# S. K. Raj Rajarshi Kumar Gaur Zhimin Yin *Editors*

# Virus Diseases of Ornamental Plants

Characterization, Identification, Diagnosis and Management



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S. K. Raj • Rajarshi Kumar Gaur • Zhimin Yin Editors

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## Tospovirus Diseases of Ornamental Plants: Characterization, Identification, Diagnosis and Management

G. Karthikeyan, K. Nagendran, Shweta Kumari, R. Priyanka, C. Senthilraja, and Betsy D. Haokip

#### Abstract

Ornamental industry is one of the commercial sectors contributing maximum for the growth of agriculture. Ornamental crops are grown for its aesthetic value and are categorized as flowering, foliage, shrubs and trees. Due to their perennial nature and availability throughout the year, they are very prone for the infection of several viruses. The Orthotospovirus is one of the most devastating viruses of ornamental plants and flower crops worldwide. It is one among the virus genera infecting ornamental crops across 46 countries belonging to the six continents. The diseases caused by orthotospoviruses (order Bunyavirales; family Tospoviridae; genus Orthotospovirus) are emerging as a significant limiting factor for the sustainable production of ornamental crops around the globe. These orthotospoviruses are known to be transmitted through thrips vector in a persistent and propagative manner. Till date 18 species of orthotospoviruses are reported to be infecting more than 600 plant species of ornamental crops belongs to all categories. Asian continent is found to show maximum diversity harboring nine different orthotospovirus species. Symptoms of orthotospovirus infections are highly variable with the host and the virus species interactions. In this chapter information on symptomatology, transmission, distribution, diagnosis, hostpathogen interactions and management of tospoviruses on ornamental plants are consolidated and presented in an organized manner.

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#### **Keywords**

 $To spovirus es \cdot Thrips \cdot Epidemiology \cdot Host-pathogen \ interaction \cdot Diagnosis \cdot Management$ 

#### 1.1 Introduction

Ornamental plants are grown for their aesthetic value. Maintaining visual appearance of ornamental plants is the major component of ornamental production facilities. There are different groups of ornamental plants, and they are classified based on their life span as annual, biennial and perennials. Similarly, based on their aesthetic part of the plant, they are grouped as flower crops, foliage/shrubs and trees. In recent days, ornamental industry has attained remarkable growth and has developed into commercial branch of modern agriculture. Economy of ornamental industry depends in sale of potted plants, tree nursery production, seed material for flower bulbs and other propagation materials and their maintenance. The Netherlands, the USA and Japan are the pioneer countries of ornamental production (Kazinczi et al. 2007).

The ornamental plants are affected by several biotic and abiotic stresses. Among them the diseases caused by viruses impose a serious threat to the ornamental industry. Tospoviruses are the plant-infecting viruses belonging to the genus Orthotospovirus under the family Bunyaviridae and are thrips transmissible. These viruses cause significant quality and quantity losses in horticultural crops including ornamentals in many parts of the world (Pappu et al. 2009). INSV infection causes crop losses in flower production of *Dendranthema* spp. (51%) and *Sinningia* speciosa (100%) (Matteoni and Allen 1989). The utilization of molecular tools enabled the characterization of tospoviruses in to 30 species grouped under five different serogroups (EPPO 2020; Chu et al. 2001). Ubiquitous nature of tospoviruses adds every day new hosts to their host range list. Due to the thrips transmissible nature, wide and overlapping host ranges of thrips and tospoviruses, lack of effective and economical management options and lack of resistant sources in hosts contribute to the repeated occurrence of tospoviruses in diverse crops both under open and protected cropping conditions (Daughtrey et al. 1997; Brown et al. 2005; Gent et al. 2006). Several reviews and book chapters extensively organized the various aspects of tospoviruses and its thrips vector in recent years (Whitfield et al. 2005; Kazinczi et al. 2007; Pappu et al. 2009; Turina et al. 2016). In this chapter, we summarized the current status of tospovirus epidemics in ornamental crops.

#### 1.2 Host Range of Tospoviruses on Ornamental Crops

The economic impact of tospoviruses are increasing every day by causing huge losses in terms of quality and quantity in a wide variety of ornamental crops. The host range of tospoviruses is highly variable between the species. In global scenario, around 204 plant species were reported to be infected by 18 different tospovirus

species (Table 1.1). Tospoviruses are infecting all kind of ornamentals such as flowering, foliage/shrubs, grasses and trees of annuals, biennials and perennials. Nearly eight different tospovirus species are affecting the cultivation of chrysanthemum in several countries. Tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV) are the two major tospoviruses threatening the ornamental crop cultivation. Both of them were known to infect around 600 species of ornamental plants (Daughtrey et al. 1997; Ullman 1998). Alstroemeria necrotic streak virus (ANSV), alstroemeria yellow spot virus (AYSV), calla lily chlorotic spot virus (CCSV) and ligustrum necrotic ringspot virus (LNRV) are the tospovirus species having very narrow host range and documented only on ornamental crop plants. The chrysanthemum stem necrosis virus (CSNV) is found to infect ornamental crops such as chrysanthemum, Callistephus spp., Eustoma grandiflorum, Gerbera and lisianthus. The INSV is having intermediate host range than the TSWV and commonly infecting annuals and perennial ornamentals. Iran and the USA are the two countries found to have wider host range for the tospovirus infection among ornamental crops. Many tospovirus species are known to infect weed species very often which are playing a major epidemiological role in disease dissemination. Tree ornamentals such as Cycas sp., Ficus benjamina, Schefflera, etc. are also infected by tospoviruses.

