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Tospoviruses on Ornamentals

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ABSTRACT

The ornamental industry has experienced tremendous growth in the past 50 years and has developed into one of the major economic branches of modern agriculture. Tospoviruses are one of the major pathogens of ornamentals all over the world, reducing their aesthetic value and profitability. The rapid emergence and marked geographic spread of tospoviruses is due to the enhancement of the international trade of propagative materials and their final products. The distribution of tospoviruses was generally preceeded by a rapid expansion of their efficient new vector, *Frankliniella occidentalis*. In this review, we give a short account of the history and distribution of tospoviruses, and describe the host range, genetic and transmission studies, isolation and detection methods. Finally conventional and transgenic means for their control are described.

Keywords: control, genetics, ornamentals, symptoms, tospoviruses, transmission

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INTRODUCTION

The ornamental industry – including vegetatively and seed propagated cut flower crops, bulb and corm crops, pot (foliage and flower) and bedding plants, and tree nursery products – has experienced tremendous growth in the past 50 years and has developed into one of the major economic branches of modern agriculture. It has become a global industry with commercial production in almost every country, and international trade has expanded rapidly. The Rabobank in The Netherlands estimated that world-wide sales of ornamental horticultural products in 1990 were more that 50 billion USA dollars. Within this total the most important category was cut flowers with retail sales of 24 billion USA

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dollars, followed by 14.3 billion in sales of pot plants, 7.6 billion of tree nursery products, 900 million of flower bulbs and 1.6 billion of other propagation material (Hack *et al*. 1992; de Kleijn *et al*. 1992) In 1990, the largest exporter of cut flowers and pot plants was Holland, with 59 and 48% of world exports, respectively (Loebenstein *et al*. 1995). In 2004, the world floriculture products was around 75 billion USA dollars. Three leading production countries are the Netherlands, 3.6 billion EURO; US, 5.18 billion USA dollars and Japan, 3.47 billion USA dollars. The proportion of different floriculture products varies greatly between countries. The main floriculture products are: Germany cut flowers (45.3%); the Netherlands cut flowers (48.5%) and potted flowers (34.8%); in the US bedding and garden plants (51.8%), but 15.7% cut flowers; in China potted flowers (39.1%) and cut flowers (31.6%), while in Columbia cut flowers dominate at 99.3% (Laws 2004). Around these three countries, three biggest producing and market regions have gradually formed: European Union-Africa region, North America region and Asia-Pacific region (Xia *et al*. 2006).

Ornamental plants are produced for their aesthetic value. Maintaining this aesthetic value is the major concern of ornamental production facilities. The major factors reducing plant quality are arthropods and disease pests, whose abundance are highly increased due to enhanced international trade of propagative materials and their final products. *Frankliniella occidentalis* is probably the most serious pest of floriculture crops in the world (Parella 1995). They damage plants directly by feeding and laying eggs on the plants, and indirectly by acting as vectors for tospoviruses (such as *Tomato spotted wilt virus*, TSWV and *Impatiens necrotic spot virus*, INSV), one of the major threats for the ornamental industry.

SHORT HISTORY OF TOSPOVIRUSES

The earliest report of a tospovirus member occurred in the 1930s, when it was demonstrated that 'spotted wilt disease' of tomato first described in Australia in 1915 (Brittlebank 1919), had a viral etiology (Samuel *et al*. 1930). This virus, named *Tomato spotted wilt virus* (TSWV) was subsequently shown to be the causal agent of a variety of other diseases, including leaf curl of gram (Reddy *et al*. 1983), brown pod of pea (Reifschneider *et al*. 1989), tomato and tobacco carcova, tobacco kromnek, pineapple yellow spot, pineapple side rot, tomato bronze leaf, Kat river disease, makhorka tip chlorosis and 'vira cabeca' (Francki and Hatta 1981). The virus was shown to be spread by thrips, a large family of minute insects (Pittman 1927). These discoveries were followed by an increase in the number of publications on the occurrence of TSWV in many countries on a large number of host plants. The first record of the disease outside of Australia was from the United Kingdom (Smith 1931), with subsequent reports from many countries in Europe, America and Africa during the 1930s (Best 1968) and recently also in Asia (Chen *et al*. 2005; Lin *et al*. 2005). From the early 1980s a rapid emergence and marked geographic spread of TSWV took place (Marchoux *et al*. 1991; Vaira *et al*. 1993) which was generally preceded by a rapid expansion of its efficient new vector, *Frankliniella occidentalis*.

Similarly, in Australia, the frequency and distribution of outbreaks increased in the 1990s due to the arrival and dispersal of *F. occidentalis* (Persley *et al*. 2006). TSWV ranks among the ten economically most important plant viruses, causing crop losses worldwide worth more than $1 billion (Goldbach and Peters 1994). In the late 1980's a new virus was recognized, *Impatiens necrotic spot virus* (INSV) (earlier TSWV-I), which was the first virus recognized as a separate tospovirus. Its name was first proposed by Law *et al*. in 1991. It was initially reported on New Guinea impatiens in the USA (Law and Moyer 1989), causing severe losses in the floral crop industry in the United States and Europe (Moyer *et al*. 1999).

DESCRIPTION OF TOSPOVIRUSES

Distribution

The tospoviruses are a highly cosmopolitan virus group now considered to have a wordwide distribution, with one or more having been recorded in over 50 different countries, representing all five continents (Gáborjányi *et al*. 1994, 1995; Mumford *et al*. 1996).

TSWV and INSV pose a major threat to the glasshouse industries of North America and Western Europe (Allen and Matteoni 1988; Hausbeck *et al*. 1992; Goldbach and Peters 1994), including The Netherlands (de Ávila *et al*. 1992), France (Marchoux *et al*. 1991), Italy (Vaira *et al*. 1993) and Spain (Lavina and Battle 1994), and recently in Asia (Adkins *et al*. 2005).

INSV has become a major pathogen on flower crops in greenhouse grown plants in the USA (Daughtrey *et al*. 1997). The distribution of other, recently developed new tospoviruses is restricted to smaller areas.

Transmission

One of the main factors contributing to the global status of the tospoviruses as pathogens, is the tenacity of their vectors. The tospoviruses are transmitted by thrips (Thysanoptera: Thripidae) with a number of species acting in this role (German *et al*. 1992).

Only 9 of the 5500 known species of thrips have been shown to be associated with any disease. Thus less than 0.2% of Thysanoptera species are known to be associated with tospoviruses. Only five of the 160 described species of *Frankliniella* genus are known vectors of tospoviruses, only three of the 280 species of the genus *Thrips*, and just one of the 9 species of *Scirtothrips* (Mound 1998). Recent studies proved that one of each genus of *Ceratothripoides* (Premachandra *et al*. 2005) and *Tenothrips* (Ghotbi and Baniameri (2006) can also be vectors.

Earlier *Thrips tabaci* was considered the major global vector of TSWV (Cho *et al*. 1989; Marchoux *et al*. 1991), but its status has now declined; later studies showed it to be incapable of transmitting some Hawaiian TSWV isolates (Cho *et al*. 1991; German *et al*. 1992).

Currently, the most important vector of TSWV is Western flower thrips (*Frankliniella occidentalis* Pergande) (Goldbach and Peters 1994). The global spread of *F. occidentalis*, a significant horticultural pest that began in the late 1970, had been previously confined to the western coast of North America. The spread across the USA and Europe was almost concurrent, aided by the huge increase in the national and international movement of plant material (Kirk and Terry 2003).

All thrips species which transmit tospoviruses occur widely accross the globe and are polyphagous in nature, infesting most ornamentals, too. One exception is *T. setosus*, occurring mainly on dahlia and narcissus and whose distribution area is limited to Japan and Korea (Umeya *et al*. 1988; Palmer *et al*. 1991).

Phylogenetically, the *Scirtothrips* genus is very distant from the first two genera (*Thrips* and *Frankliniella*) (Mound 1996). These minute, polyphagous insects form a large and diverse group with many species being regarded as serious crop pests in their own right (Lewis 1973). Transmission of plant viruses by thrips is not unique to the tospoviruses. Thrips, especially *T. tabaci*, have been shown to transmit a number of other viruses, including *Tobacco streak virus* (Sdoodee and Teakle 1987), *Prunus necrotic ringspot virus* (PNRSV) (Greber *et al*. 1991), *Maize chlorotic mottle virus* (MCMV) (Ullman *et al*. 1992) and *Sowbane mosaic virus* (SoMV) (Hardy and Teakle 1992). Although other viruses can be transmitted by thrips species, the tospoviruses are the only ones transmitted in a persistent, circulative manner (Ullman *et al*. 1992), involving a highly specific virus-vector interaction.

One of the most outstanding features of the thrips-tos-

povirus relation is the inability of adults to acquire the virus. Tospoviruses are acquired only by the first (L1) and second (L2) larval stages and can be readily transmitted in the L2 and adult stages (Sakimura 1963). For *F. occidentalis* the acquisition access period (AAP50) and inoculation access period (IAP50) needed for 50% of the thrips to acquire and inoculate TSWV are 67 min and 59 min, respectively. The median latent period (LP50) decreases with increasing temperature, and ranges between 80 and 170 hrs. Once the virus is acquired, it is passed transtadially and thrips remain infectious for life (Wijkamp and Peters 1993; Wijkamp *et al*. 1995).

Early attempts to elucidate the underlying mechanism of this differential acquisition investigated differences in gut pH and redox potential in adults and larvae. Studies recently on *F. occidentalis* have revealed that while both adults and larvae ingested the virus, hence ruling out differences in feeding behaviour as an explanation, a midgut barrier prevented the movement of the virus out of the midgut of adults (Ullman *et al*. 1992). It was found that virions accumulated within the gut epithelium of adults. The virus could not be detected by ELISA, in adults after 4 days (Cho *et al*. 1991). In contrast, the virus was detected beyond this point in larvae, being present in midgut epithelia, fat body, brain, nerve cells, and probably more importantly within the haemocoel and salivary glands (Ullman *et al*. 1992). The exact mechanism of exclusion is unknown, but analogies with other members of the Bunyaviridae with insect vectors might result in attachment and processing of virus particles (Ludwig *et al*. 1989).

The mechanism of viral penetration into thrips cells is unknown, but histological evidence suggests uptake across the apical midgut epithelial membrane occurs within smooth vesicles, indicating that the process is receptor-mediated endocytosis (German *et al*. 1992; Ullman *et al*. 1992). This hypothtesis would agree with evidence collected for other enveloped viruses that suggest an endocytotic pathway (Marsh 1984; Ludwig *et al*. 1989). The identified 55 kD protein (Bandla *et al*. 1995), which occurs in the midgut of *F. occidentalis* and which binds to TSWV glycoprotein may have a key role in this process. The full details of cellular recognition events are as yet unknown, but with advances in this area expanding rapidly, for example in the use of thrips cell culture (Hsu *et al*. 1991; Hunter and Hsu 1995), the mechanisms behind differential acquisition may soon be elucidated. This in turn will also provide an insight into other specifity mechanisms, within the thrips-tospovirus interactions, e.g. why non-vector thrips species cannot acquire the virus and how certain species differentially acquire and transmit specific tospoviruses.

Once larvae have acquired the virus, it persists within the thrips, being passed transtadially from larval to pre-pupal to pupal stages. Adult thrips remain viruliferous for their entire lives (Sakimura 1962). Evidence suggests that not only are the tospoviruses circulative, but are also propagative, with the virus replicating within the vector. Attempts to demonstrate replication in the past were unsuccessful (Best 1968), but later works provided evidence to support a propagative relationship. The evidence comes in three parts. Firstly, serological data shows that virus concentration varies within the vectors, with levels decreasing towards pupation and then increasing again post-emergence. Secondly, cDNA probes could detect both genomic and complementary RNAs within thrips. Finally, immunogold labeling has permitted detection of the non-structural protein of virus within infected *F. occidentalis* (Cho *et al*. 1991; Urban *et al*. 1991; Ullman *et al*. 1992, 1993; Wijkamp *et al*. 1993; Ullman *et al*.1995).

Transmission is primarily carried out by adult thrips, although sometimes rarely by larvae (Sakimura 1963). Once a viruliferous thrips enters adulthood, its potential for infecting plants is very high for a number of reasons. Tospoviruses persist within their vectors throughout their lives. This implies a period of between 30 to 40 days on average (Best 1968). During this period, the adults will feed actively, dispersing widely upon emergence and feeding on many different plants (Lewis 1973). The chances of the plant becoming infected are also enhanced due to a short inoculation access period, with a duration of only five minutes (Sakimura 1963), so extensive feeding on each plant visited is not required. When combined, these factors supply the potential for an individual viruliferous thrips to infect a large number of plants.

The main external influence on the virus-vector relationship is the host plant. Acquisition relies greatly on the piercing-sucking feeding behaviour of thrips (Hunter and Ullman 1992). Plant cell walls are breached and the cytoplasm ingested, along with any infecting virus. The efficiency of acquisition relies heavily upon two factors: The feeding preferences of the vector and the viral distribution within the material being fed upon. The dynamics of the thrips-plant-virus interaction are obviously far more complex and many other factors, including temperature have an important role, which must be combined with the other significant variables in order to produce a fuller epidemiological model. It may greatly help in effective disease control.

