorded as a non-vector in the past (Bonnemaison 1937).

The first thrips species described as a vector of TSWV was *T. tabaci* (Pittman 1927). Several authors were subsequently able to confirm the status of this species as a vector (Chamberlain and Taylor 1938), others were not. Three different TSWV isolates could not be transmitted by *T. tabaci*; whereas, successful transmission by *F. fusca* and *F. occidentalis* was obtained (Paliwal 1976). Strong evidence has been put forward to suggest that the competence of *T. tabaci* to transmit TSWV is restricted to one of two distinguishable subspecies of *T. tabaci* (Zawirska 1976). One subspecies, the populations of which consist of both males and females, has been designated *T. tabaci* ssp. *tabaci* and is believed to have been originally described as *T. tabaci* (Lindeman 1888). The larvae of this subspecies do not possess a comb on the IX abdominal tergite. The other subspecies, *T. tabaci* ssp. *communis* was described as *T. communis* (Uzel 1895). Outbreaks of diseases caused by TSWV in the tobacco cultures of Poland in the 1950s coincided with the spread of *T. tabaci* ssp. *tabaci*. Four *T. tabaci* populations were recently tested for their vector competence (Wijkamp et al. 1995). Three of them, devoid of males, did not transmit; whereas one population, which consisted of males and females, transmitted TSWV. These *T. tabaci* populations also failed to transmit TCSV, GRSV, and INSV (Table 3). As expected, a thrips species or population that is not
Table 3. Percentage transmission of several tospoviruses by some thrips species.

<table>
<thead>
<tr>
<th>Thrips species</th>
<th>TSWV</th>
<th>TCSV</th>
<th>GRSV</th>
<th>INSV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. occidentalis</em></td>
<td>66</td>
<td>28</td>
<td>10</td>
<td>85</td>
</tr>
<tr>
<td><em>F. schultzei</em> (dark)</td>
<td>14</td>
<td>38</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td><em>F. schultzei</em> (light)</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>F. intonsa</em></td>
<td>32</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>T. tabaci</em> (arrhenotokous)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>T. tabaci</em> (thelotokous)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* See Table 2 for abbreviations.

able to transmit a certain tospovirus species does not support its replication (Wijkamp et al. 1995). Tests on the replication of a virus in a thrips species or population may, therefore, answer the question of whether the thrips in question will transmit the virus.

*F. occidentalis* seems to be a more important vector than *T. tabaci* in many regions. This species has been recognized as a vector of TSWV since 1935 (Essig and Mischelbacher 1936). Because of its importance, most attention in vector research has been directed to this species. Its recent prominence as a vector is due to its worldwide expansion, the growing importance of horticultural crops, the emergence of INSV (a virus that is vectored exclusively by this species, Table 3), its wide host range, and its often great abundance in crops.

The epidemiology of tospoviruses
The outbreaks of tospovirus infections in new crops after the expansion of *F. occidentalis*, the failure of certain *T. tabaci* populations to transmit, and the unknown role of *F. fusca* and *F. occidentalis* in the spread of TSWV in groundnut in the southeastern United States are but a few examples of our lack of understanding of the epidemiology of vector–tospovirus relationships. The different epidemics may be due to the existence of different reservoirs, a specialization of various tospovirus species or strains to infect certain plant species, or specific adaptations of thrips populations to plant species. Introduction of new thrips species may open new niches or broaden existing ones. The success of *F. occidentalis* cannot only be explained by its worldwide expansion, but also by its efficient transmission of other tospovirus species (Table 2). This species transmits TSWV, TGSV, GRSV, and INSV at moderate to high efficiencies (Wijkamp et al. 1995).

The dynamics of virus spread are poorly understood. Reddy et al. (1983) inferred from thrips population data that primary infections played a major role in the development of bud necrosis epidemics in groundnut. Laviña et al. (1993) showed that control of thrips by insecticides did not affect the spread of TSWV in tomato.
crops. Camann et al. (1995) concluded that most infections arose in groundnut fields as a result of primary infections and that there was limited secondary spread of this virus after its establishment in the field. These cases show that primary infections prevail over intercrop spread.