#### 1.3 Symptomatology of Tospoviruses on Ornamental Crops

In ornamentals, tospovirus infection produces symptoms similar to the other biotic and abiotic stresses. Tospovirus symptoms on floral crops include concentric ring spots; line or zonal patterns, malformation and necrosis of apical shoots and flowers, necrosis of leaf petiole, reduced growth, etc. were reported (Fig. 1.1) (Kazinczi et al. 2007). Chrysanthemum plants are very often observed with chlorotic and necrotic spots on leaves and stems, stunted growth and death of young plants upon infection of TSWV and INSV (Cho et al. 1989). TSWV produces chlorotic or black ringspot and line pattern of leaves, malformed leaves and terminal bud necrosis on impatiens; chlorotic and necrotic ringspot of leaves, stem necrosis and terminal bud necrosis on Eustoma; mild mosaic and ringspot of leaves on Zinnia; and reddish concentric brown rings on the leaves and malformed flowers on *Gloxinia* (Antignus et al. 1997). TSWV infection reduces the floral fresh weight, number of petals, number of flowers per plant and inhibiting adventitious sprouting of buds in perennials (Whitfield et al. 2003). CSNV infection causes mild or severe streaks on stem, wilting of leaves and stems, chlorotic and necrotic spots and rings on leaves, necrosis of the stem and floral receptacles, necrotic lesions surrounded by yellow halo on leaves and distinct dark stem lesions on chrysanthemum (Verhoeven et al. 1996; Duarte et al. 1995; Mumford et al. 2003). Persley et al. (2006) observed ringspots, line patterns, chlorotic blotches and necrotic etching on Hoya spp. upon capsicum chlorosis virus (CaCV) infection. CCSV caused chlorotic spots on their leaves and stems of Zantedeschia species in Taiwan (Lin et al. 2006). IYSV infection in plants of Alstroemeria spp. show necrotic streak symptoms (Okuda et al. 2005).

		Number			
Continent	Country	of hosts	Hosts		
1. Alstroeme	1. Alstroemeria necrotic streak virus (ANSV)				
North America	USA	1	Hoya carnosa		
South America	Colombia	1	Alstroemeria sp.		
2. Alstroeme	eria yellow spot	virus (AYSV	· ·)		
Europe	Netherlands	1	Alstroemeria sp.		
3. Calla lily	chlorotic spot vi	rus (CCSV)			
Asia	China	1	Spider lily		
Asia	Taiwan	1	Calla lily		
4. Capsicum	chlorosis virus (	(CaCV)			
Asia	China	5	<i>Rieger begonias, Gloxinia,</i> orchids, African violet, <i>Crossandra</i>		
Asia	India	1	Hippeastrum		
Asia	Indonesia	1	Orchids		
Asia	Iran	1	Rudbeckia hirta		
Asia	Taiwan	4	Orchids, Hippeastrum, calla lily, Scadoxus multiflorus		
Asia	Thailand	1	Hymenocallis littoralis		
Australia	Australia	1	Hoya sp.		
North	USA	2	Hoya sp., Gloxinia		
America					
5. Chrysanth	nemum stem neci	rosis virus (C	CSNV)		
Asia	China	1	Chrysanthemum		
Asia	Japan	3	Chrysanthemum, Lisianthus, Eustoma grandiflorum		
Asia	South Korea	1	Chrysanthemum		
Australia	New Zealand	1	Chrysanthemum		
Europe	Belgium	1	Chrysanthemum		
Europe	Netherlands	1	Chrysanthemum		
Europe	Russia	1	Chrysanthemum		
Europe	Slovenia	2	Chrysanthemum, Gerbera		
Europe	UK	1	Chrysanthemum		
South	Brazil	4	Chrysanthemum, Lisianthus, Callistephus spp.,		
America		(95).05	Eustoma grandiflorum		
6. Groundnu	it bud necrosis vi	rus (GBNV)			
Asia	India	8	Jasmine, <i>Dahlia</i> , <i>Chrysanthemum</i>		
7. Groundnu	it ringspot orthot	ospovirus (G	RSV)		
South America	Brazil	1	Hippeastrum		
South America	Paraguay	1	Petunia		
8. Hippeastr	um chlorotic ring	gspot virus (I	HCRV)		
Asia	China	9			

