

EPPO Datasheet: *Nepovirus nicotianae*

Last updated: 2022-02-24

IDENTITY

Preferred name: *Nepovirus nicotianae*

Taxonomic position: Viruses and viroids: Riboviria: Orthornavirae: Pisuviricota: Pisoniviricetes: Picornavirales: Secoviridae

Other scientific names: *Nicotiana virus 12*, *TRSV*, *Tobacco ringspot nepovirus*, *Tobacco ringspot virus*

Common names: bud blight of soybean, necrosis of anemone, necrotic ring spot of blueberry, pemberton disease of blueberry, ring spot of soybean, ring spot of tobacco

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EPPO Categorization: A2 list

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EU Categorization: RNQP ((EU) 2019/2072 Annex IV)

EPPO Code: TRSV00



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Notes on taxonomy and nomenclature

Tobacco ringspot virus (TRSV) is the type species of the genus *Nepovirus* (Stace-Smith, 1985). Within the genus *Nepovirus*, TRSV belongs to subgroup A (Thompson *et al.*, 2017).

HOSTS

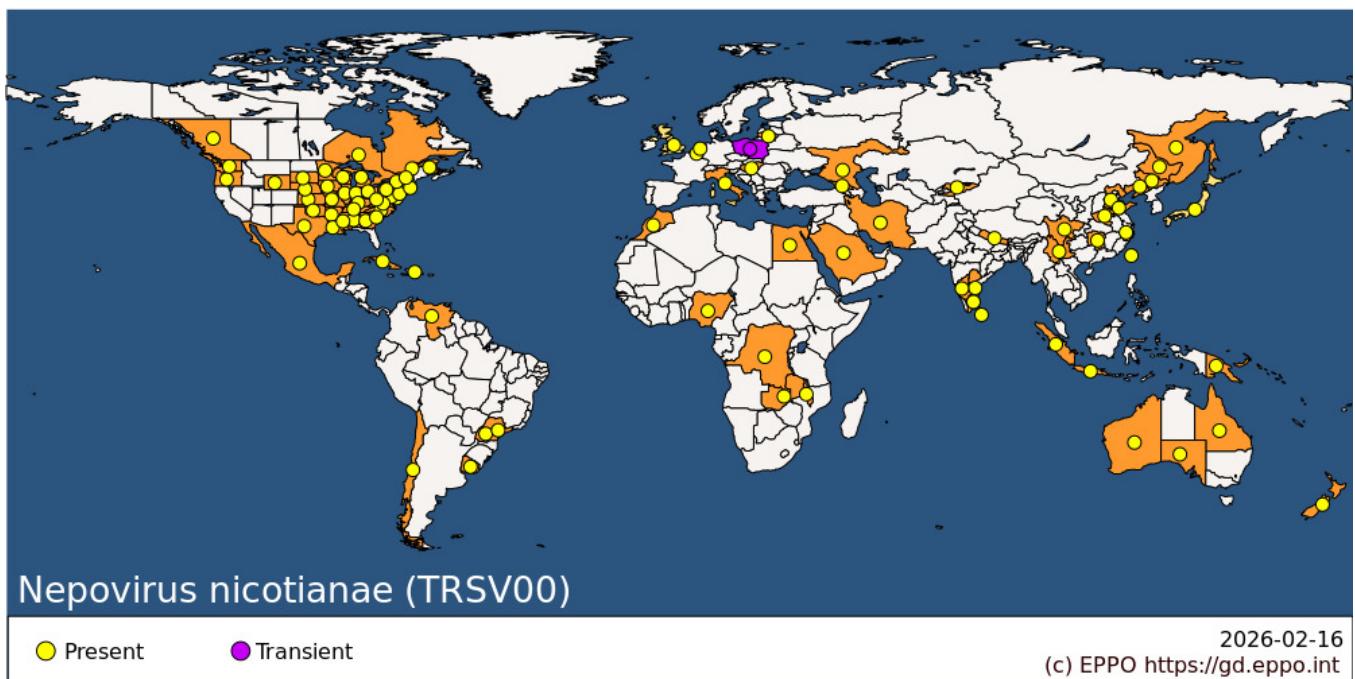
TRSV has a wide host range and has been reported in more than 100 different herbaceous and woody plant species. TRSV can cause serious diseases in blueberry (*Vaccinium corymbosum*) (Mitra *et al.*, 2021), grapevine (*Vitis vinifera*) (Walker *et al.*, 2015) and soybean (*Glycine max*) (Nyvall, 1989). Therefore, these species are considered important hosts. To a lesser extent, disease symptoms have been reported in tobacco (*Nicotiana tabacum*) (Wingard, 1928) and several species within the family *Cucurbitaceae* (Abdalla *et al.*, 2012). Infections in ornamentals and weeds often remain symptomless (EPPO, 2018b; Powell *et al.*, 1984).