The paucity of secondary spread in some crops may be explained by the failure of thrips to establish themselves. The population of thrips may also become only partial viruliferous. The nontransmitting portion of a thrips population would be composed of: all first instars born before the virus is available for acquisition; all first instars that fed on healthy parts of infected plants; all first instars that are not able to acquire an infective dose on infected plants; all first instars that acquire an infective dose, but do not transmit as an adult; all incoming adults from healthy plants; and some of the incoming adults from infected plants.

Migration of thrips from the virus reservoir to the crop depends on host-plant changes for the thrips. Host-plant changes may trigger an increased dispersal from plant to plant in the crop when the plants visited are rejected as hosts. Factors such as host-plant acceptance, movement frequency between plants, relative susceptibility of the hosts, and the probability that only probings lead to an infection will determine the success of virus spread. Knowledge on many of these factors is limited. Because viruliferous adults maintain the infectious state for their total lifespan, they are able to infect many plants as they migrate from plant to plant. But the infectivity of adults will not be the limiting factor (Wijkamp and Peters 1993). The frequency by which thrips change hosts and move between crops will be a strong facilitating factor in the spread of tospovirus to, and within, crops.

**Virus–Vector Relations**

**Transmission of tospoviruses**

Transmission is the result of the different processes that start with the ingestion of the virus on infected plants and end with the successful transmission of virus to a healthy plant. These events coincide with host-finding and feeding activities of the thrips. The rate at which thrips become viruliferous and transmit can be quantified in terms of the efficiency with which the virus can be acquired and subsequently transmitted and the length of the latent period. Its dynamics are the result of complex interactions between the triad of plants, vectors, and viruses (Table 4).

These interactions appear to be more complex as the components that compose these interrelations are more closely analyzed at the organism and population levels. The relations and interactions among the various factors that influence transmission dynamics are usually expressed as median values (Table 5).
Median acquisition access period

The virus is acquired when larvae feed on infected plants and is transmitted by second-instar larvae and adults after the virus has replicated in the vector (Wijkamp and Peters 1993, Wijkamp et al. 1993). Adults do not become viruliferous when they ingest the virus from infected plants (Ullman et al. 1992). The conversion of non-transmitters into transmitters may be blocked by the failure of the virus to pass through the intestinal tract or to replicate in the midgut cells.

Different transmission rates have been reported for various minimum acquisition access periods (AAP$_{\text{min}}$). Transmission is possible even after ingestion of the virus in feeding periods as short as 5 min (Razvyazkina 1953; Wijkamp et al. 1996b). Longer access periods are required for efficient transmission. Median values of the AAP may epidemiologically be more significant in estimating the efficiency by which the virus can be spread.

Fifty percent of $F$. occidentalis larvae (0–12 h old) were able to acquire an infective dose in 67 min with 95% fiducial limits (FL) of 39–113 min (Wijkamp et al. 1996b). The length of the median AAP may vary with the host on which the virus is acquired. The availability and distribution of the virus in the plant will affect the length of the median AAP.

Ullman et al. (1992) demonstrated that more enzyme-linked immunosorbent assay (ELISA) positive larvae were found with higher antigen titers when Datura stramonium was used as the host on which the virus was ingested as compared with Arctium lappa. Although both plant species had similar antigen, direct tissue immunoblotting showed that D. stramonium leaves displayed a more uniform antigen distribution than those of A. lappa. Even more erratic ingestion results may be obtained when feeding occurs on a plant that is only partly infected, i.e., TSWV infections in dahlia can be limited to a few leaves and INSV infections in chrysanthemum and pepper plants are also often restricted to a few leaves or to a small part of the stem.

Host suitability, which may be expressed in terms of susceptibility or resistance to thrips feeding, is another factor that governs virus acquisition. This factor can be measured by the damage caused by the feeding thrips. Because the period to induce feeding damage (silvery scars) appears to be much longer than the period required to acquire and transmit an infectious dose, it may be difficult to correlate feeding damage with the efficiency by which the virus is ingested and transmitted.