 Table 1.1 Distribution and host range of tospoviruses infecting ornamental crops

		Number	
Continent	Country	of hosts	Hosts
			Hymenocallis littoralis, Oxalis corniculata,
			Philodendron, Crinum asiaticum, Zephyranthes
			Hinpeastrum
9. Impatiens	necrotic spot vir	us (INSV)	Inpreasing
Africa	Egypt	1	Tropaeolum
Asia	China	5	Begonia orchids spider lily, Hymenocallis, Gentiana
			spp.
Asia	Japan	10	Anemone coronaria, Begonia, Chrysanthemum,
			<i>Eustoma</i> sp., <i>Gentiana</i> sp., <i>Gerbera jamesonii</i> ,
	South Karea	2	Pericallis, Exacum spp., Cyclamen, Eucharis spp.
Asia	Jron	20	A fricon violet, Impatiene, Aletro emovia, Belano enium
Asia	ITall	29	spp Cycas spp Rosa spp Scindapsus spp Ficus
			spp., Gazania spp., Erica spp., Syngonium, Gladiolus
			spp., Anthurium, Codiaeum, Cheiranthus,
			Dieffenbachia, Philodendron spp., Zinnia, Althaea
			spp., <i>Bougainvillea</i> spp., Chamomile, Marigold,
			Spathiphyllum spp. Leucanthemum vulgare Dahlia
			spp., Dianthus barbatus
Asia	Israel	3	Anemone, Impatiens, Torenia spp.
Australia	New Zealand	9	Begonia, Freesia refracta, Gardenia jasminoides,
			Gerbera jamesonii, Hibiscus rosa-sinensis, Impatiens,
			Primula, Ranunculus sp., Cyclamen
Europe	Belgium	1	Impatiens
Europe	Bosnia and	1	<i>Begonia</i> spp.
<b>.</b>	Herzegovina	1	
Europe	Bulgaria	11	Hyarangea spp.
Europe	Republic	11	<i>Columnea</i> sp., <i>Curcuma</i> sp., <i>Impatiens</i> , <i>Hippeastrum</i> , <i>Aeschynanthus</i> spp. <i>Anthurium Philodendron</i> spp.
	Republic		Osteospermum spp., Verbena spp., Saxifraga
			stolonifera, Cyrtomium falcatum
Europe	Finland	1	Lobelia spp.
Europe	Germany	2	Anemone, Impatiens
Europe	Hungary	3	Impatiens, Eustoma sp., Cyclamen
Europe	Italy	22	Antirrhinum, Scindapsus spp., Periwinkle, calla lily,
			Oncidium, Primula spp., Peperomia spp., Clarkia
			amoena, Anemone spp., Kuscus spp., Lobelia spp., Cyclamen, Cordyline, Ranunculus, Maranta spp.
			Episcia spp., Spathiphyllum spp., Isotoma axillaris
			Limonium, Dahlia spp., Delphinium spp., Dianthus
			spp.
Europe	Poland	1	Impatiens
Europe	Portugal	10	

		Number		
Continent	Country	of hosts	Hosts	
			Pelargonium spp., Gazania spp., Gladiolus spp., Sinningia spp., Penstemon, Ruscus spp., Aphelandra, Helichrysum spp., Gerbera spp., Hydrangea spp.	
Europe	Russia	4	Gloxinia, Impatiens, Ruellia, Streptocarpus	
Europe	Serbia	3	Begonia sp., Impatiens, Tulip sp.	
Europe	Spain	1	Asplenium	
Europe	The Netherlands	5	Impatiens, Iris, Lysimachia, Nerine spp., Gentiana spp.	
Europe	UK	3	Impatiens, Pericallis, Opuntia spp.	
North America	Canada	2	Begonia, Impatiens	
North America	Costa Rica	6	Antirrhinum majus, Hippeastrum sp., Impatiens, Iris sp., orchids, Plectranthus	
North America	Mexico	1	Impatiens	
North America	USA	46	Abelia spp., Antirrhinum, Begonia, Browallia speciosa, Calceolaria spp., Callistephus, Dianthus sp., Digitalis sp., Epidendrum spp., Eustoma sp., Gloxinia spp., Hosta sp., Hoya wayetii, Impatiens, Maranta leuconeura, Monarda didyma, Nemesia, Nipponanthemum, Ocimum basilicum, Lobelia spp., orchids, snapdragon, Schizanthus spp., Pelargonium spp., periwinkle, Pericallis, Primula spp., Sinningia spp., Penstemon, Exacum spp., Cyclamen, Weigela, Scaevola, Hydrangea spp., Mimulus spp., Oxydendrum arboreum, Ilex glabra, Photinia fraseri, Gaillardia spp., Franklinia spp., Halesia carolina, Rhaphiolepis indica, Calycanthus floridus	
10. Iris yello	ow spot virus (IY	SV)		
Asia	Israel	4	Lisianinus, Eustoma sp., Hippeastrum, Lilium spp.	
Asia	Japan	3	Eustoma sp., Lisianthus, Alstroemeria	
Europe	Iran	5	Chrysanthemum, Cycas spp., Pelargonium, Rosa spp., Scindapsus spp.	
Europe	Netherland	3	Iris, Eustoma sp., Impatiens spp.	
Europe	Poland	1	Chrysanthemum	
Europe	UK	2	Lisianthus, Eustoma sp.	
North America	USA	1	Eustoma sp.	
11. Lisianthus necrotic ringspot virus (LNRV)				
Asia Japan 1 Lisianthus			Lisianthus	
12. Pepper c	12. Pepper chlorotic spot virus (PCSV)			
Asia	China	1	Erigeron	
13. Tomato chlorotic spot virus (TCSV)				