Host list: *Abutilon theophrasti*, *Achillea millefolium*, *Aeonium sp.*, *Ajuga reptans*, *Althaea sp.*, *Amaranthus cruentus*, *Amaranthus hybridus*, *Amaranthus palmeri*, *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Anemone coronaria*, *Apocynum cannabinum*, *Arctium lappa*, *Argyranthemum frutescens*, *Armoracia rusticana*, *Arum orientale*, *Asimina triloba*, *Astilbe rubra*, *Bacopa sp.*, *Bacopa*, *Begonia semperflorens hybrids*, *Boerhavia coccinea*, *Boerhavia erecta*, *Brassica sp.*, *Capsicum annuum*, *Carica papaya*, *Celosia*, *Chenopodium murale*, *Chenopodium album*, *Chenopodium giganteum*, *Chrysanthemum x morifolium*, *Cichorium intybus*, *Citrullus lanatus*, *Clerodendrum thomsoniae*, *Colchicum sp.*, *Coleus sp.*, *Cornus florida*, *Cornus racemosa*, *Cornus sericea*, *Crataegus sp.*, *Crepis tectorum*, *Crocus sp.*, *Crotalaria spectabilis*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita pepo*, *Dahlia sp.*, *Daphne odora*, *Datura metel*, *Datura stramonium*, *Daucus carota*, *Echinacea purpurea*, *Echinochloa crus-galli*, *Elymus repens*, *Equisetum arvense*, *Erigeron annuus*, *Erigeron canadensis*, *Erodium cicutarium*, *Eupatorium capillifolium*, *Eupatorium purpureum*, *Forsythia ovata*, *Fraxinus americana*, *Galinsoga parviflora*, *Galinsoga quadriradiata*, *Gerbera sp.*, *Gladiolus grandiflorus*, *Glycine max*, *Gomphrena globosa*, *Gossypium hirsutum*, *Helenium amarum*, *Helianthus annuus*, *Hemerocallis sp.*, *Hibiscus cannabinus*, *Hyacinthus sp.*, *Hydrangea macrophylla*, *Hydrangea paniculata*, *Hydrangea*, *Impatiens walleriana*, *Iris ensata*, *Iris sibirica*, *Iris virginica*, *Iris x hollandica*, *Jaltomata procumbens*, *Lactuca serriola*, *Lamprocapnos spectabilis*, *Lepidium densiflorum*, *Lepidium didymum*, *Lilium sp.*, *Lilium*, *Lobelia*, *Lolium pratense*, *Lotus corniculatus*, *Lupinus polyphyllus*, *Lysimachia nummularia*, *Malus domestica*, *Malvastrum coromandelianum*, *Melilotus albus*, *Mentha x gentilis*, *Narcissus sp.*, *Nicotiana tabacum*, *Osmunda cinnamomea*, *Pelargonium x hortorum*, *Pelargonium zonale*, *Pelargonium*, *Persicaria lapathifolia*, *Petunia grandiflora hybrids*, *Phaseolus vulgaris*, *Phlox subulata*, *Physalis floridana*, *Plantago lanceolata*, *Polygonum aviculare*, *Populus tremuloides*, *Portulaca oleracea*, *Portulaca*, *Potentilla sp.*, *Prunus armeniaca*, *Prunus avium*, *Pueraria montana*, *Rosa sp.*, *Rubus allegheniensis*, *Rubus argutus*, *Rubus flagellaris*, *Rubus fruticosus*