Wijkamp *et al*. (1995) proved distinct levels of specifity in thrips transmission of tospoviruses. Transmission efficiencies were determined for four thrips species (*F. occidenttalis*, *F. schultzei*, *F. intosa*, *T. tabaci*) and four tospovirus members (TSWV, INSV, *Tomato chlorotic spot virus*, TCSV, *Groundnut ringspot virus*, GRSV) by using a petunia leaf disk assay system. *F. occidentalis* appeared to be the most effective for the four tospovirus tested. A dark form of *F. schultzei* transmitted three tospoviruses, whereas a light form of this species transmitted TSWV and TCSV rather poorly. *F. intosa* transmitted TSWV efficiently and TCSV at a very low frequency. Only one of four populations of *T. tabaci* was able to transmit TSWV at a low efficiency.

The mechanism leading to vector competence of thrips species to transmit TSWV is not well characterized. The interaction of TSWV and the non-vector species *F. tritici* was investigated. A monoclonal antibody to the non-structural protein (NSs) of the TSWV was used to detect TSWV replication within the thrips by immunofluorescence microscopy and ELISA. TSWV was aquired by *F. tritici*, replicated and moved within the alimentary canal of *F. tritici*, similar to a known vector of TSWV, *F. occidentalis*. However, the virus was not found in the salivary glands of *F. tritici*, which is prerequisite to virus transmission. Thus, the movement to the salivary glands may determine vector incompetence of *F. tritici* (Filho *et al*. 2005).

On the basis of the latest research it was proved that TSWV can activate the immune system of its main vector, *F. occidentalis*. This is the first report of the activation of an insect vector immune response by a plant virus. This may lead to a better understanding of an insect's immune responses against viruses and may help in the future development of novel control strategies against plant viruses as well as human and animal viruses transmitted by insect vectors (Medeiros *et al*. 2004).

Beside thrips transmission, the spread of tospovirus also occurs with vegetatively propagated material such as tubers, corms, cuttings, etc. of many ornamental species. Infected (not adequately tested) mother stocks may be infection sources (Wilson 2001; Adkins 2003).

TSWV persists from year to year in flower bulbs or roots. Lilies and dahlias are a common source of the virus (Swift 2003). There was a report about seed transmission via tomato and *Cineraria* seeds (Crowley 1957), while later studies suggest that seed transmission does not occur amongst tospoviruses (Mumford 1995; Antignus *et al*. 1997), neither in petunia nor in *Celosia* seeds. Therefore – as far as we know – tospoviruses are not considered seedborne. In addition, due to the highly unstable nature of tospoviruses in the plants (Best 1968), mechanical transmission and spreading by contact under normal field or glasshouse conditions is very unlikely.

Due to their wide host range (especially for TSWV) among crops, weeds and ornamentals, there are many pot-

ential sources of inoculum. Most other tospoviruses seem to have narrower host ranges and the role of alternative hosts in their epidemiology as not as clear.

Morphology and genetics of Tospoviruses

The family of Bunyaviridae is divided into five genera, based on similarities in molecular structure of their genones, biological properties and physical aspects of protein and virion morphology. The genus tospovirus contains viruses that infect plants. The four other genera, Bunyavirus, Hantavirus, Nairovirus and Phlebovirus, contain over 300 viruses that infect animals. Members of the Hantavirus genus are spread by aerosols of saliva and animal excrement while members of the other four genera have specific relationships with arthropod vectors, in which they also replicate (Nichol *et al*. 2005).

Among tospoviruses, molecular characterization was first described for TSWV and INSV, and later for other recently identified tospovirus members.

Tomato spotted wilt virus (TSWV)

The morphology of the tospoviruses is typical for members of the Bunyaviridae (Elliott 1990). Measuring 80 to 110 nm in diameter, the particles of TSWV consist of an outer membrane envelope, roughly spherical in shape. Embedded within this membrane, and projecting from its surface, are two glycoproteins denoted G1 (78 kD) and G2 (58 kD) (Tas *et al*. 1977; Mohamed 1981). Enveloped within the particle are three linear single-stranded RNAs, the S (2.9 kb), M (4.8 kb) and L (8.9 kb) RNA (de Haan *et al*. 1990, 1991; Law *et al*. 1992). Among the tripartite ssRNA genome one segment is negatively polar and the other two are ambisense. These RNAs are found in tight association with the 29 kD N protein (de Haan *et al*. 1990), forming the pseudo-circular nucleocapsid complexes (de Haan *et al*. 1989). In addition, particles also contain approximately 10 copies of the 331.5 kD L protein, the viral replicase (de Haan *et al*. 1991; van Poelwijk *et al*. 1993) (**Figs. 1, 2**).

The virus particles are unstable, with a thermal inactivation point (10 min) of 40-46°C, a longevity *in vitro* at room temperature of 2-5 hrs and a dilution end-point between 2×10^{-2} and 10^{-4}.

Small (S) RNA of TSWV

The nucleotide sequences of the three genomic RNAs of TSWV have been determined and expression strategies elucidated. When the complete nucleotide sequence of the S RNA of TSWV isolate BR-01 was determined (de Haan *et al*. 1990), it revealed the presence of two open reading frames (ORFs), linked by an A-U rich intergenic region.

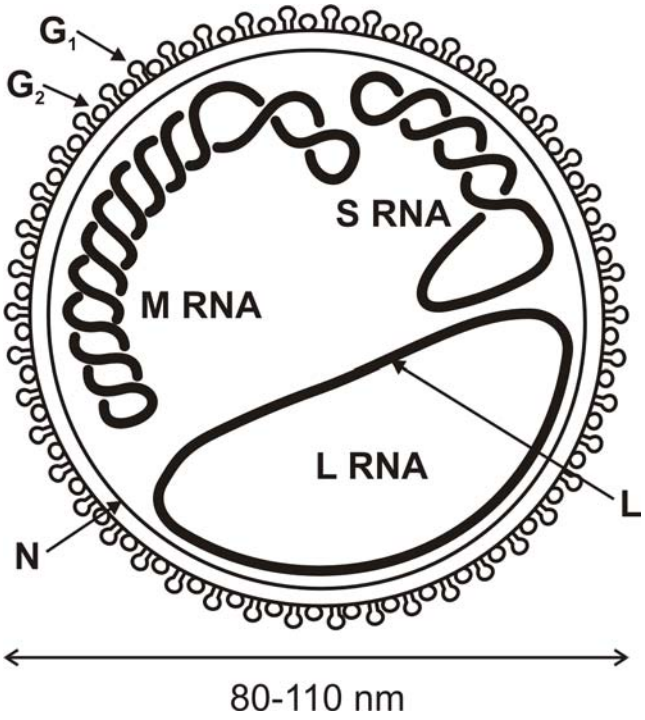

Fig. 1 Morphology of TSWV.

The genomic RNA possesses an ambisense coding strategy, whereby gene products are expressed from subgenomic mRNAs of opposite sense. The larger ORF is in the viral sense and encodes a protein with a predicted weight of 52.4 kD (the NSs protein). This non-structural protein is found in dense aggregates within the host cytoplasm, often forming fibrous or crystalline structures (Kormelink *et al*. 1991; Urban *et al*. 1991). Its function remains unclear, although the protein does appear to have a role in pathogenicity, with the levels of NSs correlating with the severity of symptoms (Goldbach *et al*. 1993). It may be involved in vector transmission. The other ORF in the complementary sense encodes for the N protein, whose predicted size (28.8 kD) compares favourably with direct measurements of its size (29 kD) determined by SDS-PAGE (Tas *et al*. 1977; Mohamed 1981). The intergenic region is predicted to have a stable secondary structure, forming a hairpin loop.

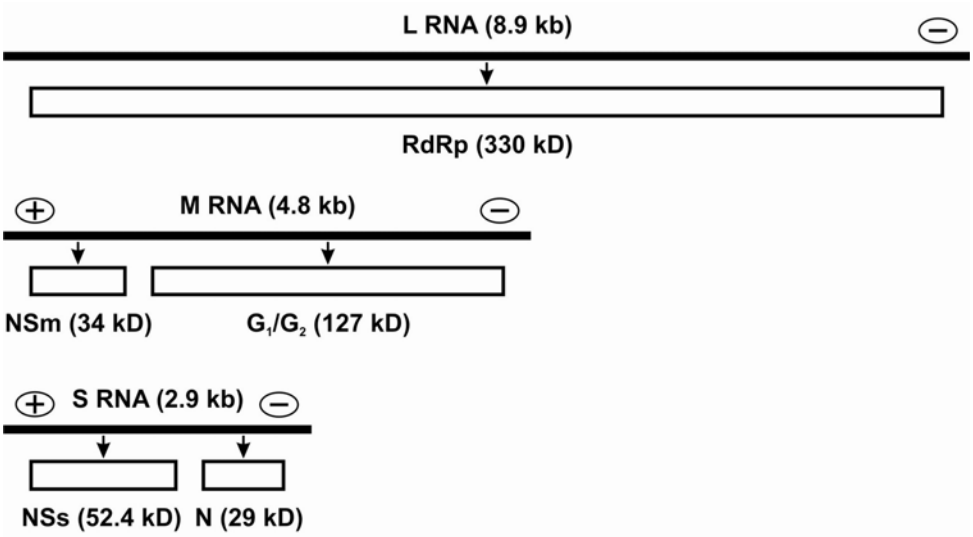

Fig. 2 Genome organization of TSWV.

S RNA of INSV, Watermelon silver mottle virus (WSMoV) and Groundnut bud necrosis virus (GBNV)

The full sequence of the S RNA of INSV (isolate NL-07) has also been determined. It is 3016 nucleotides in length and has an identical coding strategy and gene structure to TSWV, with marginal differences in size and variation in sequence (de Haan *et al*. 1992). The S RNAs of WSMoV (Yeh and Chang 1995) and GBNV (Satyanarayana *et al*. 1995) are 3534 and 3057 nucleotides in length, respectively while the S RNA of the GBNV sequenced by Maiss (1995) is 3536 nucleotides in length. All have the characteristic coding strategy, although some have unusually long intergenic regions. The features of twin ORFs expressed by an ambisense coding strategy and intergenic hairpin structure, which are found in the tospovirus S RNA, are also found in members of the Phlebovirus genus, an animal Bunyaviridae group (Elliott 1990).

Medium (M) RNAs of TSWV and INSV

The complete RNA sequences have also been obtained for the M RNAs of TSWV and INSV (Kormelink *et al*. 1992; Law *et al*. 1992) and reveal many features in common with their respective S RNAs. The M RNA contains two ORFs, which are separated by an A-U rich intergenic region, which forms a stable hairpin. These two ORF are expressed via subgenomic mRNAs, in an ambisense manner (Kormelink *et al*. 1992; Law *et al*. 1992). This double ambisense arrangement (used for both and M RNAs) is unique within the Bunyaviridae, with all the other members possessing a negative-strand M RNA (Elliott 1990). Of the two ORFs on the M RNA, the complementary (negative) sense ORF encodes a single, large gene product with predicted sizes of 127.4 and 124.9 kD, for TSWV and INSV, respectively. This product is assumed to be the precursor of the glycoproteins, G1 and G2.

The other ORF in the viral sense encodes a small 33.6 and 34.1 kD protein for TSWV and INSV, respectively, designated by NSm (Kormelink *et al*. 1994). The function of the NSm protein deduced from several lines of evidence is considered to be in cell-to-cell movement (Kormelink *et al*. 1994). From these evidences a putative model for the role of NSm in viral transport has been suggested, the NSm binding to uncoated nucleocapsid, in the cytoplasm, but once at the plasmodesmata, uncoating to form a tubular structure, passing through into the neigbouring cell via the plasmodesmata, through which the nucleocapsid travels (Goldbach *et al*. 1993).

Large (L) RNAs of TSWV and INSV

The L RNAs of TSWV and INSV have also been fully sequenced (de Haan *et al*. 1991). The segment contains a large, single ORF, with a negative polarity, a feature of all Bunyaviride members (Elliott 1990). This ORF is transcribed into a subgenomic RNA, from which the putative viral replicase is translated. Estimates of this protein's size, approximately 200 kD, taken from SDS-PAGE studies (Tas *et al*. 1977; Mohamed 1981), vary considerably from that predicted from the sequence, 331.5 kD and 330.3 kD for TSWV and INSV, respectively. When the L RNA ORF of TSWV is expressed in bacteria a 330 kD protein is produced. When this protein was used to raise an antiserum, it was found that this cross-reacted with the purified virus (van Poelwijk *et al*. 1993) and as no protein processing was observed (the antiserum only reacted with the full-sized protein), it can be assumed that the earlier estimates of 200 kD were anomalies generated by the SDS-PAGE system used.

