# Introduction

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The International Symposium on Tospoviruses and Thrips of Floral and Vegetable Crops, held 7–10 November 1995 in Taiwan, attracted scientists active in virology, entomology, and resistance breeding. This blend of expertise provided much new information. The last international meeting on tospoviruses and their interactions with thrips and plants took place 5 years ago in Beltsville, Maryland, USA. During the past 5 years, tospovirus research has greatly expanded, particularly in the area of molecular biology. The meeting in Taiwan demonstrated that the Asia–Pacific region has become an important area for tospovirus research. This section summarizes the salient points that arose during the individual sessions.

**Biodiversity and Taxonomy of Tospoviruses**

Only a few years ago, tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV) were the sole established members of the *Tospovirus* genus in the Bunyaviridae family of arthropod-borne viruses. Some preliminary data suggested the presence of other distinct species of tospoviruses. It was not until the Taiwan meeting that: scientists agreed on the descriptors to be used to distinguish tospovirus species; and an updated list of tospoviruses became available. During a roundtable discussion on the classification and nomenclature of tospoviruses, the forum agreed on several descriptors for tospovirus classification (Table 1).

Although the International Committee on Taxonomy of Viruses (ICTV) only recognizes family, genus, and species as formal taxa, the participants agreed to maintain **serogroup** as an informal taxon to help avoid premature classification of distinct tospovirus species. Tentative species can be provisionally classified and described as a serogroup member or as a new serogroup, based on serological characterization using polyclonal antibodies against the nucleocapsid (N) protein, while awaiting further characterization. To become a distinct, established species, the N-protein sequence should show less than 90% amino-acid sequence identity with that of any other tospovirus species that has been described. However, the biological characters (vector specificity and host range) should also be determined to improve characterization and to help understand the ecology of the species.

A seven-person committee was formed during the symposium to review proposed species names. The current members are: D.V.R. Reddy, J. Moyer, D. Peters,

**Table 1.** Suggested descriptors for tospovirus classification.

| Level (taxon) | Descriptor |
|---|---|
| Genus | Virion morphology |
| | Genome organization |
| | Thrips transmission |
| Serogroup | Serological affinity (based on N protein) |
| Species | N-protein sequence (identity less than 90%) |
| | Vector specificity |
| | Host range (not symptoms) |

**Table 2.** List of established tospovirus species.[a]

| | | Descriptors[b] | | | |
|---|---|---|---|---|---|
| Serogroup | Species | Serological affinity | N-protein sequence[c] | Vector specificity | Host range |
| I | Tomato spotted wilt virus (TSWV) | + | 100% | + | + |
| II | Tomato chlorotic spot virus (TCSV) | + | 76% | + | + |
| II | Groundnut ringspot virus (GRSV) | + | 78% | + | + |
| III | Impatiens necrotic spot virus (INSV) | + | 55% | + | + |
| IV | Watermelon silver mottle virus (WSMV) (including isolate Tospo-to) | + | 29% | + | + |
| | Groundnut bud necrosis virus[d] (GBNV) | + | 29% | + | + |
| | Melon spotted wilt virus (MSWV) | + | 35% | + | + |
| | Groundnut yellow spot virus (GYSV) | + | ? | ? | + |
| | Isolates Chry1 and BR-11t (no species name given yet) | + | 65% | ? | ? |
| | Isolate BR-09[z] (no species name given yet) | + | 63% | ? | ? |

[a]More extensive descriptions of the ten established species and the four unassigned isolates can be found in various papers included in these proceedings.
[b]+ established; ? not yet determined.
[c]Percentage of homology with TSWV.
[d]Also reported as peanut bud necrosis virus (PBNV).

R. de O. Resende, R. Milne, S. Tsuda, and S.D. Yeh. The committee will evaluate the proposed name and confer with ICTV to seek approval. It was recommended that an informal isolate code (c.f. Chry 1, BR-11t, and BR-09z used for the Brazilian tospovirus isolates in Resende et al. in these proceedings) be used in publications until the proposed name has been approved.

By using the descriptors and the species names already introduced in the literature, a list of 10 definitive species was established (Table 2). It was also agreed that four additional tospovirus isolates [(**BR-l0o**: host, onion; origin, Brazil), **Tospo-PD2**: host, groundnut; origin, Taiwan), (**TSWV-W**: host, watermelon; origin, India), and (**PCFV**: host, groundnut; origin, Taiwan)] still need further characterization to establish their taxonomic positions.