Besides host suitability and the distribution and concentration of the virus, the infected plant will also quantitatively affect the acquisition and transmission of the virus because $F$. occidentalis feed preferentially on TSWV-infected plants (Bautista et al. 1995).
Table 4. Interactions among tospoviruses, thrips, vectors, and host plants.

<table>
<thead>
<tr>
<th>Virus-vector relations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval stage which acquires virus</td>
</tr>
<tr>
<td>Larval stage which transmits virus</td>
</tr>
<tr>
<td>Transmission efficiency</td>
</tr>
<tr>
<td>Length median acquisition access period ($AAP_{50}$)</td>
</tr>
<tr>
<td>Length median latent period ($LP_{50}$)</td>
</tr>
<tr>
<td>Length median inoculation period ($IAP_{50}$)</td>
</tr>
<tr>
<td>Pathologic effect of virus on vector</td>
</tr>
<tr>
<td>Vertical transmission</td>
</tr>
<tr>
<td>Vector specificities (variability between thrips populations)</td>
</tr>
<tr>
<td>Virus specificities</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant-vector relations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host adaption/acceptance</td>
</tr>
<tr>
<td>Feeding behavior</td>
</tr>
<tr>
<td>Penetration/shallow feeding</td>
</tr>
<tr>
<td>Saliva ejection</td>
</tr>
<tr>
<td>Ingestion</td>
</tr>
<tr>
<td>Quality of infected plant as food source</td>
</tr>
<tr>
<td>Abiotic conditions</td>
</tr>
<tr>
<td>Genotype/phenotype of plants</td>
</tr>
<tr>
<td>Diversity in thrips species populations</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Virus-plant relations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host range</td>
</tr>
<tr>
<td>Susceptibility</td>
</tr>
<tr>
<td>Sensitivity</td>
</tr>
<tr>
<td>Reaction of plants as virus source</td>
</tr>
<tr>
<td>Availability of virus</td>
</tr>
<tr>
<td>Length of incubation time</td>
</tr>
<tr>
<td>Distribution of virus in leaf</td>
</tr>
<tr>
<td>Distribution of virus throughout the plant</td>
</tr>
</tbody>
</table>

**Development of infectivity**
The latent period expressed as the median $LP_{50}$ has been established for TSWV and INSV in *F. occidentalis* (Wijkamp and Peters 1993). Newborn larvae (0–4 h old) were allowed to feed for 24 h on infected impatiens plants. INSV was transmitted more efficiently than TSWV. INSV was transmitted by 93%, 82%, and 82% of the thrips when the virus was acquired at 20, 24, and 27°C, respectively. These values were 55%, 46%, and 43% for TSWV. This study also showed that most thrips transmit virus when they are second-instar larval. At 20, 24, and 27°C,
Table 5.  Kinetics of tospovirus transmission in thrips vectors.

<table>
<thead>
<tr>
<th>Median acquisition access period (AAP&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>TSWV 67 min (FL 95% 39–113 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median latent period (LP&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>TSWV 171 h, 109 h, and 84 h at 20°C, 24°C, and 27°C</td>
</tr>
<tr>
<td></td>
<td>INSV 157 h, 103 h, and 82 h at 20°C, 24°C, and 27°C</td>
</tr>
<tr>
<td>Median inoculation period (IAP&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>TSWV 59 min (FL 95% 44–77 min) petunia</td>
</tr>
<tr>
<td></td>
<td>TSWV 133 min (FL 95% 82–232 min) Datura</td>
</tr>
</tbody>
</table>

The percentage of thrips that transmitted during the second instar stage was 80, 70, and 63 for INSV, and 53, 41, and 33 for TSWV. These data show that only a few of the thrips that did not transmit as larvae, transmitted when they were adults. These results suggest that larvae kept at higher temperatures transmit at a lower rate than those kept at lower temperatures. This may indicate that the larvae develop relatively faster at higher temperatures than their infectivity. However, the time left between becoming infectious and pupation may be too short for some individual vectors to transmit the virus successfully when they develop at higher temperatures.