Comparisons of the L protein with that of other Bunyaviridae shows a surprising trend. While studies with other RNA segments have shown the tospoviruses to be most closely related to the phleboviruses, the L RNA does not follow this pattern. When aligned, the highest level of homology is found with sequence data for Bunyamwera virus (Elliott 1989), the prototype bunyavirus, with 27% identity at the amino acid level (de Haan *et al*. 1991). Little homology was found between the predicted amino acid sequences of TSWV L protein and those of Rift Valley fever and Uukuniemi phleboviruses (Elliott *et al*. 1992).

Nucleotide sequence comparisons with other RNA polymerases suggest that the product of the L ORF is the viral RNA dependent RNA polymerase (RdRp). Several amino acid motifs are present which are also present in a range of putative polymerases from RNA viruses (Poch *et al*. 1989). These motifs may be involved in various aspects of polymerase function and the RNA replication process. In addition, Jin and Elliott (1993) have shown recombinant vaccinia virus, expressing the L protein from Bunyamwera virus, has both RNA transcriptase and replicase activity. Finally, detergent-disrupted virions have been shown to have RdRp activity (German *et al*. 1995).

Iris yellow spot virus (IYSV)

Serological and molecular characterization of IYSV was done by Cortes *et al*. (1998). The N protein of IYSV had an estimated size of 30 kD. In RNA analysis, the size of S, M and L RNA corresponds to 2.9, 4.8 and 8.9 kb, respectively. Molecular cloning and sequence determination, characteristics and predicted gene products, and expression of the vcORF of IYSV S RNA were also described.

Chrysanthemum stem necrosis virus (CSNV) and Zucchini lethal chlorosis virus (ZLCV)

Molecular cloning, sequence determination and analysis of CSNV and ZLCV N protein genes were done by Bezzera *et al*. (1999). Analysis of the genomic RNAs of CSNV and ZLCV revealed the presence of three RNA segments with sizes corresponding to those of RNAs of TSWV. The nucleotide sequences of the nucleoapsid (N) genes of both viruses contain 780 nucleotides encoding for deduced proteins of 260 amino acids, with a predicted size of 29 kDa. The 3′ untranslated region (UTR) of the S RNA of ZLCV was revealed to be much longer (220 bases) than that of all the other tospovirus species (approximately 130 bp).

Melon yellow spot virus (MYSV)

Kato *et al*. (2000) did the molecular and serological analysis of MYSV. Viral nucleocapsid (N) was purified with two protective reagents, and RNA extracted from the viral nucleocapsid was used for genomic analysis. The virus had a genome consisting of three single-stranded RNA molecules. The ORF on the viral complementary strand, located at the 3′ end of the viral S RNA, encoded the N protein. The molecular mass was estimated to be 31.9 kDa and comprised 279 amino acids. The 3′ terminus of the S RNA contained an eight-nucleotide sequence similar to the conserved sequence at the 3′ end of genomic RNA molecules of tospoviruses.

Peanut chlorotic fan-spot virus (PCFV)

To characterize the virus, viral S double-stranded RNA was extracted from PCFV-infected *Chenopodium quinoa* and used for cDNA cloning and sequencing. The full length viral strand of the S RNA was determined to be 2833 nucleotides, with an inverted repeat at the 5′ and 3′ ends and two open reading frames in an ambisense arrangement. The 3′ terminal sequence (5′-AUUGCUCU-3′) of the viral S RNA is identical to that of other tospoviruses, indicating that PCFV is a member of the tospovirus genus. The N and the NSs proteins of PCFV share low amino acid identities with other tospoviruses. The N ORF was 810 nts, encoding a predicted protein of 270 amino acids, with a calculated molecular mass of 31.1 kDa. The ORF started at nucleotide

253 with an AUG start codon and terminated at nucleotide 1943 with an UAA stop codon. The phylogenetic dendrogram of the *N* gene of PCFV compared with those of other tospoviruses indicated that PCFV is a distinct tospovirus. In hybridization analyses, and *N* gene cDNA probe of PCFV did not react with viral RNAs of other tospoviruses and vice versa (Chu *et al*. 2001).

S RNA of Calla lily chlorotic spot virus (CCSV)

DNA fragments corresponding to the S RNA of CCSV were amplified from total RNA extracted from CCSV-infected plants, cloned and sequenced. The full-length viral strand of the S RNA was determined as 3172 nucleotides in length, with an inverted repeat at the 5′ and 3′ ends and two open reading frames in an ambisense arrangement. The 3′ terminal sequence (5′-AUUGCUCU-3′) of the viral S RNA was found to be identical to that of other tospoviruses, confirming that CCSV belongs to the genus tospovirus. The N and the NSs proteins of CCSV share low amino acid identities, 20.9 to 65.1% and 19.9 to 66.1%, respectively, with those of reported tospoviruses (TSWV, GRSV, TCSV, CSNV, ZLCV, INSV, WSMoV, PBNV, WBNV, CaCV, PSMV, MYSV, IYSV, TYFRV, PYSV and PCFV). The phylogenetic dendrogram of the N protein of CCSV compared with those of other tospoviruses in WSMoV serogroup indicates that CCSV is a distinct tospovirus. Thus, based on the results of molecular characterization of S RNA, it was concluded that CCSV is a new tospovirus species belonging to the WSMoV serogroup (Lin *et al*. 2006).

Within the tospovirus genome, several regions have been found to have a putative role in gene regulation. The first of these are inverted complementary repeat sequences found at the termini of the RNA molecules (de Haan *et al*. 1989). These 65-70 nt sequences are thought to bind together, non-covalently forming the linear molecules into circular nucleocapsids (hence pseudo-circular), once encapsidation has occurred. A similar process, modulated by equivalent terminal sequences, has been recorded in other Bunyaviridae (Elliott 1990). These terminal sequences may also contain other motifs that control replication (de Haan *et al*. 1989). Sequence data from the S, M and L RNAs of TSWV (de Haan *et al*. 1990, 1991; Kormelink *et al*. 1992) and S and M RNAs of INSV Law *et al*. 1991, 1992) revealed a consensus terminal sequence of 8 nt (GAUUGC UC-3′) which should function in this role. Again, similar but distinct genus-specific consensus termini have been recorded throughout the Bunyaviridae (Boulay 1991).

In addition to viral sequences with regulatory roles, non-viral sequences have also been found spliced onto the 5′ termini of viral mRNAs (Kormelink *et al*. 1992). These 12-20 nt sequences are presumed to act as primers, initiating the transcription of the mRNAs. This process, known as 'cap-snatching' (where the primers are generated by cleavage from capped host mRNAs), is found amongst animal viruses, including the Bunyaviridae (Jin and Elliott 1993), but is unique amongst plant viruses (Kormelink *et al*. 1992). Comparison with the Bunyaviridae has also revealed a putative transcription termination consensus. In the Punta Toro phlebovirus S RNA, a small sequence at the peak of the intergenic hairpin loop is known to mark the point where the transcription of the subgenomic mRNAs terminates (Emery and Bishop 1987). Tospovirus S RNA species also feature such a secondary structure, as mentioned earlier. A consensus (CAAUUUGG) has been found which coincides with the peak of the hairpin, and has been designated as a putative termination motif (de Haan *et al*. 1992).

Naturally occurring defective forms of tospoviruses

These are found as two distinct types: Morphologically defective forms (Ie 1982; Verkleij and Peters 1983) and defective-interfering isolates (Resende *et al*. 1991).

Morphologically defective forms

Morphologically defective forms are characterized by their lack of a viral envelope, with an amorphous appearance under EM, being visualised as dark, diffuse aggregates in the host cell cytoplasm (Ie 1982; Verkleij and Peters 1983). In addition, membrane glycoproteins cannot be detected in plants infected with such isolates (Verkleij and Peters 1983). Morphologically defective forms are generated by serial mechanical inoculation through host plants (Ie 1982), although Resende *et al*. (1991) found that only mechanical inoculation onto *Nicotiana benthamiana* and not *N. rustica*, produced envelope-deficient particles, strongly suggesting an important role for the host in the generation of such mutants. Similar mutant isolates have also been obtained from INSV (Lawson *et al*. 1993), although these were generated by repeated mechanical transmission, using plants kept at elevated temperatures (24-27°C). Although the mechanism involved in triggering the generation of such defective forms is unknown, the cause is likely to be a mutation within the M RNA. Verkleij and Peters (1983) found that the M RNAs of defective isolates was smaller than those of normal isolates. This would suggest that possibly deletions had occurred within the glycoprotein ORFs, leading to a loss of at least one glycoprotein, and hence an inability to assemble the viral envelope. While Resende *et al*. (1991) did not find any changes in the size of the M RNA of the morphologically defective isolate that they characterised, an accumulation of point mutations, including ones leading to frameshifts, was detected (Goldbach and Peters 1994). This evidence suggests that the glycoprotein gene is no longer under selective pressure, providing circumstantial evidence for the hypothesis that the glycoproteins (and lipid envelope) are only involved in thrips transmission, with no role in the plant host (Goldbach and Peters 1994).

Defective interfering (DI) isolates

Similar to other plant viruses, DI RNAs have also been found in the Bunyamwera Bunyavirus (Patel and Elliott 1992). In TSWV and INSV, isolates possess truncated L RNAs, of 3.2 and 3.0 kb respectively, over 5 kb shorter than in non-mutant forms. In *N. rustica* plants infected with the DI isolate the DI RNA is preferentially produced, although some full length copies can be detected (Resende *et al*. 1991). While the isolates still form enveloped particles and the level of nucleocapsid protein detected was the same as in non-DI infected plants, the severity of symptoms was highly reduced (Resende *et al*. 1991). The causes and mechanisms behind DI RNAs are as yet unknown although further characterization of DI L RNA segments from TSWV show that, despite large internal deletions of between 60% and 80%, both 3′ and 5′ termini are retained (Resende *et al*. 1992). It is assumed that these termini contain all the required signals for replication and translation, but that the removal of most of the polymerase ORF prevents the production of a functional polypeptide (Resende *et al*. 1992).

Recent studies are about the evolution of tospoviruses and phylogenetic analysis based on sequence information for different isolates of the same virus (Mound 1998; Pappu *et al*. 2000). Dietzgen *et al*. (2005) showed that genetic variability among Australian isolates of TSWV was small. Direct sequencing of a 587 bp region of the nucleoprotein gene (S RNA) of 29 isolates from diverse crops and geographical locations yielded a maximum of only 4.3% nucleotide sequence difference. Phylogenetic analysis revealed no obvious groupings of isolates according to geographic origin or host species. Minimal differences were found between the *N* gene amino acid sequences of Australian isolates and these were most closely related to a clade of northern European isolates (Persley *et al*. 2006).

Little or no information is available on the prevalence of TSWV strains that differ in pathogenicity. Information on the biological variability of TSWV may be useful in developing more durable TSWV resistant crops (Mandal *et al*. 2006). Balukiewicz *et al*. (2005) found no considerable dif-

ferences in biological properties of five TSWV isolates from chrysanthemum plants.

Host range and symptoms with special regards to ornamentals – tospovirus relations

General

Many viruses included in the genus have very wide host ranges and can cause problems in an extensive range of food and ornamental crop species. Tospoviruses are capable of inflicting devastating losses and have been responsible for a number of very serious epidemics (Mumford *et al*. 1996). The ability of the tospoviruses to cause such severe losses on a broad range of crops, places them amongst the most economically important plant pathogens in the world at present.

TSWV has one of the largest host ranges of any plant virus, with hosts representing over 70 different families of both monocot and dicot species (Goldbach and Peters 1994). In 1987, the largest published host list contained nearly 250 susceptible species (Cho *et al*. 1987), although the number of reported hosts has continued to grow rapidly and now far exceeds this total. An unpublished list (cited in Mumford *et al*. 1996) is reported to contain over 650 different species as hosts of tospoviruses, including over 250 species from the Solanaceae and Compositae alone (Goldbach and Peters 1994). Solanaceae is the plant family containing the largest number of TSWV hosts (Cho *et al*. 1987; German *et al*. 1992). Adkins *et al*. (2005) reported over 800 hosts of TSWV including 80 plant families.

Peters (2003) showed a host list of tospoviruses including over 1050 species, among which more than 900 species are hosts to TSWV. Host ranges for the other tospoviruses are by no means as comprehensive. Evidence exists suggesting that there is a degree of host specialization within the tospoviruses. Most tospoviruses have a moderate or small host range. IYSV has a relatively restricted host range and is commonly found only in monocots such as onion, chive and leek. INSV has a more intermediate host range but is most commonly found infecting annual and perennial ornamental crops. It is the dominant tospovirus in the glasshouse and rarely infects field crops, where TSWV is dominant (Chamberlain *et al*. 1992).

Although host ranges tend to vary from virus to virus, *N. benthamiana* is a good assay host for most tospoviruses.

The symptoms induced by the tospoviruses are highly varied. Symptoms may be present on leaves, stems, petioles and flower petals. On non-systemic hosts, symptoms are generally restricted to local lesions, with chlorosis and necrosis in some instances. With systemic hosts, the list is far greater. Descriptions include ring spots, line patterns, tip necrosis, veinal necrosis, wilting, stunting, silvering, mottling, bronzing, chlorosis, necrosis, stunting and a range of leaf and stem lesions (Francki and Hatta 1981; German *et al*. 1992). Obviously the main variable affecting symptom expression is the particular combination of virus and host, but environmental factors, host cultivar, and age of plants also have an important role (Allen *et al*. 1991). Differences in symptoms have also been noted between different isolates of the same virus (TSWV) on the same hosts, held at the same conditions (de Ávila *et al*. 1992). Therefore symptoms caused by tospoviruses are highly variable and of little diagnostic value.