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		Number	
Continent	Country	of hosts	Hosts
North	USA	4	Periwinkle, Marsdenia floribunda, Hoya wayetii,
America			Schlumbergera truncata
South America	Argentina	1	Eustoma
South America	Brazil	5	Bouvardia, Dieffenbachia, Mirabilis jalapa, Spilanthes oleracea, Eustoma
14. Tomato	spotted wilt virus	s (TSWV)	
Asia	China	10	Argyranthemum, Bidens, Chrysanthemum, Dahlia, Geranium, Iris, Platycodon grandiflorus, Tropaeolum majus, Zinnia, Campanula spp.
Africa	Egypt	1	Chrysanthemum
Asia	India	4	Antirrhinum majus, Callistephus, Chrysanthemum, marigold
Asia	Iran	30	Althaea, Anthurium, Asplenium, Calendula spp., Canna indica, Codiaeum variegatum, Cupressus sempervirens, Cycas spp., Dahlia spp., Dieffenbachia amoena, Dracaena spp., Erica spp., Ficus benjamina, Gazania spp., Jasmine, Mirabilis jalapa, orchids, Pericallis, Philodendron spp., Pteris cretica, Rosa spp., Saintpaulia ionantha, Rudbeckia spp., Salvia spp., Schefflera, Scindapsus spp., Strelitzia reginae, marigold, Torenia fournieri, Zinnia
Asia	Israel	2	Eustoma, Pittosporum tobira
Asia	Japan	11	Arctotis, Callistephus, Chrysanthemum, Pericallis, Valeriana fauriei, Dahlia spp., Dimorphotheca sinuate, Eustoma, Felicia amelloides, Ranunculus spp., Verbena spp.
Asia	Jordan	2	Gerbera, marigold
Asia	Saudi Arabia	1	Periwinkle
Asia	South Korea	11	Angelica gigas, Anthurium, Impatiens, Brugmansia sp., Chrysanthemum, Eustoma, Gerbera, Hoya sp., Peperomia, Ranunculus, Nasturtium
Africa	Zimbabwe	2	Chrysanthemum, Gypsophila
Australia	Australia	5	Spider lily, Agapanthus, Gladiolus spp., Lupinus spp., Tanacetum spp.
Australia	New Zealand	9	Chrysanthemum, <i>Cineraria</i> sp., <i>Dahlia</i> , <i>Hoya</i> sp., <i>Impatiens</i> , <i>Ocimum</i> , <i>Pinellia tripartita</i> , marigold, <i>Ornithogalum</i> spp.
Europe	Belgium	1	Lobelia spp.
Europe	Bosnia and Herzegovina	2	Chrysanthemum, Gloxinia
Europe	Bulgaria	14	Leuzea carthamoides, lisianthus, Adenium, Althaea, Calendula spp., Helichrysum bracteatum, Hydrangea spp., Inula helenium, Physostegia spp., Salvia spp., marigold, Thymus vulgaris, Verbena spp., Zinnia

Continent	Country	Number of hosts	Hosts
Europe	Czech Republic	3	Campanula spp., Columnea, Gazania spp.
Europe	France	4	Dahlia, Arum spp., Dimorphotheca sinuate, Salvia spp.
Europe	Germany	1	Lysimachia
Europe	Greece	9	Aristolochia clematitis, Periwinkle, Dimorphotheca sinuate, Fuchsia spp., Gazania spp., Justicia brandegeeana, Portulaca spp., Salvia spp., marigold
Europe	Hungary	3	Aristolochia clematitis, Echinacea purpurea, Heuchera sanguinea
Europe	Italy	21	Chrysanthemum, Coprosma repens, Gerbera, Iberis semperflorens, Anemone coronaria, Arctotis, Arum spp., Asclepias, Dimorphotheca sinuate, Euphorbia, Eritrea, Eustoma, Gazania spp., Limonium perezii, Nerium oleander, orchids, Polygala myrtifolia, Primula spp., Ranunculus spp., Ruscus racemosus, Verbena spp.
Europe	Lithuania	2	Dicentra Formosa, Lilium spp.
Europe	Montenegro	6	Aquilegia sp., Calceolaria sp., Chrysanthemum, Gerbera, Petunia, Primula sp.
Europe	Poland	5	Chrysanthemum, Ocimum, Dieffenbachia amoena, Hippeastrum spp., Hydrangea spp.
Europe	Portugal	11	Anthurium, Aphelandra, periwinkle, Fuchsia spp., Gazania spp., Gerbera, Gladiolus spp., Helichrysum bracteatum, jasmine, Penstemon spp., Sinningia speciosa
Europe	Russia	1	Streptocarpus
Europe	Serbia	7	Brugmansia sp., Chrysanthemum, Dahlia, Gerbera, Impatiens, Petunia, Sinningia speciosa
Europe	Spain	1	Ficus benjamina
Europe	The Netherlands	11	Adenium, Anthurium, Columnea, Dahlia spp., Hippeastrum spp., Impatiens spp., Iris, Lobelia spp., Lysimachia spp., Nerine spp., Schizanthus spp.
Europe	Turkey	1	Ranunculus
Europe	UK	4	Impatiens, Delphinium spp., Paeonia spp., Verbena spp.
North America	Canada	7	Periwinkle, Cyclamen persicum, Fuchsia spp., Gaillardia spp., Impatiens spp., orchids, Verbena spp.
North America	Mexico	1	Zinnia
North America	USA	76	Orchids, Chrysanthemum, Dahlia, Eustoma, Hoya sp., Lobelia spp., Matricaria, Pittosporum tobira, Stokesia, Tulbaghia violacea, Adenium, Anemone coronaria, Aeschynanthus, Aglaonema, Anthurium, Antirrhinum, Aphelandra, Arum spp., Begonia, Calceolaria spp., Callistephus chinensis, periwinkle,