The length of the LP<sub>50</sub> also appears to depend on temperature. These periods were 169 h, 118 h, and 98 h for INSV and 176 h, 116 h, and 103 h for TSWV when the larvae were kept and tested at 20, 24, and 27°C. The latent period is completed in most larvae prior to prepupation. It is obvious that second-instar larvae will not carry the virus over large distances, but they may be active transmitters if plants are touching each other. The age of the plants, the age at canopy closure, and other cropping properties such as plant population or density will determine the infection pressure that is exerted by viruliferous larvae.

**Median inoculation access period (IAP)**

The inoculation period also affects the efficiency of tospoviruses transmission. Inoculation periods as short as 5–30 min have been recorded. When larvae 0–12 h old were fed for 3 days on infected <i>D. stramonium</i>, 6.3% of the thrips (adults) could infect petunia leaf disks in 5 min, and 16.7% transmitted when placed on <i>D. stramonium</i> seedlings (Wijkamp et al. 1996b).

Fifty percent of the petunia leaf disks were infected in 59 min with 95% fiducial limits of 44–77 min. The median number of <i>D. stramonium</i> seedlings became infected in 133 min with 95% fiducial limits of 82–232 min. The median IAP was longer for <i>D. stramonium</i> plants than for petunia leaf disks; whereas, the transmission rate, when IAPs of 5 min were given, showed a reverse tendency. The successful transmission in IAPs of different lengths may be due to differences in feeding behavior (Wijkamp et al. 1996b).
Restriction of virus acquisition by first-instar larvae

Acquisition feeding may last throughout the entire larval period because the larvae are confined to the host on which they develop. In addition, it is generally believed that both larval stages can acquire the virus. The capacity of each larval stage to acquire and transmit the virus has been analyzed (Wetering et al. 1996). The results showed that the capacity to become a transmitter was restricted to the first-instar larvae. This capacity rapidly decreased in this stage and became almost nil by the end of the first-instar stage. The second-instar larvae failed to develop transmission capability after virus ingestion. Virus replication was detected after virus uptake in first-instar larvae, but not in second-instar larvae.

Because only first-instar larvae will develop into transmitting thrips, there is only a small window in the whole lifespan of thrips that is open for virus acquisition. This may partially explain the limited number of thrips that become viruliferous on a recently infected plant. The number will be restricted to those that develop after the virus becomes available for ingestion. The number of thrips that becomes viruliferous will be even lower than the number that ingest the virus. This may be due to a number of factors: not all first-instar larvae will ingest the virus; not all larvae that ingest the virus will replicate the virus; not all larvae that ingest and replicate the virus will become viruliferous; and not all larvae will survive.

An estimation of the viruliferous thrips population on a plant can be made by assaying the number of thrips in which the virus has been replicated. Replication can be detected using antiserum to the nonstructural (NSs) protein, which is expressed in detectable quantities during the replication of the virus in thrips (Wijkamp and Peters 1993). These assays will give a better estimate of the infection pressure of the thrips population on an infected plant than the use of antiserum to the whole virus or N protein. In the latter case, detectable amounts of viral antigens will be found in the majority of the population living on an infected plant, but it will overestimate the potential infection pressure.

Feeding behavior of thrips

Ingestion and transmission of virus occur when a thrips selects its food source or feeds on it. Host selection and food ingestion are distinguished by different activities (Table 6). Sakimura (1962) recognized two types of feeding activities. In the first type (shallow), penetration is limited to the epidermal tissues or a few adjacent cells; this activity is restricted to a small area and no scars are produced.

The second type (penetrating) is characterized by swallowing of the content of disrupted cells and results in the well-known silvery scars on the leaves. As suggested by Sakimura (1963) successful inoculations might result from shallow feedings. However, proof is lacking to demonstrate the type of feeding activity during which virus ingestion and inoculation occurs, and at what efficiency.
Table 6. Feeding behavior and its relation to the ingestion and transmission of tospoviruses.