Ornamentals

In ornamentals, tospovirus symptoms may mimic symptoms and injuries caused by other biotic and abiotic stresses, such as nutrient deficiencies (Daughtrey *et al*. 1995). Tospoviruses, however may be present even though the plants show no symptoms, or only long time after infection. Cyclamen for example can show no symptoms for up to two months after infection (Allen and Matteoni 1988).

The symptoms of tospovirus infections in floral crops are listed below (Daugherty and Casey 1998; Chatzivassiliou *et al*. 2000): brown, black or white spots; necrosis of the leaf petiole; yellow mottling and variegation; death of the young plants or death of terminal meristems of older plants; stunting; brown or black cankers on the stem; veinal necrosis; concentric ring spots; mosaics; line or zonal patterns; malformation and necrosis of the flowers (**Figs. 3, 4**).

TSWV symptoms in chrysanthemum plants are frequently distinct and they consist of chlorotic and necrotic spots or large necroses on leaves and stems as well as stunting and death of the young plants. Similar symptoms were observed due to INSV infection (Cho *et al*. 1989). Infected chrysanthemums may also remain symptomless, which is typical for many cultivars (Matteoni and Allen 1989; Bo *et al*. 1999).

Besides destroying the aesthetic value, TSWV infection significantly reduces flower fresh weight, petal number, the number of flowers on the stem and inhibits adventitious bud sprouting in perennials (Whitfield *et al*. 2003). Crop losses in flower production of *Dendranthema* spp. can reach up to 51% (Matteoni and Allen 1989). Some growers of *Sinningia speciosa* have had 100% losses due to INSV infection.

There is great variability regarding the susceptibility of chrysanthemum (*Dendranthema grandiflora*) cultivars to TSWV, e.g. in 'Fiji Yellow', 'Jewel Time' and 'Reagan White Elite' TSWV was not found, while a high (25-39%) infection rate was observed with 'Blanch', 'Divalis Intrepid', 'Sheena Yellow' and 'Puma' cultivars (Balukiewicz and Kryczynski 2005).

Basis on the findings of Antignus *et al*. (1997) the following symptoms were discerned on ornamentals due to TSWV infection. On new Guinea impatiens: chlorotic or black ringspot and line patterns on leaves, black lesions on stems, stunted plants, malformed leaves and terminal dieback; on *Asclepias tuberosa*: severe leaf vein necrosis, necrotic ringspot on leaves, severe necrosis on stem and terminal dieback; on *Eustoma*: chlorotic and necrotic leaf spots, stem necrosis, necrotic terminals, color break and flower distortion; on *Zinnia*: mild mosaic and ringspot on leaves; on *Gloxinia*: reddish spots and/or concentric brown rings on the leaves and malformed flowers.

Symptoms on *Sinningia speciosa* (gloxinia) due to INSV and TSWV were as follows: chlorotic-necrotic spots and rings on inoculated leaves; systemic chlorosis to necrosis; young plants may die, older plants may recover. On *Dendranthema grandiflorum* (chrysanthemum) due to INSV: chlorotic spots with necrotic flecks on inoculated leaves (not systemic), stunting, top necrosis, due to TSWV: chlorotic to necrotic spots on inoculated leaves; systemic symptoms: leaf collapse, veinal necrosis, stem necrosis, flower tilts to side, may kill young plants. *Petunia hybrida* due to INSV and TSWV: small necrotic pots on inoculated leaves (not systemic). On *Impatiens wallerana* due to TSWV and INSV: chlorotic to necrotic spots or rings on inoculated leaves, systemic chlorotic to necrotic spots (Cho *et al*. 1989; Daughtrey *et al*. 1997).

CSNV (which is on the list of quarantine pests of the European Plant Protection Organization (EPPO) caused typical tospovirus symptoms on host plants, inducing necrotic-chlorotic local lesions and concentric rings on the inoculated leaves, followed by vein necrosis, mosaic or mottling and leaf deformation. Severe symptoms on inoculated chrysanthemum plants were observed within 2 weeks after infection, eventually leading to the death of most plants. The experimental host range of CSNV was broad infecting 19 different hosts in seven families, including *Zinnia elegans* as a systemic host among ornamentals. CSNV causes mild or severe streaks on the chrysanthemum stem, wilting of leaves and stems, chlorotic and necrotic spots and rings on some leaves in The Netherlands, however CSNV can result in complete necrosis of the stem (Verhoeven *et al*. 1996). In Brazil, symptoms were described as necrotic lesions surrounded by yellow areas on leaves followed by necrosis on stems, penducles and floral receptacles (Duarte *et al*. 1995). In the British outbreak, distinct dark stem lesions with some

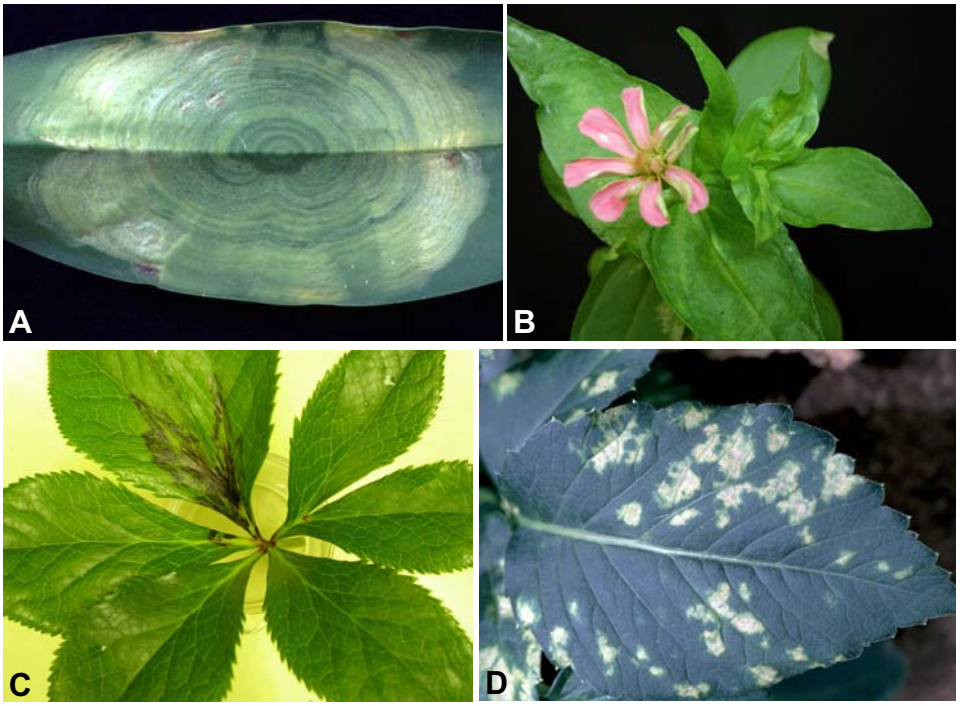

Fig. 3 TSWV symptoms on ornamentals. (**A**) *Phalaenopsis* spp.; (**B**) *Zinnia* spp.; (**C**) *Helleborus* spp.; (**D**) *Dahlia* spp. All photos taken courtesy of Margery Daughtrey (Cornell University, Department of Plant Pathology, USA).

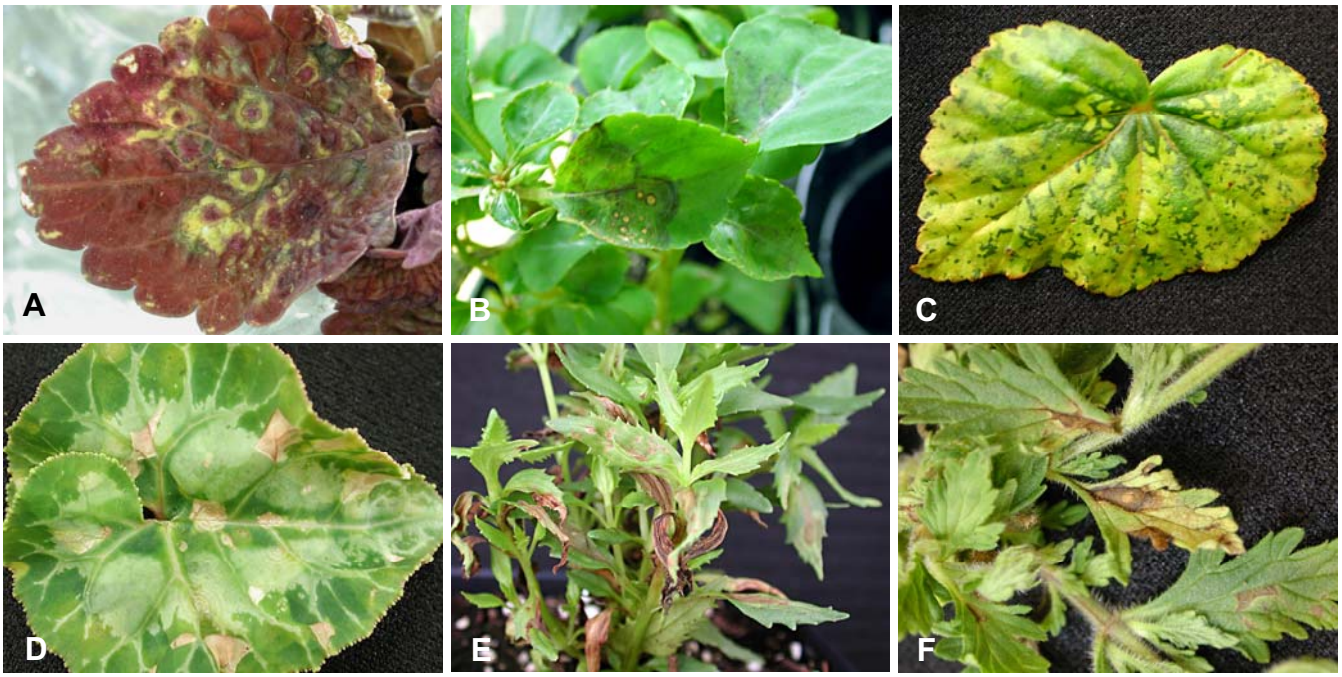

Fig. 4 INSV symptoms on ornamentals. (**A**) *Coleus* spp.; (**B**) *Impatiens wallerana*; (**C**) *Begonia* spp.; (**D**) *Cyclamen* spp.; (**E**) *Nemesia* spp.; (**F**) *Verbena* spp. All photos taken courtesy of Margery Daughtrey (Cornell University, Department of Plant Pathology, USA).

leaf necrosis can be seen (Mumford *et al*. 2003).

CCSV, a new tospovirus infecting *Zantedeschia* species in Taiwan, caused chlorotic spots on their leaves and stems (Lin *et al*. 2006). Symptoms on naturally infected calla lilies (*Zantedeschia* spp.) appeared mostly on the middle leaves of plants. Symptoms were mostly chlorosis with a few yellow spots about 2 mm in diameter radiating from the midrib toward the edge of the leaves. Inoculation of healthy calla lily seedlings with inocula prepared from CCSV-infected *N. benthamiana* produced chlorotic spots similar to those on naturally infected plants. Larger plants were however not susceptible to mechanical inoculation. After mechanical inoculation, systemic invasion of CCSV was observed only in *Benincasa hyspida*, *Gomphrena globosa* and *Zantedeschia* spp. (Chen *et al*. 2005).

Capsicum chlorosis virus (CaCV) infection results in ringspots, linepatterns, chlorotic blotches and necrotic etching on *Hoya* spp. (Persley *et al*. 2006).

Less than 20% of greenhouse flower crop tospovirus problems have been identified as TSWV, and INSV predominates (Hausbeck *et al*. 1992; Daughtrey 1996). Roses and poinsettias are the only major greenhouse crops not susceptible to INSV. On the basis of other reports TSWV and INSV are considered as main viruses of ornamental plants, both of them infecting 600 species of ornamental plants (Daughtery *et al*. 1997; Ullman 1998). INSV was the first virus to be recorded from a fern (the glasshouse ornamental *Asplenium nidus-avis*). It is important to know that not all plants susceptible to TSWV and INSV are hosts for *F. occidentalis*.

Virological surveys of ornamentals were done recently in many countries, including the USA (Daughtrey *et al*.