## Virus–Vector Interactions

It was emphasized that it is of great importance to collect voucher specimens when working with thrips and studying their virus-vectoring parameters. This will ensure that the correctness of species identities can be verified. The importance of this was illustrated during the meeting when it was pointed out that the family Thripinae encompasses more than 1400 species that are grouped into more than 20 genera. Thrips species that are known to vector tospoviruses belong to the genera *Frankliniella* and *Thrips*. These two genera are the largest within the Thripinae; therefore, there are potentially many more thrips species that may vector tospoviruses. Based on the data presented during the symposium, the participants agreed on a list of established thrips vectors and their specificity (Table 3).

There are claims that *Scirtothrips dorsalis* transmits TSWV and that *Frankliniella flavus* (close to *T. palmi*) transmits a tospovirus in India; however, the data are insufficient to regard these as vector species. During the meeting, it became evident that virus–vector interactions have almost exclusively been studied for *F. occidentalis* transmitting TSWV. Although *T. tabaci* has generally been regarded as the main vector for TSWV, data presented during the symposium indicate that, in general, this species may be a poor vector. TSWV was a problem in Europe long before *F. occidentalis* was introduced during the 1980s. Papers from southern and eastern Europe suggest that biotypes may occur within given thrips species and that these biotypes may be further specialized in terms of host preference and vector activity.

A number of well-illustrated contributions were made on the cell biology of virus–thrips (*F. occidentalis*) interactions. Detailed immunolocalization studies indicated that tospoviruses actively replicate in their insect vector. More insight was also obtained on viral uptake and routing through the insect. Using in vitro approaches, a thrips protein (50 kDa) capable of binding to the virus has been

**Table 3.** Reported thrips vectors and their specificity.

| Thrips species | Viruses vectored[a] |
|---|---|
| 1. *Frankliniella occidentalis* | TSWV, TCSV, GRSV, INSV |
| 2. *F. schultzei* | TSWV, TCSV, GRSV |
| 3. *F. fusca* | TSWV, INSV |
| 4. *F. intonsa* | TSWV, TCSV |
| 5. *Thrips tabaci* | TSWV |
| 6. *T. setosus* | TSWV |
| 7. *T. palmi* | GBNV, WSMV, MSWV |

[a]See Table 2 for abbreviations.

identified. This may be the midgut receptor protein involved in viral uptake. These observations need in vivo confirmation because this protein is present throughout all developmental stages of the insect. Another contribution demonstrated that only $L_1$ larvae of *F. occidentalis* can successfully acquire the virus and become transmitting adults.

## Molecular Biology of Tospoviruses

A number of presentations provided an overview of the advances that have been made in the molecular biology of tospoviruses. At the meeting in 1990, only the full sequence of the S RNA segment (2.9 kb) of TSWV was known. Now, complete sequences of the entire genome of both TSWV and INSV are available, and full sequence data for two of the three genomic RNA molecules (M and S RNAs) were presented for GBNV. Furthermore, for a growing number of TSWV isolates and for two isolates of WSMV, complete sequences of the S RNA have been determined.

From these data it can be concluded that the ambisense character of two genome segments (S and M) is a unique generic character that is not found among other (animal-infecting) bunyaviruses. The overall genome and coding strategy is the same for all tospoviruses that have been analyzed to date. The nucleoprotein (N) and a nonstructural protein (NSs) are encoded by the S RNA, and the common precursor to both envelop glycoproteins (Gl and G2) and a second nonstructural protein (NSm) are encoded by the M RNA. Firm evidence was presented for the NSm having a role in cell-to-cell movement of the virus during systemic infection of plant tissue. However, the role of NSs remains enigmatic because no further progress was reported in the analysis of this protein, which may form paracrystalline or filamentous inclusions in both infected plant and insect cells. From the sequence data of NSs that is currently available, it has become clear that NSs represents the least conserved tospoviral protein. Therefore, a role in a basic process such as genome replication seems unlikely.