Continent	Country	Number	Heate	
Continent	Country	of nosts		
			Celosia cristata, Centranthus ruber, Clarkia amoena, Cordyline terminalis, Cosmos spp., Cyclamen persicum, Delphinium spp., Digitalis spp., Dracaena spp., Eucharis grandiflora, Exacum spp., Gardenia jasminoides, Gerbera, Gladiolus spp., Gomphrena globosa, Helichrysum bracteatum, Hosta spp., Hydrangea spp., Impatiens spp., Lupinus spp., Melampodium divaricatum, Opuntia spp., Osteospermum, Pedilanthus, Penstemon spp., Peperomia spp., Pericallis, Petunia spp., Phlox spp., Pinus spp., Pittosporum tobira, Plectranthus australis, Portulaca grandiflora, Primula spp., Ranunculus spp., Rhododendron spp., Rohdea spp., Rudbeckia spp., Schlumbergera truncate, Sedum spp., Lychnis chalcedonica, Sinningia speciose, Stephanotis floribunda, Streptocarpus spp., Tropaeolum majus, Verbena spp., Veronica spp.,	
South America	Argentina	2	Alstroemeria, Eustoma	
South America	Brazil	2	Eucharis grandiflora, Campanula spp.	
South America	Ecuador	1	Chrysanthemum	
South America	Venezuela	2	Chrysanthemum, Gerbera	
15. Tomato	yellow ring virus	(TYRV)		
Asia	Iran	22	Tropaeolum, Cineraria, Gazania, Chrysanthemum, Alstroemeria, Anemone, Antirrhinum spp., Althaea spp., Beaucarnea recurvata, Bougainvillea spp., Dahlia, Dieffenbachia, Dracaena, Ficus benjamina, Impatiens, Jasminum, Pelargonium, Pericallis hybrida, Rosa spp., Spathiphyllum, Syngonium, marigold	
16. Tomato	zonate spot virus	(TZSV)		
Asia	China	3	Bidens, Crinum asiaticum, Iris	
17. Waterme	elon bud necrosis	virus (WBN	VV)	
Asia	India	1	Chrysanthemum	
18. Waterme	18. Watermelon silver mottle virus (WSMoV)			
Asia	Taiwan	1	Calla lily	

Source: NCBI database and Sastry et al. 2019



chrysanth

m by TSWV infection on chrysant

ringspots by GBNV streaks and chlrotic infection omum loovos (Courtesy: R. Aravintharaj, India)

Fig. 1.1 Symptom variation of tospovirus infection on different ornamental crops

#### 1.4 **Transmission of Tospoviruses**

Vectors play a major role in dissemination of tospoviruses and escalating them as devastating pathogen in the global status. The tospoviruses are transmitted by thrips (Thysanoptera: Thripidae) in a persistent, circulative and propagative manner (German et al. 1992). Among the 5500 known thrips species, nine species (Frankliniella sp., Thrips sp. and Scirtothrips sp.) were reported to be associated with tospoviruses (Mound 1998). All thrips species transmitting tospoviruses are polyphagous in nature and distributed worldwide causing the tospovirus a menace to ornamentals. Transmission of tospoviruses by thrips undergoes specific virus-vector interaction. One of the most interesting features is adults thrips were not able to acquire the virus in the thrips-tospovirus interaction. Tospoviruses acquired by first and second instar larval stage alone can able to transmit virus in their adult stage (Ullman et al. 1992; Sakimura 1963). Once thrips acquire the virus, it remains infectious throughout its life span because of its transstadial transmission nature (Wijkamp and Peters 1993; Wijkamp et al. 1995).

Frankliniella occidentalis (western flower thrips), a notorious insect pest, exists globally and is the most important vector of TSWV (Goldbach and Peters 1994). In petunia, F. occidentalis appeared to be the most effective in transmitting four tospoviruses tested (TSWV, INSV, tomato chlorotic spot virus (TCSV) and groundnut ringspot virus (GRSV)) in comparison to other thrips species through leaf disk assay. Also, one among four populations of T. tabaci was able to transmit TSWV in a low transmission efficiency (Wijkamp et al. 1995). Upon, ingestion of tospovirus by both adults and larvae of F. occidentalis, barrier in midgut prevents the virus movement out of the midgut of adults. Virions accumulated within epithelium gut of adults, whereas virions are seen in the midgut epithelia, fat body, brain, nerve cells, haemocoel and salivary glands of larvae (Ullman et al. 1992). Nagata and De Avila (2000) studied the transmission efficiency of CSNV by *F. schultzei* (78.1%), *F. occidentalis* (65.1%) and *T. tabaci* (0.0%). High CSNV titre was found in 75.9% of *F. schultzei* and 97.4% of *F. occidentalis* adults. Studies of Sharman et al. (2020) showed *Thrips palmi*, *F. schultzei* and *Microcephalothrips abdominalis* were transmitting CaCV, while no transmission was achieved with *F. occidentalis* upon acquiring TSWV. Beside thrips transmission, the dissemination of tospovirus also occurs through vegetatively propagated material such as tubers, corms, cuttings, etc. in many ornamental species (Wilson 2001; Adkins 2003). TSWV persists in flower bulbs and roots of lilies and dahlias (Kazinczi et al. 2007). Transmission of tospovirus through seeds does not occur among ornamentals (Mumford et al. 1996; Antignus et al. 1997). Major vectors of tospoviruses infecting ornamental crops are described in Table 1.2.