Table 1 Ornamental hosts of TSWV and INSV

Plant species	TSWV	INSV
Aconitum spp.		Daughtrey *et al*. 1997
Ageratum spp.	Cho *et al*. 1989; Kormelink *et al*. 1998	
Ageratum houstonianum		Roggero *et al*. 1999
Alstroemelia spp.	Cho *et al*. 1989; Kormelink *et al*. 1998; Chatzivassiliou *et al*. 2000; Latham and Jones 1997	Daughtrey *et al*. 1997
Amaryllis spp.	McDonough *et al*. 1999	McDonough *et al*. 1999
Anemone spp.	Gera *et al*. 1998b	Daughtrey *et al*. 1997
Anemone coronaria	Chatzivassiliou *et al*. 2000	
Antirrhinum spp.		Daughtrey *et al*. 1997
Antirrhinum majus	Chatzivassiliou *et al*. 2000	
Anthurium andreanum	Cho *et al*. 1989; Kormelink *et al*. 1998	McDonough *et al*. 1999
Aphelandra spp..	Bo *et al*. 1999	
Aralia japonica	Chatzivassiliou *et al*. 2000	
Asia japonica		Daughtrey *et al*. 1997
Asparagus sprengeri	Mertelik *et al*. 1996	
Aster spp.	Swift 2003; Ramasso *et al*. 1994; Chatzivassiliou *et al*. 2000; Latham and Jones 1997	
Aster ericoides	Bo *et al*. 1999	
Begonia spp.	Cho *et al*. 1989; Kormelink *et al*. 1998; McDonough *et al*. 1999; Chatzivassiliou *et al*. 2000	Daughtrey *et al*. 1997; McDonough *et al*. 1999
Begonia tuperhybrida		Daughtrey *et al*. 1997
Beloperone guttata	Chatzivassiliou *et al*. 2000	
Benincasa hyspida	Chatzivassiliou *et al*. 2000	
Bouvardia spp.		Daughtrey *et al*. 1997
Bromelia spp.	McDonough *et al*. 1999	
Browallaria spp.		McDonough *et al*. 1999
Calendula officinalis	Latham and Jones 1997; Chatzivassiliou *et al*. 2000	
Callistephus spp.		Daughtrey *et al*. 1997
Callistephus chinensis	Ramasso *et al*. 1994; Chatzivassiliou *et al*. 2000	
Capsicum spp.		Roggero *et al*. 1999
Catharanthus roseus	Chatzivassiliou *et al*. 2000	
Celosia cristata	Chatzivassiliou *et al*. 2000	
Chrysanthemum spp.	Cho *et al*. 1989; Mertelik *et al*. 1996; Latham and Jones 1997; Kormelink *et al*. 1998; Ravnikar *et al*. 2003	Daughtrey *et al*. 1997; Ravnikar *et al*. 2003
Chrysanthemum morifolium	Mertelik and Mokra 1998	
Cineraria spp.	Swift 2003	
Coleus barbatus	Chatzivassiliou *et al*. 2000	
Coleus blueri		Roggero *et al*. 1999
Columnea spp.		Daughtrey *et al*. 1997
Cordyline terminalis		Roggero *et al*. 1999
Cosmos spp.	Latham and Jones 1997	
Cyclamen spp.	Cho *et al*. 1989; Kormelink *et al*. 1998	Daughtrey 1996; Ravnikar *et al*. 2003
Cyclamen persicum		Daughtrey *et al*. 1997; Tóth *et al*. 2007
Dahlia spp.	Cho *et al*. 1989; Kormelink *et al*. 1998; Vicchi and Bellardi 1996; Latham and Jones 1997; McDonough *et al*. 1999	Daughtrey *et al*. 1997; Roggero *et al*. 1999; McDonough *et al*. 1999
Dahlia hybrida	Chatzivassiliou *et al*. 2000	
Delphinium spp.	Latham and Jones 1997	
Dendranthema spp.	Chatzivassiliou *et al*. 2000	
Dendranthema murifolium cv. Dignity; Palisade; Southing Sun	Bo *et al*. 1999	
Dendranthema × *grandiflorum*		Daughtrey *et al*. 1997
Dianthus chinensis	Chatzivassiliou *et al*. 2000	Roggero *et al*. 1999
Dieffenbachia spp.	McDonough *et al*. 1999; Chatzivassiliou *et al*. 2000	
Dimorphotheca sinuata	Ramasso *et al*. 1994; Chatzivassiliou *et al*. 2000	
Dracaena spp.	McDonough *et al*. 1999	McDonough *et al*. 1999
Echinacea purpurea	Tóth *et al*. 2007	
Episcia capreata		Roggero *et al*. 1999
Eustoma (*Lisianthus*) spp.	Bo *et al*. 1999	
Eustoma grandiflorum		Daughtrey *et al*. 1997; Tóth *et al*. 2007
Eustoma russelianum		Daughtrey *et al*. 1997
Exacum affine		Daughtrey *et al*. 1997
Fuchsia hybrida	McDonough *et al*. 1999; Chatzivassiliou *et al*. 2000	
Gazania spp.	McDonough *et al*. 1999; Chatzivassiliou *et al*. 2000	McDonough *et al*. 1999
Geranium spp.	Chatzivassiliou *et al*. 2000; McDonough *et al*. 1999	McDonough *et al*. 1999
Gerbera spp.	Cho *et al*. 1989; Kormelink *et al*. 1998; Bo *et al*. 1999	Daughtrey *et al*. 1997
Gerbera jamesonii	Ramasso *et al*. 1994; Mertelik *et al*. 1996; Chatzivassiliou *et al*. 2000	
Gladiolus spp.	Latham and Jones 1997	Daughtrey *et al*. 1997
Godetia grandiflora		Roggero *et al*. 1999
Gomphrena globosa		McDonough *et al*. 1999
Heuchera sanguinea	Tóth *et al*. 2007	
Hosta spp.		McDonough *et al*. 1999
Hoya spp.		McDonough *et al*. 1999
Hydrangea spp.	McDonough *et al*. 1999	McDonough *et al*. 1999

Table 1 (Cont.)

Plant species	TSWV	INSV
Impatiens spp.	Cho *et al*. 1989; Wijkamp *et al*. 1995; Mertelik *et al*. 1996; Kormelink *et al*. 1998; McDonough *et al*. 1999	Wijkamp *et al*. 1995; McDonough *et al*. 1999
Impatiens balsamina	Chatzivassiliou *et al*. 2000	
Impatiens petersiana	Chatzivassiliou *et al*. 2000	
Impatiens walleriana		Daughtrey *et al*. 1997; Tóth *et al*. 2007
Impatiens New Guinea hybrids		Daughtrey *et al*. 1997; McDonough *et al*. 1999
Iris spp.	Chatzivassiliou *et al*. 2000	
Iris hollandica	Chatzivassiliou *et al*. 2000	
Lilium aurtum	Chatzivassiliou *et al*. 2000	
Kalanchoe spp.	McDonough *et al*. 1999	McDonough *et al*. 1999
Limonium spp.		Daughtrey *et al*. 1997
Limonium (Goniolimon) tataricum	Ramasso *et al*. 1994	
Lobelia spp.	McDonough *et al*. 1999	Daughtrey *et al*. 1997; McDonough *et al*. 1999
Maranta leuconeura		Roggero *et al*. 1999
Matthiola incana	Chatzivassiliou *et al*. 2000	
Melampodium divaricatum	Holcomb and Valverde 2000	
Nasturtium spp.	Swift 2003	
Nemisia spp.	Vicchi and Bellardi 1996	
Ocimum basilicum	Chatzivassiliou *et al*. 2000	Tóth *et al*. 2007
Oleander spp.	Ramasso *et al*. 1994	
orchids	Swift 2003	
Pelargonium spp.	Ravnikar *et al*. 2004; Chatzivassiliou *et al*. 2000	Ravnikar *et al*. 2003
Pelargonium zonale	Ramasso *et al*. 1994	
Peperomia obtusifolia		Roggero *et al*. 1999
Pericallis hybrida cineraria		Daughtrey *et al*. 1997
Petunia spp.	McDonough *et al*. 1999; Swift 2003	McDonough *et al*. 1999
Petunia hybrida	Chatzivassiliou *et al*. 2000	
Phalaenopsis spp.	McDonough *et al*. 1999	
Phlox spp.		McDonough *et al*. 1999
Pittosporum spp.	Cohen *et al*. 1999	Daughtrey *et al*. 1997
Portulaca grandiflora	Chatzivassiliou *et al*. 2000	
Primula spp.	McDonough *et al*. 1999	Hausbeck *et al*. 1992; Daughtrey *et al*. 1997
Ranunculus spp.	Gera *et al*. 1999; Chatzivassiliou *et al*. 2000	Daughtrey *et al*. 1997
Ranunculus asiaticus		Daughtrey *et al*. 1997
Saintpaulia ionantha	Chatzivassiliou *et al*. 2000	
Salpiglossis spp.	Swift 2003	
Salvia spp.	McDonough *et al*. 1999	McDonough *et al*. 1999
Salvia splendens	Chatzivassiliou *et al*. 2000	
Scindapsus aureus		Roggero *et al*. 1999
Schefflera spp.		McDonough *et al*. 1999
Schizanthus spp.	Swift 2003	
Senecio cruentus	Chatzivassiliou *et al*. 2000	Daughtrey *et al*. 1997
Sinningia speciosa gloxinia	Cho *et al*. 1989; Kormelink *et al*. 1998	Hausbeck *et al*. 1992; Daughtrey *et al*. 1997
Snapdragon spp.	Latham and Jones 1997	
Solanum capsicastrum	Chatzivassiliou *et al*. 2000	
Stapelia spp.	Ramasso *et al*. 1994	
Statice spp.	Latham and Jones 1997	
Stephanotis floribunda	Chatzivassiliou *et al*. 2000	
Tagetes erecta	Chatzivassiliou *et al*. 2000	
Torenia fournieri		Roggero *et al*. 1999
Tradescantia albiflora	Chatzivassiliou *et al*. 2000	McDonough *et al*. 1999
Tropaeolum majus	Chatzivassiliou *et al*. 2000	
Verbena spp.		McDonough *et al*. 1999
Viola tricolor	Chatzivassiliou *et al*. 2000	
Zantedeschia spp.	Mertelik *et al*. 1996; Mertelik and Mokra 1998; Chatzivassiliou *et al*. 2000; Swift 2003	
Zantedeschia aethiopica		Ramasso *et al*. 1994 ; Daughtrey *et al*. 1997
Zinnia spp.	Latham and Jones 1997; Swift 2003	McDonough *et al*. 1999
Zinnia elegans	Chatzivassiliou *et al*. 2000	

1997), Brazil (Bo *et al*. 1999), Greece (Chatzivassiliou *et al*. 2000), Poland (Balukiewicz and Kryczynski 2005), the Czech Republic (Mertelik and Mokra 1998; Mertelik *et al*. 1996), Hungary (Takács *et al*. 2006; Tóth *et al*. 2007), Slovenia (Vozelj *et al*. 2003; Ravnikar *et al*. 2003), Italy (Roggero *et al*. 1999), and Iran (Ghotbi *et al*. 2005).

Table 1 shows lists of ornamentals subject to infection with tospoviruses. As can be seen in **Table 1**, ornamentals are considered as hosts mainly of TSWV and INSV. Besides these two tospoviruses, other tospoviruses can also infect them, such as CSNV on *Chrysanthemum*, *Gerbera* and *Zinnia s*pecies (Mumford *et al*. 2003; Vozelj *et al*. 2003; Ravni-

kar *et al*. 2003); CCSV on *Benincasa hispida*, *Gomphrena globosa* and *Zantedeschia* spp. (Chen *et al*. 2005); CaCV on *Hoya* spp. (Persley *et al*. 2006); IYSV on *Eustoma russelianum*, *E. grandifolium* and *Iris hollandica* (Cortes *et al*. 1998; Kritzman *et al*. 2000); TCSV and GRSV on *Impatiens* spp. (Wijkamp *et al*. 1995); TYRV on *Alstroemelia*, *Impatiens*, *Gazania* spp. and *Tropaeolum majus* (Mehraban *et al*. 2005).

Tospoviruses have occurred sporadically in Iran in the past, however since 2000, outbreaks of tospoviruses have been recorded every year. Three years surveys were carried out on ornamentals between 2000 and 2002. TSWV, INSV and *Tomato Varamin virus* (ToVV), a new tospovirus from Iran were frequently detected in samples of many ornamentals and often in mixed infections, whereas IYSV only in a few samples (Ghotbi *et al*. 2005).

Complex infections, e.g TSWV+INSV on *Gerbera* spp. (Vicchi and Bellardi 1996) and on *Pittosporum tobira* (Ramasso *et al*. 1994) had been already observed earlier.

Thrips transmission of TSWV was observed to *Symphoricarpos albos* and *Weigelia florida*, while mechanical transmission to *Buddlea davidii*, *Gleditschia triacanthos*, *Hydrangea* spp., *Ligustrum vulgare*, *Lonicera japonica*, *Ulmus pumila* var. *arborea* (Mertelik *et al*. 1996).

In Australia, flowering ornamentals were sampled from 6 sites and samples from 2698 plants were tested. Out of them 296 samples were infected with TSWV. The frequency of infection was 81, 65, 57, 32, 30 and 28% in Chinese aster, calendula, *Statice*, *Alstroemeria*, *Chrysanthemum* and dahlia species, respectively. *Cosmos*, *Delphinium*, *Gladiolus*, *Antirrhinum and Zinnia* species were less infected. INSV was not detected in the samples (Latham and Jones 1997).