, *Rubus occidentalis*, *Rubus* sp., *Rumex crispus*, *Rumex obtusifolius*, *Salix nigra*, *Salvia rosmarinus*, *Sambucus* sp., *Schefflera* sp., *Senecio vulgaris*, *Sinapis arvensis*, *Solanum diphylum*, *Solanum elaeagnifolium*, *Solanum lycopersicum*, *Solanum melongena*, *Solanum nigrum*, *Sonchus arvensis*, *Sonchus oleraceus*, *Sophora microphylla*, *Stellaria* sp., *Tagetes patula*, *Tanacetum vulgare*, *Taraxacum officinale*, *Trifolium pratense*, *Tulipa* sp., *Ulmus americana*, *Vaccinium corymbosum*, *Vicia* sp., *Vigna radiata*, *Vigna unguiculata* subsp. *unguiculata*, *Vitis aestivalis*, *Vitis vinifera*, *Xanthium strumarium*, *Zamia furfuracea*

GEOGRAPHICAL DISTRIBUTION

Tobacco ringspot disease was first observed in the state of Virginia, USA, in 1917. TRSV was described as the causal agent of the disease in 1927 (Wingard, 1928). From its origin in central and eastern North America, TRSV has spread worldwide. Nowadays, there are scattered records from many countries, most of which are associated with material originating from North America. Since TRSV infections may remain symptomless in many hosts, there is uncertainty concerning its current distribution within the EPPO region (e.g. in the EU; EFSA PLH, 2019).



EPPO Region: Belgium, Georgia, Hungary, Italy (mainland), Kyrgyzstan, Lithuania, Morocco, Netherlands, Poland, Russian Federation (Far East, Southern Russia), United Kingdom

Africa: Congo, The Democratic Republic of the, Egypt, Malawi, Morocco, Nigeria, Zambia

Asia: China (Hebei, Heilongjiang, Henan, Hunan, Jilin, Liaoning, Shandong, Sichuan, Yunnan, Zhejiang), India (Andhra Pradesh, Karnataka, Tamil Nadu), Indonesia (Java, Sumatra), Iran, Islamic Republic of, Japan, Kyrgyzstan, Nepal, Saudi Arabia, Sri Lanka, Taiwan

North America: Canada (British Columbia, New Brunswick, Ontario, Québec), Mexico, United States of America (Alabama, Arkansas, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming)

Central America and Caribbean: Cuba, Dominican Republic

South America: Brazil (Parana, Sao Paulo), Chile, Uruguay, Venezuela

Oceania: Australia (Queensland, South Australia, Western Australia), New Zealand, Papua New Guinea

BIOLOGY

TRSV generally occurs in all plant parts of its host species, including seeds.

Local spread is mainly attributed to transmission by its nematode vectors and through seeds of weed hosts. In addition, TRSV can be spread by machinery by movement of soils containing viruliferous nematodes and/or infected seeds. In its native range, TRSV is transmitted by nematodes from the species complex *Xiphinema americanum* *sensu lato*, of which *X. rivesi* is present in the EPPO region (Brown *et al.*, 1995; EFSA PLH, 2018). Although experimental transmission by a European population of *X. rivesi* was shown, this nematode species has never been reported as being associated with the spread of TRSV under field conditions in the EPPO region (EFSA PLH, 2019). Under experimental conditions, the virus is acquired within 24 h and transmitted by both adult and larval stages (Stace-Smith, 1985). The virus can persist inside the vector for a long period. *X. americanum* was shown to be able to transmit TRSV after an 11 months period during which it did not have access to plants (Brown *et al.*, 1995; Taylor & Robertson, 1975). Since the virus can survive in the soil in its vector as well as in seeds of its (weed) hosts, TRSV can be transferred to newly-grown plants and crops after a longer period of time in the absence of host plants.

TRSV can spread over longer distances via seeds, by grafting of woody hosts, by vegetative propagation of herbaceous hosts, and via adherent soil containing viruliferous nematode vectors and/or infected seeds. Infected seeds and (grafted) plants can establish new infections when they are introduced in soils where the vector is present. Seed transmission of TRSV has been reported in multiple hosts including *Cucurbitaceae* and soybean (Sastry, 2013) but probably occurs in more hosts than reported (Stace-Smith, 1985). For soybean, seed-transmission rates up to 100% have been reported under experimental conditions (Sastry, 2013). Weed species for which seed transmission has been reported, include *Amaranthus hybridus*, *Gomphrena globosa*, *Senecio vulgaris* and *Taraxacum officinale* (Harrison & Murant, 1996; Sastry, 2013; Stace-Smith, 1985).