Virus	Vector
ANSV	Frankliniella occidentalis
AYSV	Thrips tabaci
CCSV	T. palmi
CaCV	Ceratothripoides claratris, F. schultzei, T. palmi
CSNV	F. gemina, F. occidentalis, F. intonsa, F. schultzei
GBNV	F. schultzei, Scirtothrips dorsalis, T. palmi
GRSV	F. gemina, F. intonsa, F. occidentalis, F. schultzei
HCRV	Unknown
INSV	F. fusca, F. intonsa, F. occidentalis, F. schultzei
IYSV	F. fusca, T. tabaci
LNRV	Unknown
PCSV	Unknown
TCSV	F. intonosa, F. occidentalis, F. schultzei
TSWV	F. bispinosa, F. cephalica, F. fusca, F. gemina, F. intonsa, F. occidentalis,
	F. schultzei, T. setosus, T. tabaci
TYRV	Microcephalothrips abdominalis, T. tabaci
TZSV	F. occidentalis
WBNV	T. palmi
WSMoV	T. palmi

 Table 1.2
 Vectors of orthotospovirus species infecting ornamental crops

Source: (EPPO 2020)

#### 1.5 Occurrence and Distribution of Tospoviruses on Ornamental Crops

Tospoviruses are a highly cosmopolitan virus genus to have a worldwide distribution. The tospoviruses infecting ornamental crops are reported in 46 countries distributed representing all 6 continents (Fig. 1.2). TSWV and INSV are predominant tospovirus species infecting wide host range and are distributed across 38 and 26 countries on ornamental crops, respectively. TSWV is found to be prevalent among six continents and INSV in five continents and are ubiquitous infecting varieties of crops. INSV has become a serious pathogen on flower crops cultivated in greenhouse conditions in the USA (Daughtrey et al. 1997). Among the 18 ornamental-infecting tospoviruses, nine viruses, viz. CCNV, GBNV, HCRV, LNRV, PCSV, TYRV, TZSV, WBNV and WSMoV, seem to be restricted only to Asian countries. Totally 14 tospovirus species are infecting ornamental crops of Asian continent. Interestingly, GBNV is found on eight ornamental plants only in India, while HCRV infects nine plants only in China. In the Australian continent, four species (CaCV, CSNV, INSV and TSWV) are infecting different plant species such as Hoya sp., chrysanthemum, Begonia, Cyclamen, Freesia, Gardenia, Gerbera, Hibiscus, Impatiens, Primula, Ranunculus, spider lily, Agapanthus, Gladiolus, Lupinus, Tanacetum, Cineraria, Dahlia, Ocimum, Pinellia, marigold and Ornithogalum. Among the different countries, maximum of eight different tospovirus species are distributed across the China followed by six in the USA and five in Iran, Japan and the Netherlands (Table 1.1).



Fig. 1.2 Geographical distribution of tospoviruses species infecting ornamentals in different continents

#### 1.6 Diagnostics of Tospoviruses

In this omics era, several sophisticated methods were utilized to detect tospoviruses. Exploiting viral properties such as morphology, transmission ability, serological property and nucleotide sequence identity is employed for tospovirus identification and characterization.

#### 1.6.1 Visual Observation

Tospoviruses produces wide range of symptoms on ornamental plants upon infection. But some peculiar symptoms such as chlorotic or necrotic lesions, ringspots with concentric rings, green island mosaic on leaves, bronzing, chlorosis, tip necrosis and wilting are commonly seen across the hosts irrespective of tospovirus species infected. As they are infecting extensive hosts, symptom expression varies according to the hosts and virus interaction (Mumford et al. 1996). It is not feasible to identify the virus species based on the symptomatology, and therefore the serological and molecular tools are extensively used in the tospovirus detection.

#### 1.6.2 Morphology

Use of electron microscope (EM) in the visualization of plant viruses was first demonstrated with tobacco mosaic virus (Kausche et al. 1939). EM has been extensively used to view the characteristic morphology of enveloped quasi-spherical particles of tospoviruses. Tospovirus-like particles of 90–120 nm in diameter were detected from the leaf extracts of chrysanthemum when negatively stained with 2% uranyl acetate (Duarte et al. 1995; Verhoeven et al. (1996). Gera et al. (1999) visualized INSV from the leaf-dip preparations prepared from leaf samples of *Anemone coronaria* plants through transmission electron microscopy (TEM). EM is used as a preliminary detection method of tospoviruses, and it does not satisfy their complete identification.

#### 1.6.3 Bioassay

Mechanical inoculations on indicator plants such as *Nicotiana* sp., *Vigna unguiculata* (cowpea), etc. are used for the generic detection of tospoviruses. They produce characteristic local or systemic symptoms on indicator hosts. Inoculation of TSWV from chrysanthemum produced local chlorotic, concentric rings that later turned to necrotic spots 6 days post inoculation (DPI) on cowpea and pig weed (Renukadevi et al. 2015). Though it does not lead to identification at species level, it can be used for subsequent identification by other techniques.

#### 1.6.4 Serological Methods

Serological method detects the presence of virus particle directly through the interaction between coat protein of virus (antigen) and the immunoproteins raised against the antigen in warm-blooded animals (antibody). Enzyme-linked immunosorbent assay (ELISA) is a very commonly used serological assay for the detection of tospovirus. Variants of ELISA such as double antibody sandwich [(DAS)-ELISA] and triple antibody sandwich [(TAS)-ELISA] are performed using antibody that has been raised against the specific tospovirus species. Based on the same principle, other methods such as dot immuno-binding assay, lateral flow assay, tissue immunobinding assay, etc. are being carried out. As there are five sero-groups prevalent among the tospoviruses, cross reaction of particular antibody with other tospovirus members will occur commonly in the serological assays. Through this method, presence or absence of tospovirus can be ascertained, but the species level identity cannot be confirmed. To confirm the identity of tospovirus species, application of molecular method will be of more helpful. Shahraeen et al. (2002) utilized the TAS-ELISA assay to detect INSV among the various ornamental plants grown in commercial nurseries and field in Iran. Renukadevi et al. (2015) used DAS-ELISA technique to detect the TSWV in chrysanthemum.