Tospovirus members

For many years, TSWV was considered to be unique amongst known plant viruses and remained as the sole member of the *Tomato spotted wilt virus* group (Matthews 1982). However, as interest in TSWV has increased in recent years, it has become clear that this position was wrong. Firstly TSWV was reclassified as the type member of the tospovirus genus, created to contain the plant-infecting members of the family Bunyaviridae (Francki *et al*. 1991). Secondly, a number of other tospoviruses, distict from TSWV, have been added to the tospovirus genus, including Impatiens necrotic spot virus (INSV), *Tomato chlorotic spot virus* (TCSV) and *Groundnut ringspot virus* (GRSV) (de Ávila *et al*. 1993).

In the late eighties and early nineties new members with a narrower host range as compared to TSWV and INSV were added to the genus based on their serological and molecular characterization, and isolated from different hosts (de Ávila *et al*. 1993). From these serological differences, a serogroup classification system was established (de Ávila *et al*. 1992). These serogroups were further characterised by analysis of nucleotide sequences of the *N* gene from members of each serogrup (de Ávila *et al*. 1993). It was proposed that a virus should be designated as a tospovirus initially on the basis of virion morphology, genome organization and thrips transmission. Tospovirus species are distinguished on the basis of N protein serology, N protein sequences and vector specifity (Goldbach and Kuo 1996).

There are currently 18 ICTV-recognised or proposed as tospovirus species (Persley *et al*. 2006).

Distinction between viruses within a serogroup should then be made on the basis of N protein sequence homology. Only if there is sufficient difference between the sequence of the N protein of the new virus and that of previously-described viruses should a virus be regarded as distinct (less than 90% homology has been suggested). On the basis of this proposed classification, the list of distinct and possible tospoviruses is indicated in **Table 2**.

As indicated above, INSV is serologically distinct from TSWV (Law and Moyer 1990; de Ávila *et al*. 1992), with little or no serological cross-reactivity between the N proteins of the two viruses, when using either polyclonal or monoclonal antibodies. This lack of similarity can be explained by comparing N protein gene nucleotide sequences from TSWV and INSV (Law *et al*. 1991; de Haan *et al*. 1992), which only possess a 62% identity of the N ORF, corresponding to an amino acid sequence homology of 55%. It should be noted, however, that conserved domains do exist, a fact borne out with the production of monoclonal antibodies that react to the N proteins of both INSV and TSWV (Hall and Moyer 1992). In contrast to the N protein, the envelope glycoprotein of the two viruses shows a greater level of epitope conservation with glycoprotein-specific antisera being cross-reactive to both viruses (Law and Moyer 1990). Sequence comparisons between the M RNA segments of TSWV and INSV support the serological evidence that the glycoproteins show a relatively high degree of conservation (Kormelink *et al*. 1992; Law *et al*. 1992).

Relative to INSV, TCSV and GRSV are less well characterised. Although again serologically distinct from TSWV, the level of variation is much less, with a greater degree of cross-reactivity between the three viruses (de Ávila *et al*. 1990). This greater level of serological relatedness can be attributed to a higher degree of similarity at the amino acid level, with 76% and 78% homology between the N proteins of TCSV and TSWV and GRSV and TSWV, respectively. Between the N proteins of TCSV and GRSV, the amino acid homology is 81%.

The nucleotide sequence of the *N* gene of WSMoV has been determined, revealing a nucleic acid sequence homology of around only 55% compared with all the other tospovirus members and even less (40-46%) at the amino acid level (Yeh and Chang 1995).

Comparisons of WSMoV and GBNV isolates have indicated that these two viruses are closely related. They share a high level of serological cross-reaction and also both possess an N protein of 30-32 kD, compared to 29 kD for TSWV and INSV (Adam *et al*. 1993). The N protein of GBNV has 86% identity with that of WSMoV, but approximately 30% homology with that of TSWV.

A recently characterized new tospovirus *Capsicum chlorosis virus* (CaCV) is most closely related to WSMoV, a serogroup four (IV) tospovirus (Persley *et al*. 2006).

To clarifiy the serological relationship between CCSV and WSMoV, rabbit polyconal antibody (PAb) to CCSV and mouse monoclonal antibodies (MAbs) to WSMoV NP or CCSV NP were produced, using purified nucleocapsid protein (NP) as immunogens. The sequence identities of CCSV and other viruses proved that CCSV is a new species in the genus tospovirus and belongs to the WSMoV serogroup (Lin *et al*. 2005).

The S RNA of a new tospovirus, TYRV was cloned recently and its 3,061 nucleotide long sequence showed features characteristic of tospoviral S RNA segments. The N protein with a predicted Mr of 30 kDa showed closest relationship to the N protein of IYSV (74% sequence identitiy) (Mehraban *et al*. 2005).

Table 3 shows the amino acid sequence identities (%) of 15 tospovirus N proteins.

ISOLATION AND IDENTIFICATION

Detection and identification of tospoviruses can be achieved in a number of ways including observation of disease symptoms and host reactions as a preliminary indication, followed by electron microscopy (EM), serological and nucleic acid-based assays.

Nicotiana benthamiana is a very susceptible diagnostic host for most tospoviruses, but it is a poor propagation host, as it survives for only a short time following symptoms expression.

Symptomatology has been frequently used for diagnosis and detection. Tospoviruses are sap-transmissible and can be mechanically inoculated into indicator plants (Best 1968). A large number of indicators have been recorded (Francki and Hatta 1981), one of the most favoured is garden petunia

Table 2 Tospovirus members.

Serogroup	Virus	Distribution	Host range	References	Vectors
I (monotypic)	*Tomato spotted wilt virus* (TSWV), type member	Worldwide	Over 1000 hosts, almost half of the susceptible species mainly Solanaceae and Asteraceae plants	Goldbach and Peters 1994; Murphy *et al*. 1995; Parella *et al*. 2003; Peters 2003	*F. occidentalis* (Gardner *et al*. 1935); *F. schultzei* (Samuel *et al*. 1930, Wijkamp *et al*. 1995); *F. fusca* (Sakimura 1963); *F. intosa* (Wijkamp *et al*. 1995); *F. bispinosa* (Sherwood *et al*. 2000); *T. tabaci* (Pittman 1927); *T. setosus* (Fujisawa *et al*. 1988); *T. palmi* (Fujisawa *et al*. 1988); *Tenothrips frici* (Ghotbi and Baniameri 2006); (Francki and Grivell 1970; De Haan *et al*. 1989; Dewey *et al*. 1993; Goldbach and Peters 1994)
II	*Tomato chlorotic spot virus* (TCSV)	Brazil	Tomato, pepper	De Ávila *et al*. 1990; 1993	*F. occidentalis*, *F. schultzei*, *F. intosa* (Goldbach and Peters 1994; Wijkamp *et al*. 1995)
II	*Groundnut (peanut) ringspot virus* (GRSV)	Brazil, Argentine, South-Africa, Asia	Groundnut, tomato	De Ávila *et al*. 1990; 1993; Dewey *et al*. 1993	*F. occidentalis*, *F. schultzei* (Goldbach and Peters 1994; Wijkamp *et al*. 1995)
III Monospecies serotype	*Impatiens necrotic spot virus* (INSV)	USA, Europe, Asia	Mainly under glasshouse, on ornamentals (e.g. *Begonia*, *Dahlia*, *Gloxinia*, *Sinningia*, *Impatiens* spp.)	Law and Moyer 1990; de Ávila *et al*. 1992; Marchoux *et al*. 1991; Vaira *et al*. 1993	*F. occidentalis* (De Angelis *et al*. 1993; Wijkamp and Peters 1993); *F. fusca* (Best and Katekar 1964; Goldbach and Kuo 1996); *T. tabaci* (Ghotbi and Baniameri (2006)
IV	*Watermelon silver mottle virus* (WSMoV) type member	Taiwan, Thailand, Japan	Cucurbits, tomato, *Hoya* spp.	Iwaki *et al*. 1984; Yeh and Chang 1995; Heinze *et al*. 1995	*T. palmi* (Yeh *et al*. 1992; Hall *et al*. 1993; Chen *et al*. 2006)
IV	*Peanut (groundnut) bud necrosis virus* (GBNV)	India, South-East-Asia, South America, Africa	Groundnut, other grain legumes, tomato, pepper	Reddy *et al*. 1992 Adam *et al*. 1993; Satyanarayana *et al*. 1996	*T. palmi* (Palmer *et al*. 1991); *Scirtothrips dorsalis*, *F. schultzei* (Vijayalakshmi 1994)
IV	*Capsicum chorosis virus* (CaCV)	Australia, Thailand, Taiwan	Pepper, tomato, peanut, *Hoya* spp. *Sinningia* spp.	Lee *et al*. 2002; Persley *et al*. 2006	*T. palmi*, *F. schultzei*, *Ceratothripoides claratris* (McMichael *et al*. 2002; Premachandra *et al*. 2005)
IV	*Melon spotted wilt virus* (MSWV)	Japan	melon	Heinze *et al*. 1995; Kato 1995	*T. palmi*
IV	*Calla lily chlorotic spot virus* (CCSV)	Taiwan	*Zantedeschia* spp., some cucurbits	Hsu *et al*. 2002; Chen *et al*. 2005; Lin *et al*. 2005	*T. palmi* (Chen *et al*. 2005; Lin *et al*. 2005)
IV	*Watermelon bud necrosis virus* (WBNV)	India	watermelon	Singh and Krishnareddy 1996	*T. palmi* (Jain *et al*. 1998)
V	*Iris yellow spot virus* (IYSV), type member	Brazil, Japan, Israel, The Netherlands, Slovenia, USA, Australia	Onion, chive, leek, *Iris hollandica*, *Amaryllis*, *Hippeastrum*, *Eustoma russelianum* *Dendranthema grandiflora*	Cortes *et al*. 1998; Gera *et al*. 1998a; Pozzer *et al*. 1999; Kritzman *et al*. 2000; Schwartz *et al*. 2002; Balukiewicz and Kryczynski 2005; Persley *et al*. 2006	*T. tabaci* (Nagata and Almeida 1999)
V	*Tomato yellow (fruit) ring virus* (TYRV)	Iran	*Impatiens*, *Gazania* spp., *Tropaeolum majus*, *Alstoemeria* spp. *Nicotiana* spp., tomato, *Pisum*, *Vigna* spp.	Mehraban *et al*. 2005	Not known
V	*Tomato Varamin virus* (ToVV)**	Iran		Ghotbi *et al*. 2005	Not known
Monospecies serotype	*Peanut (groundnut) yellow spot virus* (PYSV)	India, Thailand	peanut	De Haan *et al*. 1990; Reddy *et al*. 1990; Satyanarayana *et al*. 1998	Not known
Monospecies serotype	*Melon yellow spot virus* (MYSV)	Japan, Taiwan	melon	Kato *et al*. 2000	*T. palmi* (Kato *et al*. 2000)
Monospecies serotype	*Physalis severe mottle virus* (PSMV)*	Thailand	*Physalis minima*	Cortes *et al*. 2001	*T. palmi* (Kato *et al*. 2000)
Monospecies serotype	*Peanut (groundnut) chlorotic fan-spot virus* (PCFV)	Thaiwan	Peanut	Chen and Chiu 1995; Chu *et al*. 2001	*Scirtothrips dorsalis* (Chen and Chiu 1995)
Monospecies serotype	*Zucchini lethal chlorosis virus* (ZLCV)	Brazil	cucurbits	Resende *et al*. 1996	*F. zucchini* (Bezerra *et al*. 1999)
Monospecies serotype	*Crysanthemum stem necrosis virus* (CSNV)	Brazil, the Netherlands	*Chrysanthemum* spp., tomato, *Gerbera* spp.	Nagata *et al*. 1998; Ravnikar *et al*. 2003; Verhoeven *et al*. 1996	*F. ocidentalis*, *F. schultzei* (Bezerra *et al*. 1999)
	unidentified	Australia	*Pterostylis* spp.	Gibbs *et al*. 2000	Not known

* it is considered as an isolate of MYSV (Jan *et al*. 2003)
** it is presumed the same as TYRV

Table 3 Amino acid sequence identities (%) of tospoviral N proteins (after Mehraban *et al*. 2005)

Viruses	TSWV	TCSV	GRSV	INSV	CSNV	ZLCV	PBNV	WSMoV	WBNV	MYSV	PCFV	PYSV	CaCV	IYSV	TYRV
TSWV	100	76	78	55	75	72	25	28	26	26	18	19	29	30	30
TCSV		100	81	52	72	71	27	27	26	27	19	20	28	29	31
GRSV			100	52	73	75	27	29	28	27	19	19	29	29	31
INSV				100	53	50	27	27	26	24	21	21	27	26	29
CSNV					100	80	26	28	24	29	20	20	28	31	29
ZLCV						100	26	26	26	26	19	19	27	29	29
PBNV							100	86	85	60	21	20	84	42	40
WSMoV								100	86	58	20	20	86	41	39
WBNV									100	58	19	20	82	42	39
MYSV										100	19	19	59	47	45
PCFV											100	59	20	18	17
PYSV												100	20	18	20
CaCV													100	43	40
IYSV														100	74
TYRV															100

which gives characteristic brown, necrotic local lesions 3 days after inoculation. Results obtained from sap-transmission tests are often unreliable. Tospoviruses are highly unstable *in vitro*, and even when using carefully composed inoculation buffers to aid retention of virus viability (Best 1968), sap preparations become rapidly uninfective. The implications of this are that the lack of symptoms on an indicator does not necessarily imply a lack of virus in the sample tested. Future complications are also observed due to variable symptom expression. This is often related to the environmental factors (especially light conditions) of plants before and after inoculation (Best 1968).