Pollen transmission of TRSV has been reported but data are limited: The virus has been found to be transmitted through pollen in *Nicotiana* spp. (Zadeh & Foster, 2004). In addition, a citation by Card *et al.* (2007) reports pollen transmission for *Cucumis sativus*, *Glycine max*, *Solanum* spp. and *Vaccinium* spp., although scientific evidence is lacking. In soybean infected pollen have been shown to impair fertilisation, thereby eliminating virus transmission (Yang & Hamilton, 1974).

Transmission of TRSV by arthropods has been extensively studied in soybean, since the observed spread in the field was more rapid than could be expected from a nematode vector. Transmission of TRSV has been reported for aphids, beetles, grasshoppers, thrips species and spider mites. Generally, the transmission efficiency of these vectors was low and their significance unclear (Hill & Whitham, 2014). Additionally, TRSV has been reported to systemically spread and propagate within European honeybees *Apis mellifera* (Li *et al.*, 2014). Since these results have been debated, the relevance of this insect species as a vector remains unclear (Cornman, 2017; Miller *et al.*, 2014).

Besides natural spread, TRSV can also be transmitted by grafting of woody hosts, by vegetative propagation of herbaceous hosts, and via (adherent) soil containing viruliferous nematode vectors or infected seeds.

DETECTION AND IDENTIFICATION

Symptoms

The type and severity of symptoms caused by TRSV depend on the virus isolate, host species and cultivar, as well as the environmental conditions. TRSV infections may be symptomless or produce symptoms ranging from mild to severe. A concise description of the most prominent symptoms in its major hosts is given below. Note, that especially in woody hosts, similar symptoms can be caused by tomato ringspot virus and other nepoviruses, and often will result from mixed infections. Infections in most ornamentals and weeds generally remain symptomless (Martin *et al.*, 2012; Stace-Smith, 1985).

On blueberries

Infected plants show stunting, twig dieback and chlorotic or necrotic spots and rings or line patterns on the leaves. Other leaf symptoms include red blotches, mosaic and deformation. Fruits may remain small and poorly ripened (Martin *et al.*, 2012; Mitra *et al.*, 2021).

On grapevines

Infected plants show decline. New growth is weak and sparse, internodes are shortened, leaves are small, mottled and distorted, and plants are stunted. A limited number of grapes are produced, which develop unevenly and are deformed. In colder climates, buds can become more sensitive to frost (EFSA PLH, 2019; Rowhani *et al.*, 2017; Walker *et al.*, 2015).

On soybeans

Infections in soybean are indicated by its disease name ‘bud blight’. Symptoms are most severe when plants are infected at a young stage (less than 5 weeks old or from seed). Early infection results in severely stunted plants due to shortened internodes and fewer nodes. When plants become infected before bloom, the characteristic symptom of bud blight occurs, i.e. curving of the terminal bud to form a crook, called ‘shepherds crook’. Other buds become brown, necrotic and brittle and the plants produce little to no seed. Brown streaks can be seen in the pith of stems and branches, and occasionally on petioles and leaf veins. Infection after flowering results in poorly filled pods that often drop early. Plants infected by TRSV usually remain green when uninfected plants turn brown, e.g. after a killing frost (Hill & Whitham, 2014; Nyvall, 1989).

On cucurbits

Infected plants are malformed and show yellowing and mottling on the leaves. Fruit set is decreased, and fruits are deformed and smaller in size. In mature plants, leaves become tattered and necrotic while internodes are shortened. This results in a compact plant with brittle leaves and stems (Abdalla *et al.*, 2012; Murant *et al.*, 1996). Recently, multiple cultivars of *Cucurbita pepo* were found to be asymptotically infected. Since these melons originated from multiple states in the USA and were infected by different isolates of TRSV, the virus may be more widespread in melons than is currently known (Tabara *et al.*, 2021).

On tobacco

Infected plants show concentric rings, chlorotic or necrotic ringspot and line patterns on the leaves. Spots located in interveinal regions are circular and the lines are necrotic. Spots centered near larger veins are irregular and follow the veins and their branches. Infection results in stunting of the plant (Murant *et al.*, 1996).