#### 1.6.5 DNA Technology

The development of PCR and nucleic acid-based assays such as RT-PCR and quantitative (q) RT-PCR provides a simple, quick and precise means for tospovirus detection. According to the ICTV nomenclature, species are defined on the coat protein (N) sequence with amino acid identity of >90% than that of previously described tospovirus species called as new species (Plyusnin et al. 2011). Therefore, sequencing of tospovirus genome is indispensable for its proper identification. For generic identification, conventional RT-PCR assays using universal degenerate primers are used. Several utilized degenerate primers for the preliminary detection of tospoviruses in ornamental plants (Chu et al. 2001; Renukadevi et al. 2015; Huang et al. 2018; Basavaraj et al. 2020). Naidu et al. (2007) documented the natural infection of impatiens necrotic spot virus on Monarda didyma in Washington State by molecular method. RT-PCR assay using species-specific primer followed by sequencing of amplified fragments allows confirmation of tospovirus at species level. Uga and Tsuda (2005) developed multiplex RT-PCR for the simultaneous detection of five tospoviruses (WSMoV, TSWV, INSV, MNSV and IYSV) in ornamental crops. Charoenvilaisiri et al. (2014) standardized a multiplex RT-PCR-ELISA to detect and differentiate four tospovirus species found in Thailand, viz. CaCV, MYSV, TNRV and WSMoV. Targeted viral genomes amplified and labelled with digoxigenin (DIG) in a single RT-PCR reaction followed by distinguishing four species by parallel hybridizations with species-specific biotinylated probes in streptavidin-coated microtiter wells were used in ELISA detection. Assay sensitivity

was 10 to 1000-fold higher than conventional RT-PCR. Real-time RT-PCR assay based on TaqMan probe has been demonstrated for the detection and quantification of TSWV in infected plant samples. Assay detected TSWV in 500 fg of total RNA and sensitized TSWV to tenfold higher than ethidium bromide staining (Roberts et al. 2000).

#### 1.7 Molecular Biology of Tospoviruses

Tospoviruses are having a tripartite genome of ssRNA molecules comprising a total of five open reading frames (ORFs) enveloped inside the quasi-spherical particles with a diameter of 80-120 nm. The three single-stranded RNA (ssRNA) segments are denoted as S, M and L RNAs with 2.9 kb, 5 kb and 8.9 kb in size, respectively (Fig. 1.3). The L RNA is of negative polarity and encodes a putative 331-kDa RNA polymerase that may be associated with the ribonucleoprotein complex. The other two genomic RNAs use an ambisense coding strategy. The M RNA encodes a nonstructural (NSm) protein (34 kDa) in the viral (v) sense and a protein (155 kDa) to serve as the precursor for the Gn (95 kDa) and Gc (58 kDa) glycoproteins in the viral complementary (vc). The NSm protein may be involved in cell-to-cell movement of nonenveloped ribonucleocapsid structures. Tubular structures are specifically formed when the NSm gene is expressed in both plant and insect cell systems. The Gn and Gc glycoproteins are believed to form spikes on the viral envelope which help in virus acquisition and transmission by thrips. The S RNA encodes in the 'v' sense a 52-kDa nonstructural (NSs) protein that forms filamentous inclusion bodies, and in the 'vc' sense, a 29-kDa nucleocapsid protein (NP) encapsidates viral RNAs (Adkins et al. 1996; de Haan et al. 1990, 1992; Kormelink et al. 1992; van Poelwijk et al. 1997; Chu and Yeh 1998; Law et al. 1992; Satyanarayana et al. 1996; Whitfield et al. 2005). RNA molecules are generally seen as pseudocircular because of highly conserved and complementary first eight nucleotides (5'-AGAGCAAU and 3'-UCUGCUUA) at the termini of all RNA segments. These sequences act as promotor region for viral RNA replication and protein transcription (Turina et al. 2016; Kormelink 2011).



Fig. 1.3 Genome organization of tospovirus

#### 1.8 Host-Pathogen Interaction

Host-pathogen interaction is a multifaceted process, mediated by the pathogen- and host-derived molecules which mainly include proteins, sugars and lipopolysaccharides. Plant viruses move cell to cell via plasmodesmata through the vascular system. Plant viruses encoding nonstructural proteins such as movement protein are specifically required for movement within their hosts. Movement proteins can able to increase the plasmodesmal size exclusion limit (SEL) to facilitate cell-to-cell movement of virions (Boevink and Oparka 2005). In the tospovirus infection cycle, only very few host factors involved were studied at various stages in plants. Interaction between DNA-J protein of host with NSM (movement protein) of TSWV suggested the movement of infectious ribonucleoproteins possible involvement of a mechanism that requires Hsp-70 (Soellick et al. 2000; von Bargen et al. 2001). Also, another chaperone protein (At-4/1) which shares homology with  $\alpha$ -helical domains of ankyrin-, myosin- and kinesin-like proteins showed to be involved in both intra- and extracellular movement of TSWV virions (Paape et al. 2006; Morozov et al. 2014). Investigation on changes in the jasmonic acid (JA) and salicylic acid (SA) pathways upon TSWV inoculation through thrips revealed upregulation in the SA pathway in the infected plants than the healthy plants. Although feeding of thrips normally induces JA pathway, virus infection counteracts and suppresses the JA (antiherbivore response) pathway. These facilitate the thrips to get more attracted towards the infected plants rather than the healthy plants. This interaction supports the dissemination of the TSWV by thrips (Maris et al. 2004; Abe et al. 2012; Turina et al. 2016). Though few of the factors involved in tospovirushost interaction were studied in model plants such tobacco and Arabidopsis, no factors were understood with the interaction of tospovirus-ornamental plants.