Verhoeven *et al*. (1996) suggested the use of *Datura stramonium* as a suitable indicator host to differentiate CSNV from other tospoviruses as only CSNV causes stem necrosis after mechanical inoculation. *Physalis floridana* can also be used for separation of CSNV from TSWV as only the latter evokes systemic symptoms.

Light microscopy of viral inclusion bodies is also useful for tospovirus diagnosis (Adkins *et al*. 2005). Cytopathological differences have been observed between TSWV and INSV. Both are able to form dense inclusions, consisting of NSs protein, within the cytoplasm of host cells. But in TSWV-infected cells the inclusions take the form of loose aggregates of fibres (Kormelink *et al*. 1991), while in INSV cells NSs is formed into highly ordered paracrystalline entities, so-called Z structures (Urban *et al*. 1991).

As the tospoviruses have a morphology that is unique amongst the plant viruses, their particles can be directly identified using electron microscopy (EM) (Black *et al*. 1963). Disadvantages of this method are that sample preparation is expensive and examination is time-consuming. At a low titre, often only individual particles can be seen. In isolation, tospovirus particles can resemble plant membranous debris, such has chloroplast or mitochondrial fragments, which often make reliable diagnosis impossible. EM of plant sap preparations of tospoviruses can be unreliable, as the membrane-bound particles are easily degraded unless fixed, and can be confused with other membranous structures. ISEM has been successfully applied, and can be enhanced by gold labelling (Kitajima *et al*. 1992). The use of immunoelectron microscopy (IEM) has proved successful, even for the detection of morphologically defective isolates (Kitajima *et al*. 1992). However IEM suffers from the limitations described for TEM and is only applicable for small numbers of samples.

The detection and diagnosis of tospoviruses is based on serologically based procedures, mainly on ELISA with high quality polyclonal and monoclonal antibodies (Gonsalves and Trujillo 1986). ELISA allows the detection of TSWV within individual thrips (Cho *et al*. 1988; Bandla *et al*. 1994). Now, many antisera are available to a wide range of tospoviruses. Polyclonal antisera have been prepared to purified whole virions or nucleocapsids, to *in vitro* expressed viral proteins and to synthesized peptides from the NSs protein. Monoclonal antibodies and recombinant single chain variable fragment antibodies have also been produced. With the availability of a range of good antisera to various tospoviruses, ELISA and other serologically-based assays, such as lateral flow devices or dip-sticks have become the standard diagnostic methods. Other serological techniques have also been developed, including dot-blot immunoassay (DBIA; Huguenot *et al*. 1990) and direct tissue blotting (Hsu and Lawson 1991), although neither appear to offer any significant advantages over ELISA. Tissue blot assays are also effective. Whitfield *et al*. (2003) used tissue blot immunoassay (TBIA) to detect TSWV in *Ranunculus asiaticus* tubers and other ornamentals.

A recent approach is for the detection of tospoviruses based upon molecular biology techniques. Nucleic acid hybridization techniques have been developed and evaluated for their use in the detection and diagnosis of tospoviruses. Both riboprobes (Huguenot *et al*. 1990) and cDNA probes (Ronco *et al*. 1989; Rice *et al*. 1990) have been used. The sensitivity of these tests are often not greater than that recorded for ELISA, but probes avoid the problems associated with serological tests (Mumford 1995). Tests based upon the use of the polymerase chain reaction (PCR) have also been developed (Mumford *et al*. 1994). By using reverse transcription (RT-PCR) on plant total RNA extracts, it is possible to detect and distinguish between TSWV and INSV, by using different virus-specific primers (Mumford *et al*. 1994), and using universal primers, to detect all tospoviruses (Wood *et al*. 1995).

The first report of a RT-PCR for a tospovirus (TSWV) was by Mumford *et al*. (1994), who used primers specific for *L* gene sequences. Later, Dewey *et al*. (1996) and Mumford *et al*. (1996) described a tospovirus genus-specific primer pair UNIV S1 and UNIV S2, specific for the conserved sequences in the *N* gene and 3′ untranslated region, respectively. These primers were used successfully to detect TSWV, TCSV, INSV and GRSV, the known tospoviruses at that time. McMichael *et al*. (2002) used primers specific to the S RNA serogroup IV tospoviruses (Jain *et al*. 1998), to characterize CaCV, a novel virus previously identified by ELISA.

Cortes *et al*. (2001) used a primer targeted to the conserved sequence at the 3′ end of the S RNA together with a primer to the tracts of adenosine bases present in the highly homologous sequences of the intergenic region. Using this approach with MYSV, either the whole S RNA or the N and NSs genes separately, could be amplified. A one-step RT-PCR system for the simultaneous detection and identification of multiple tospoviruses in plants has been recently

Table 4 Primers used for tospovirus detections.

Primer/primer pairs	Viruses	What they encode	Cycling conditions	References
L1, L2	TSWV	L RNA fragment	Denaturation 94°C 1 min Annealing 37°C 1 min Extension 72°C 1 min	Mumford *et al.* 1994; Balukiewicz *et al*. 2005
N3534c	WSMoV	S RNA	Denaturation 94°C 1 min	Lin *et al*. 2005
N3101	MYSV, IYSV, WSMoV	Conserved regions of N genes	Annealing 50°C 30 s	
Ter398s, 398S-NSs3', 398S-ter3', 398N354c, 398S-ter5'	CCSV	S RNA	Extension 72°C 1 min	
18TNS gL3637 gL4435c	Tospoviruses	Conserved region of the L genes	-	Chen *et al*. 2005
WN2645K, WN3469cKS	WSMoV	N gene		
J13	Physalis tospovirus isolate	S RNA	-	Cortes *et al*. 2001
UHP		N, NSs protein genes		
Actin1, actin 2 Forward primers, Reverse primers	TSWV	mRNA sequence of actin gene	Denaturation 94°C 1 min Annealing 54°C 1 min Extension 72°C 1 min	Boonham *et al.* 2002
Primer1	Tospovirus isolates	3'end of S RNA	Denaturation 92°C 0 s	Dewey *et al*. 1996
Primer2		N gene ORF	Annealing 52°C 0 s Extension 72°C 15 s	
Primer 1	TSWV	Coding region of the NP gene	Denaturation 96°C 30 s	Jain *et al*. 1998
Primer2		L RNA	Annealing 42°C 2 min Extension 72°C 1 min	
S6t7, S12-166, S12-155, S6t3, anchor primer, downsream primer	PCSV	Intergenic and terminal regions	-	Chu *et al.* 2001
Universal primer (TOS-R15)	Tospoviruses		-	Uga and Tsuda 2005
IYSV-837, 459	IYSV			
TSWV-709	TSWV			
INSV-589	INSV			
WSMoV-524, 848	WSMoV			
MYSV-433, 511, 834	MYSV			
L1, L2	TSWV	Central region of L RNA	Denaturation 94°C 30 s	Mumford *et al.* 1996
S1 INSV, S2 INSV	INSV, TSWV	S RNA	Annealing 55°C 1 min	
S1 UNIV	TSWV, INSV, GRSV, TCSV	S RNA	Extension 72°C 1 min	
S1 TSWV, S2 TSWV	TSWV	N gene		
SN2 INSV	INSV	N gene		
TSW.1, TSW.2	TSWV		Denaturation 94°C 45 s Annealing 45°C 45 s Extension 72°C 1 min	Dietzgen *et al.* 2005

-, not known

described (Uga and Tsuda 2005). Chu *et al*. (2001) designed two pairs of genus-specific RT-PCR primers which allowed amplification of tospoviruses from five different serogroups. Real-time RT-PCR has also been developed and shown to be a sensitive and reliable method of detection of TSWV in a range of plant species and thrips vectors (Roberts *et al*. 2000; Boonham *et al*. 2002; Dietzgen *et al*. 2005) (**Table 4**).

To investigate tospoviruses, isolation of the encoded products of viruses is difficult because of their genomic complexity and particle instability. In the last decade, new techniques using plant viruses as vectors for *in planta* expression of proteins and efficient purification procedures of the expressed proteins from plants have been developed. The entire open reading frame (ORF) of N or NSs protein encoded by S RNA of WSMoV was inserted in between the P1 and –Pro genes of the *Zucchini yellow mosaic virus* (ZYMV) vector for expression *in planta*. Six histidine residues and an additional NIa protease cleavage site were introduced to facilitate the purification and the process of free-form proteins, respectively. Symptoms of mosaic and leaf distortion on zucchini squash were induced by the chimeric viruses ZWSMoV-N and TWSMoV-NSs carrying the N and NSs ORFs of WSMoV, respectively. The expressed N and NSs proteins were purified from infected tissues by the Ni^{2+}-NTA affinity column. Results indicated that the constructed ZYMV vector can efficiently express tospoviral proteins in cucurbits. The same vector may have a potential application for expression of plant derived vaccines for mammalian infecting viruses of the same family such as the viruses in the genera Bunyavirus, Hantavirus, Nairovirus and Phlebovirus (Chen *et al*. 2006).

CONVENTIONAL MEANS FOR THEIR CONTROL AND ERADICATION

Effective antiviral chemical therapy is not available for almost all viral diseases, plant or animal, including tospovirus diseases.

The reasons for the difficulty in controlling tospoviruses include the wide host range of TSWV, the most widespread member of the genus, thus providing a very large number of alternative weed and crop hosts. Thrips vectors have the capacity to rapidly develop resistance to many insecticides. Control through resistant cultivars has been hampered by the lack or resistance to tospoviruses among many important crop hosts and the ineffectiveness or lack of durability of some sources when used (Boiteux and Nagata 1993; Cho *et al*. 1996).

To be effective, control measures should be based on epidemiological principles. There is a considerable body of information on the complex interactions between tospoviruses, their hosts and vectors, particularly for TSWV (Jones 2004).

We must rely on preventive methods. When considering plant virus diseases, two main options are available: the use of resistant plants and to limit transmission and spread. Useful sources of resistance for a variety of crops have now been identified, including tomato (Boiteux and Giordano 1993), pepper (Boiteux and de Ávila 1994), lettuce (Wang *et al*. 1992) and tobacco (Kennedy and Nielsen 1993).

While efforts are being made to produce resistant plants, the only practical means of controlling tospovirus induced diseases is to reduce their spread. This is mostly achieved

by controlling thrips populations.

Managing *F. occidentalis*, a dominant species in glasshouses is difficult (Robb and Parella 1995; McDonough *et al*. 1999) because:

- Short feeding period (1-2 hrs) is required for transmission;
- *F. occidentalis* is very small and easy to overlook;
- Spend part of their life cycle in the soil;
- Prefer to feed on flower parts, where systemic insecticides do not reach;
- Like to hide in flowers, flower and leaf buds making them hard to spot and reach with pesticides;
- Live on a wide variety of host plants;
- Feed on other insects;
- Reproduce rapidly in warm greenhouses. The development time from egg to adult is 7 to 13 days when temperatures range from 17 to 37°C (Robb 1989).
- Hide in plant materials and transported worldwide;
- Transmit tospoviruses to a range of plant species;
- They are resistant to a number of insecticides.

Physical exclusion is a very effective control method. In order to prevent *F. occidentalis* entering greenhouses Robb and Parella (1995) suggest the following:

- Install screens on all external openings, paying particular attention to areas where air is drawn into the glasshouse. The use of screens (mesh size <135 nm) prevent entry of thrips into glasshouse;
- Limit access to the greenhouse and install double doors at all entrances;
- Do not move *F. occidentalis* or virus-infested plants from propagation areas to production areas.

With this method the application of pesticide was reduced by 40% in poinsettia stock and New Guinea impatiens production areas (Hall 1995).

Cultural methods are the following according Robb and Parella (1995):

- Remove weeds and flowering plants growing near glasshouses because the plants can harbor *F. occidentalis* and tospoviruses;
- Avoid continuous cropping, and alternate crop plants with non-susceptible plants;
- Remove all plant debris.

If soil is present under greenhouse benches, the soil sterilization must be done periodically or the soil must be treated with a pesticide to eliminate the developmental stages of *F. occidentalis*. Some growers apply a layer of hydrated lime thick enough to cover the soil beneath the benches because hydrated lime reduces algae, weeds and insects in this critical area (Lindquist 1998). As an alternative to soil sterilization, greenhouse vents should be closed, and the house should be kept fallow for seven days between crops to accelerate the development of *F. occidentalis* pupae in the soil, and eliminate the emerging adults. Heating the greenhouse to temperatures in excess of 39°C for two days will kill adult thrips (Parella 1995).