<table>
<thead>
<tr>
<th>Method</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light microscopy (Sakimura 1962)</td>
<td></td>
</tr>
<tr>
<td>Shallow type of feeding</td>
<td>Inoculation (?)</td>
</tr>
<tr>
<td>Penetration (swallow) type of feeding</td>
<td>Ingestion (?)</td>
</tr>
<tr>
<td>Electrical penetration graph technique (Harrewijn et al. 1996)</td>
<td></td>
</tr>
<tr>
<td>Penetrations (single cells)</td>
<td>Inoculation (?)</td>
</tr>
<tr>
<td>Salivation</td>
<td>Inoculation (?)</td>
</tr>
<tr>
<td>Ingestion (several cells)</td>
<td>Ingestion (?)</td>
</tr>
</tbody>
</table>

Recent studies on feeding behavior using the electrical penetration graph technique have revealed three basic waveforms (Harrewijn et al. 1996). These could be correlated with stylet penetration into separate cells, salivation, and ingestion. The first cell penetrations, characterized by P waves, last 6–7 s and are not necessarily followed by ingestion. Continuous ingestion may occur when the S pattern is observed. The content of several cells is ingested during this feeding type. The significance of these patterns for acquisition and inoculation has not yet been established. However, the short single-cell penetrations are probably homologous to the probing activity of Homoptera. Continuous feeding is unlikely to result in successful virus transmission because empty cells do not support virus replication. Therefore, it is unlikely that these cell penetrations by viruliferous thrips will result in infection. However, continuous feeding may be required to ingest an infectious dose of virus.

Pathological effects of tospoviruses on the vector

Possible pathological effects of tospoviruses on the vector are a matter of dispute. Sakimura (1963) was unable to find any deleterious effect; however, Robb (1989) suggested that thrips that feed for their whole larval development on TSWV-infected Nicotiana rustica plants showed a high mortality. An adverse effect was also demonstrated on the survival, reproduction, and development of thrips grown on INSV-infected lobelia plants (DeAngelis et al. 1993). The life parameters of viruliferous and nonviruliferous thrips that had fed as larvae (0–4 h old) for 6 h on TSWV infected D. stramonium leaves, and of noninfected thrips that had fed only on healthy plants were compared (Wijkamp et al. 1996a). A short AAP was chosen to exclude the possible deleterious effects of the infected plant on the physiology of the thrips. No differences were found between egg production, mean development time from egg to adult, preadult mortality, and longevity for all the three groups of thrips. Therefore, replication of TSWV in thrips does not have any deleterious effect on the physiology or morbidity of the viruliferous thrips. The adverse effects reported in previous studies may have been due to feeding of thrips on infected plants that had reduced food quality.
Transmission of tospovirus to offspring

Transovarial transmission is not uncommon among the bunyaviruses. This type of transmission can assure the maintenance of tospoviruses under adverse conditions or during absence of infected plants. However, the large number of host species, the vegetative propagation of infected plant material, the abundance of thrips, and their year-round reproduction in most climates are factors that diminish the need for vertical transmission of tospoviruses. This idea was confirmed when offspring raised from viruliferous *F. occidentalis* thrips were tested for their ability to transmit TSWV to leaf disks. Analysis of the offspring by ELISA demonstrated the complete absence of viral antigen (Wijkamp et al. 1996a).

Conclusion

Integration of our knowledge on thrips biology, the transmission of the virus, the rate at which the adult thrips migrate from the virus reservoir to a crop and from plant to plant in the crop, the feeding behavior of the thrips on the plant, and the susceptibility of the plant should provide a base for an understanding of TSWV epidemics. The transmission of the tospoviruses seems to be characterized by a number of features that are unique to circulatively transmitted plant viruses. The acquisition access and inoculation access periods are short, which is explained by the location of the cells from which the virus can be ingested and transmitted. These short periods promote the spread of the virus. However, because only the first-instar larvae can acquire the virus and subsequently develop into transmitters, the spread of the tospovirus is limited. The considerable spread, or the development of epidemics, must therefore to be explained by the development of large populations of viruliferous thrips on other plants species. The development of such populations, the potential of natural vegetation to act as virus reservoirs, and the impact of feeding preferences must still be explored. Results of these studies will increase the possibilities for better management of tospovirus diseases.


Pittman, H.A. 1927. Spotted wilt of tomatoes. Preliminary note concerning the