#### 1.9 Management of Tospoviruses in Ornamental Crops

Early detection gives rise to early implementation of effective management strategies that can limit crop damage and economic loss. Several management strategies were demonstrated under open field and controlled conditions grown crops against tospoviruses across agricultural and horticultural crops. But information on tospovirus management in ornamental crops are limited. In general, management of tospoviruses can be achieved through cultural, biological, chemical, host plant resistance and biotechnological interventions. These interventions may target tospovirus as well as its thrips vector. Monitoring crops for thrips using yellow or blue sticky traps, checking every 7 days for thrips in foliage or flowers by tapping over a white surface or blowing lightly into buds or open flowers to draw thrips out for detection is helpful in controlling the thrips population. Proper maintenance of greenhouses plays an important role in the management strategy of tospoviruses in ornamental crops. All plant materials should be inspected before it is brought into the greenhouse and preferably kept in a separate section for a week or more before incorporating the new material into the production area, to assure that thrips are not

introduced into the main production area. The greenhouse should be kept free of weeds inside and outside since both thrips and tospoviruses are easily harboured on greenhouse weed plants. Virus indicator plants can also be employed in the greenhouse. Growing crops with proper isolation distances from the TSWV source of inoculum provided there are no weed plant playing reservoir host in between will contain the disease spread (Coutts et al. 2004). Several cultural practices demonstrated to slow down the spread of tospoviruses are periodical removal of virus sources, avoidance of side-by-side and continuous plantings, planting of non-host barrier crops, growing under thrips proof net, mulching with black-silver polythene sheet, installation of yellow sticky traps, etc. under open field conditions (Jones 2004; Priyanka et al. 2019).

Use of insecticides in the management of thrips vector is the primary strategy for management of tospoviruses. Generally, the insecticides used in the thrips management can be grouped as broad-spectrum insecticides (pyrethroids, neonicotinoids, organophosphates and carbamates) and narrow-spectrum insecticides (pyridalyl and lufenuron) (Mouden et al. 2017). Regular use of broad-spectrum insecticides invites resistance development in thrips and detrimental to the natural enemies. Spinosad and spinetoram are being extensively used to manage thrips efficiently due to its tendency to be compatible with natural enemies (Gao et al. 2012; Li et al. 2016). However, the use of insecticides will ultimately lead the development of resistance in insect vectors. Therefore, it is necessary to use the insecticides accurate, precise and safe (He et al. 2020).

Biological means of tospovirus management can be achieved by targeting either the virus or the insect vector. Yoon et al. (2020) developed a novel approach in the management of TSWV in chrysanthemum by combining application of soildwelling predatory mite (Stratiolaelaps scimitus) @ 60/m<sup>2</sup> placed in soil along with foliar spray of four essential oil mixtures (cinnamon oil, cinnamon bark oil, oregano oil and thyme oil). These treatments were toxic to eggs, larvae and adults of F. occidentalis. Treatment has reduced the incidence of TSWV to 0.93% in comparison to chemical insecticides (32.05%) and untreated controls (84.85%) in chrysanthemum. Amblyseius cucumeris, another predatory mite, gave excellent control of F. occidentalis on Impatiens and reduced the spread and severity of TSWV under glasshouse conditions in the UK (Bennison et al. 2002). Another approach in management of tospoviruses in ornamental crops is development of resistant varieties. Some of the resistance genes such as Tsw and Sw-5 were identified in solanaceous vegetables and are deploying resistance against TSWV (Turina et al. 2016). Unfortunately, resistance source identification against tospoviruses among the ornamental crops was in infant stage. Biotechnological interventions such RNAi, clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) and exogenous application of dsRNA to mediate resistance against tospovirus have been demonstrated by several workers (Tabein et al. 2020; Carbonell et al. 2019). Though these findings were demonstrated in other crop plants, these tospoviruses are also known to infect ornamental crops too. Therefore, these biotechnological tools are inviting major attention among the researchers to manage the tospoviruses.

#### 1.10 Conclusion

The literature clearly indicates the prospective of tospovirus to cause damage to the ornamental crops worldwide. Though there are several reports for the infection of tospoviruses on ornamental crops, the coexistence of tospovirus with other viruses in combinations has to be studied in detail. When the vector is considered, most of the research findings of other crops are being considered for ornamental crops. However, it is essential to explore the involvement and distribution of thrips species in different ornamental crops and tospovirus interactions. Further, understanding of genome diversity, biology, geographical distribution and molecular and serological diagnostics of different tospoviruses in ornamental crops can contribute for the development of effective management strategies to prevent further spread of tospoviruses.

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