The use of horticultural oils, insecticidal soaps and film-forming agents showed a significant reduction of TSWV transmission (Allen *et al*. 1993). UV reflective and plastic mulches acted to reduce thrips' access to the growing medium, where they pupate (Greenough *et al*. 1990).

Good sanitation controls tospoviruses

Three common sources of tospovirus outbreaks in the greenhouse are: Asymptomatic carry-over crops such as holiday cactus (*Schlumbergera* spp.); weeds growing in the greenhouse and infected stock plants, which serve as bridges for virus survival (Daughtrey 1997).

Monitoring (the use of indicator plants and coloured sticky cards) is essential, because early warning is critical to the contol of *F. occidentalis* and to the prevention of tospovirus infections. Indicator plants must be more attractive to the pest than the producing crops, the pest or pathogen must develop faster on the indicator plant, the indicator must show feeding damage (or virus symptoms) more readily, and finally indicator plants should not contribute to the spread of the virus being monitored (Allen and Matteoni 1991; Powell and Lindquist 1997). Petunias (*P. hybrida*) are excellent early indicators for the presence of *F. occidentalis* feeding and transmission of tospoviruses because petunias are not systemically infected with either TSWV or INSV (Ullman 1998). In response to a tospovirus infection, petunia show a hypersensitive response – a rapid death of plant tissues that also kills the invading virus (Robb *et al*. 1998). Daughtrey *et al*. (1995) and Robb *et al*. (1998) suggest the following cultivars for tospovirus detection: 'Calypso', 'Super Blue Magic', 'Blue Carpet', 'Cascade Blue', 'Summer Madness', 'Burgundy Madness', 'Red Cloud', 'Super Magic Coral'.

Use of petunia indicator plant/directional trap system alerts the grower to the presence of infective thrips and helps locate their source.

Beside petunias, fava beans as indicator plants are also suggested for growers (Pundt 2001).

Biological control

Biological control may be effective if used as part of the integrated pest management (IPM) scheme, including other non-chemical methods, such as general glasshouse hygiene.

There are increasing demands for minimal pesticide use on both edible and ornamental plants.

Thus many growers of greenhouse crops use biological control methods for thrips within IPM (Bennison 2004). The use of spinosad (a new insecticide derived from the fermentation of *Saccharpolyspora spinosa*) and *Orius insidiosus* in combination to control *F. occidentalis* on greenhouse grown pot chrysanthemum seems to be promising (Ludwig and Oetting 2001). The main elements of biological control are:

1. Predatory mites. *Neoseilus* (*Amblyseilus*) *cucumeris* and *N. degenerans* by feeding on larval thrips gives a good control of *F. occidentalis* on a wide range of greenhouse ornamental crops including pot and bedding plants, cut flowers and nursery stock. UK growers of ornamentals using *A. cucumeris* against thrips have very few problems with TSWV and INSV. The mites are released every week if using the broadcast method, or every six weeks if using the 'controlled relase' sachet method. As *A. cucumeris* feeds only on young thrips larvae, it is important to start releases preventively, before thrips are seen. *Hypoaspis miles* and *H. aculeifer* are ground-dwelling predatory mites, which were originally marketed for control of sciarid flies (fungus gnats). These mites may supplement the control of thrips by *A. cucumeris* (Higgins 1992; Cook *et al*. 1995).

2. Predatory bugs. Although *Orius* spp. (*O. insidiosus*) have potential for use on ornamental crops, they are little used. The bugs need a pollen food source in addition to prey such as thrips, and they can be slow to establish (Higgins 1992; Cook *et al*. 1995).

3. Entomopathogen nematodes (Bennison 2004). Grower trials with foliar applications of *Steinernema feltiae* against *F. occidentalis* have led to successful commercial uptake of this product on certain ornamental crops including chrysanthmum and saintpaulia, which hosts are very susceptible to *F. occidentalis* damage and are crops on which *A. cucumeris* does not give a reliable control. Other nematodes, such as *Thripinema nicklewoodi* also seem promising.

4. Entomopathogenic fungi. *Verticillium lecanii* and *Beauveria bassiana* species and their strains are promising in this respect, attacking all *F. occidentalis* life stages and killing in 2 to 14 days.

4. "Push-pull" strategy. There is increasing interest in developing the "push-pull" strategy (Bennison 2004), where thrips are pushed from the cultivated plants using a repellent or antifeedant, and "pulled" to trap plants attractive to thrips. The trap plants could be then be replaced regularly, or could be used to target control agents such as entomopathogenic nematodes, thus making them more cost-effec-

tive than use on the whole crop. In a previous project, flowering pot chrysanthemums 'Swingtime' were sown to be very attractive to *F. occidentalis* adults when placed amongst a pot chrysanthemum crop in the vegetative stage. A semiochemical thrips lure based on (*E*)–β-farnesene (EBF) increased the attraction to the trap plants and a plant-derived antifeedant helped to "push" the thrips from the crop plants. Beside this, flowering verbenas e.g. cv. 'Sissinghurst Pink' have also been shown to be effective trap plants for *F. occidentalis* in bedding plant crops (Bennison 2004).

Chemical control

There are many chemicals available to eliminate *F. occidentalis* but overuse of pesticides can lead to resistance in the *F. occidentalis* populations. Classes of insecticides registered for use on *F. occidentalis* include organophosphates, carbamates, pyrethrinoids, insect growth regulators, chlorinated hydrocarbons, chloronicotinyls, endosulfan, chlorpyrifos, bendiocarb, spinosyns, macrocyclic lactone, microbials and horticultural oils (Parella 1995; Robb 1998). For controlling *F. occidentalis* only the organophosphates proved successful by keeping the thrips population at an acceptable low threshold (Helyer and Brobyn 1992). A number of serious problems are raised by this action. These compounds are highly toxic and environmentally undesirable. These agents act in a non-specific manner, and this also results in the killing of insect predators, which are often introduced in the glasshouses to act as biological agents of other pests. Finally, control of TSWV via reducing thrips numbers can only be effective if treatments are performed regularly, to prevent populations reestablishing themselves. Eggs and pupal stages are unaffected by pesticide sprays, so we must make sure that the spraying program lasts long enough to include emerging larvae and adults. Rotating the use of insecticides among different classes of pesticides remains the single best approach in dealing with insect resistance.

Recently developed chemicals such as the microbial insecticide Spinosad®, a natural macroyclic lactone with a unique mode of action and law mammalian toxicity, offer greater promise as part of IPM strategies (Eger *et al*. 1998). Acibenzolar-methyl (Actigard®) induces systemic aquired resistance against a broad range of pathogens and has reduced TSVW incidence.

Alternative means of modifying thrips behaviour include UV reflective mulch (Geer and Dole 2003).

Suitable post-harvest management can reduce overwintering thrips populations, a proposed major source of initial virus inoculum for the following growing season, but to very lower levels due to the influx of viruliferous thrips from outside or other untreated areas.

A different approach to reducing thrips transmission, without directly reducing vector numbers, is to use thrips-resistant cultivars in groundnut (Amin 1985) and tomato (Krishna *et al*. 1993), but not in ornamentals. Broadbent *et al*. (1990) suggested that for chrysanthemum, host resistance was epidemiologically more significant than vector resistance.

Besides thrips, the spread of tospovirus also occurs via the movement of infected plant material, such as cuttings, used in the vegetative propagation of many ornamental species. This type of spread is most effectively controlled by the use of certified virus-free material.

Flower and vegetable transplants are often produced together in the same greenhouse, providing an opportunity from transmission of tospoviruses from vegetatively propagated ornamentals to field grown vegetables.

Winter and summer annual and perennial weeds as virus reservoirs and hosts of thrips vectors may be as primary infection sources, therefore effective weed control methods are necessary not only to prevent a direct harmful effect of weeds on crops but also from a virus epidemiological point of view (Cho *et al*. 1986; Gáborjányi *et al*. 1995; Jenser *et al*. 2005; Takács *et al*. 2006). *Stellaria media* and *Galinsoga parviflora* frequently occur in the glasshouses and they are also considered good hosts of *F. occidentalis* and tospoviruses (Mertelik and Mokra 1998).

An adequate method of controlling tospovirus infection is to start production from *in vitro* obtained stock material. Growing chrysanthemums from meristem-tip culture proved to be a good method for eliminating TSWV (Balukiewicz and Kryczynski 2001).

TRANSGENIC MEANS FOR TOSPOVIRUSES CONTROL AND ERADICATIONS

Transgenic plants resistant to TSWV infections have been developed using pathogen-derived resistance approaches. Resistant cultivars are currently part of the control strategies for TSWV mainly in capsicum, peanut and tomato (Cho *et al*. 1996; Roggero *et al*. 2002; Culbreath *et al*. 2003). It is recognized that TSWV can adapt and overcome resistance genes relatively easily. Therefore there is an urgent need to identify new sources of resistance to TSWV and other tospoviruses with a view to broadening the genetic base through gene pyramiding and other strategies.

Genetic transformation of plants with the N or NSm gene has been achieved in several important hosts of TSWV (tomato, tobacco, peanut). These approaches should provide greater opportunity to develop durable resistance to tospoviruses, particularly in the many hosts where conventional resistance genes have not been found.

Initial studies reported the production of transgenic tobacco, expressing the TSWV N gene (Pang *et al*. 1992), and showing a significant level of resistance to TSWV infection. Transgenic plants expressed viral nucleocapsid protein which was assumed to mediate resistance. Subsequent studies showed that the resistance is in fact mediated by the expression of viral RNA and not viral protein (Kim *et al*. 1994).

In a recent study Xu *et al*. (2006) proved that transgenic plants expressing a single-chain Fv antibody to TSWV are resistant to TSWV infection. Functional antibodies against plant viruses could be produced in tobacco plants and these transgenic tobacco plants expressing these anti-virus antibodies were resistant to infection by the homologous virus. The level of virus resistance was correlated with the level of the transgene RNA transcript and scFv antibody production and inherited into the R_1 generation. This principle of antibody-mediated protection may be useful for protecting plants against plant viruses.

Breeding for virus resistance in floricultural crops has traditionally been hampered by the diversity of the species, the lack of identified sources of resistance genes, the overriding importance of horticultural traits and the rapid turnover of popular cultivars (Daub *et al*. 1997). Given these constraints genetic engineering seems to be the best potential for the development of resistance to tospoviruses in floricultural crops, particularly given the extensive studies on the effectiveness of genetic engineering for tospovirus resistance in tobacco and vegetable crops (Accotto *et al*. 2005).

Although techniques for genetically engineering floricultural crops have logged behind those for major food crops, significant efforts have already been made. Many of the major flower crops (rose, chrysanthemum, carnation, lily, tulip, gebera and geranium) have been successfully transformed, resulting in altered phenotypes (Hsu and Lawson 1991; Davies and Schwinn 1997; reviewed extensively in Teixeira da Silva 2006).

To date there are few reports of genetically engineered disease resistance in floricultural crops. N gene transformation and conferment of resistance has now been achieved in a number of commercially-important crops, including chrysanthemum (Urban *et al*. 1994; Gonsalves *et al*. 1995; Sherman *et al*. 1995), New Guinea impatiens (*Impatiens* spp.) and *Osteospermum ecklonis* (Vaira *et al*. 2000).

Sherman *et al*. (1998) employed chrysanthemum as a model system for exploring the feasibility of using genetic-

ally engineered resistance to TSWV in ornamentals. They developed an efficient *Agrobacterium*-mediated transformation system that allowed for the recovery of transgenic plants from three cultivars of chrysanthemum. They transformed the highly TSWV susceptible chrysanthemum 'Polaris' with the TSWV nucleoapsid (N) protein gene. Resistant plants were identified following challenge by viruliferous thrips that vectored a highly virulent heterologous isolate of TSWV.

An alternative or complementary resistance strategy is through host resistance to thrips (Jericho and Wilson 2003).

FUTURE PERSPECTIVES AND CHALLENGES

The enhancing of the international trade of propagative materials and final floricultural products favours for the rapid distribution of pests and pathogens – including tospoviruses and their vectors also. Therefore more severe plant protection control of the planting materials is necessary at the borders in order to avoid the introduction of new (exotic) pests and pathogens. International cooperations and projects regarding tospovirus-ornamentals relations are necessary and future international symposiums on virus diseases of ornamental plants are the best possibilities to exchange latest research results in this respect.

The use of chemicals on flower crops is not regulated as in food crops. The large amounts of chemicals pose a threat to the environment and wildlife, water contamination and the health for the workers. The most effective and environmentally safe method against viruses is the breeding for virus resistance. Genetical methods will allow for the introduction of novel characters, such as new colors, manipulated growth, and also to strengthen the plant's responses to biotic and abiotic stresses. Genetically engineered virus resistance in floricultural crops must be a new challenge in the future, with an alternative or complementary resistance strategy is through host resistance to thrips. Organically-grown flowers are another solution to overcome the use of potentially dangerous chemicals (Jericho and Wilson 2003, Xia *et al*. 2006).

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