The sequence of the single open-reading frame in the largest (L) RNA segment (negative-stranded, 8.9 kb) of TSWV and INSV indicates that this part of the genome encodes the viral polymerase that is involved in virus replication and transcription. Evidence showed that initiation of transcription of the tospoviral genome involves cap-snatching, as is found in the animal-infecting members of the Bunyaviridae. During the symposium, it was shown that viral mRNAs contain 12–21 nonviral nucleotides at the 5'-end; whereas, enzymatic data illustrated that purified TSWV virus preparations contain an endonucleolytic activity that is able to cleave the capped leader sequence from added brome mosaic virus (BMV) RNA 4. These purified preparations were also shown to possess high in vitro transcription activity and even putative helicase (double-stranded RNA unwinding) activity.

Because *reverse genetics* (i.e., inability to recover infectious virus from cloned genomic sequences) is still not possible with tospoviruses (a general problem for negative-strand RNA viruses), the genetic analysis of viral genes and their products has been hampered. However, during the meeting, two alternative approaches for genetic analysis were presented: study tospoviral gene functions through transgenic expression of individual viral proteins in a host plant (as exemplified by the NSm gene); and use genome reassortment to map important phenotypic characters on the three individual RNA segments.

**Control of Tospovirus Diseases**

Three lines of research were presented in the area of management of tospovirus diseases: development of reliable and sensitive diagnostics and detection protocols; breeding and genetic engineering for host-plant resistance; and thrips management using biological and chemical agents.

The main problems with respect to diagnostics and detection have been solved during the past 5 years. For serology, high quality and specific antisera (both mono- and polyclonal) have become available and some of them are commercially available. The results of a ring test using various antibodies against geographically diverse tospovirus isolates illustrated that there is variability even within a given tospovirus species. RT-PCR diagnosis protocols have also been developed using either specific or degenerate universal primers.

With respect to the host-plant resistance to tospoviruses, natural resistance genes in the genera *Capsicum* and *Lycopersicon* have been found. Promising levels of field resistance were also reported for some groundnut genotypes in the United States. Two presentations dealt with the so-called "Stevens resistance" (*Sw-5*), which originates from *Lycopersicon peruvianum* and is introgressed into cultivated tomato (*L. esculentum*). Useful molecular markers were obtained that can be time-

saving tools in resistance-breeding programs, and these may pave the way to future cloning of the resistance gene. Some discrepancies in molecular mapping of *Sw-5* on the long arm of chromosome 9 were obtained. However, it was concluded that the different mapping of *Sw-5* was due to the use of (partly) different markers and genotypes. Although *Sw-5* has been regarded as a durable resistance gene, the occurrence of isolates that can break *Sw-5* resistance was reported from South Africa and Hawaii.

The lack of suitable resistance genes in most vegetable and floral crops has been a challenge to developing *pathogen-derived resistance*, i.e., to combat the pathogen with its own (transgenically expressed) genes or sequences. A broad overview of current strategies for tospoviruses and other viruses was presented. The resistance obtained by gene transformation with the viral N gene was found to be based on transcriptional expression rather than translational expression. Further experimental data, including nuclear run-on transcription analyses, gave first indications that the RNA-resistance phenotype is based on the specific breakdown of viral RNA sequences in the transgenic plants, similar to the phenomenon of *cosuppression* or *gene silencing* that has been reported for plants transformed with additional copies of endogenous genes. This N-protein gene-mediated resistance has been achieved in tobacco, tomato, lettuce, and chrysanthemum. N-gene mediated resistance is virus specific; however, it was demonstrated that this restriction can be circumvented by transforming host plants with multiple copies of N genes derived from various tospoviruses. Furthermore, it was reported that RNA-mediated resistance can only be induced by N gene and NSm gene sequences.

A series of papers on the prospects for biological control and integrated pest management of thrips formed an important part of the symposium. Potential parasites (e.g., entomopathogenic fungi) and predators (e.g., *Orius* spp.) and their effectiveness were discussed, as was the application of chemical volatiles from chrysanthemum to influence the behavior of *F. occidentalis* or its predators. However, more knowledge on the epidemiology of tospoviruses and thrips, including the role of weeds in the spread of tospovirus diseases, needs to be generated. This information is needed not only in view of consumer demand for decreased use of pesticides, but because it is clear that insecticides alone do not work and that they can even be counter-productive to the control of thrips and tospoviruses.