Morphology

Virus particles are isometric and about 28 nm in diameter. The protein shell is formed by 60 copies of a single coat protein of approximately 56 kDa, which encapsidates the genome of TRSV that consists of two positive-sense, single-stranded RNA molecules (RNA1 and RNA2). Virus particles sediment in sucrose density gradients as three components, i.e. empty protein shells and two nucleoproteins containing different amounts of RNA. One of the nucleoproteins contains a single copy of RNA2 while the other nucleoprotein can either contain a single copy of RNA1 or two copies of RNA2 (Stace-Smith, 1985). RNA1 is around 7.5 kb and encodes a polyprotein of 255.5 kDa. This polyprotein is subsequently cleaved by the viral protease into five mature proteins: proteinase cofactor (P1A), helicase (Hel), genome linked protein (VPg), protease (Pro) and the RNA-dependent RNA polymerase (Pol). RNA2 is around 3.9 kb and encodes for a polyprotein of 122.2 kDa which is cleaved into three mature proteins: P2A, which is involved in RNA replication at the N-terminus, the movement protein (Mp) and the coat protein (Cp) (Chandrasekar & Johnson, 1998; Murant *et al.*, 1996; Zhao *et al.*, 2015).

Detection and inspection methods

Field inspections can be used to detect TRSV infections in blueberries, grapevines and soybeans, since these infections generally show clear symptoms. A procedure for inspection of places of production of *Vitis* plants for planting is provided in Standard PM 3/85 (EPPO, 2018a). In addition, the virus can be detected in symptomatic and symptomless hosts by mechanical inoculation of test plants. However, since TRSV symptoms in both symptomatic hosts and test plants cannot be distinguished from those caused by other (nepo-) viruses, serological and/or molecular tests are essential for its identification. Therefore, these tests are also often used as a first test. Several antisera are available as well as multiple conventional RT-PCR tests and real-time RT-PCR tests. Further details on tests for TRSV are described in PM 7/2 *Tobacco ringspot virus* (EPPO, 2017).

PATHWAYS FOR MOVEMENT

Movement of infected vegetatively propagated plants, grafts and seeds form the most efficient ways of spreading TRSV over longer distances. In this respect the trade of symptomless infected hosts, such as many ornamentals and tolerant genotypes of blueberries and grapevines, pose a high risk of spreading the virus. Most records of TRSV outside North America are associated with the movement of plant material from this region. In addition, TRSV and its vectors may also be introduced in new areas via adherent soil or growing media of imported plants from infested areas (section Biology).

PEST SIGNIFICANCE

Economic impact

The economic impact of TRSV consists of decline and/or yield losses in its major hosts. The most serious disease caused by TRSV is bud blight in soybean, which can result in yield losses of 25-100%. Although frequently detected in Canada and the USA in the past (Tu, 1986), the virus is not generally widespread in soybean (Hill & Whitham, 2014). On blueberries, TRSV causes blueberry necrotic ringspot disease in susceptible cultivars. Infected bushes show a slow and steady decline, which may eventually lead to plant death. The disease has been an important factor in blueberry production in the USA since the 1950s and losses up to a few million USD a year have been reported (Chodorska *et al.*, 2012; Martin *et al.*, 2012; Mitra *et al.*, 2021). On grapevines, TRSV can cause decline, especially in *Vitis vinifera* (EFSA PLH, 2019). Therefore, the virus is considered a significant risk to grapevines in the EPPO region. In the USA it is considered of minor economic importance because *Vitis vinifera* is relatively rarely grown in the affected region. Instead, mostly interspecific hybrids are grown that show some resistance or tolerance to TRSV. Additionally, rootstocks are used that are more tolerant or resistant to *X. americanum sensu lato*, thus preventing infection by TRSV (Maliogka *et al.*, 2015; Oliver & Fuchs, 2011; Rowhani *et al.*, 2017). The impact of TRSV on cucurbits, fruit crops other than blueberries and grapevines, ornamentals and tobacco is of minor importance.

Control

To control TRSV in vegetatively propagated crops, mother plants should be tested in order to establish virus-free nuclear stocks, from which plants or grafts can be propagated and distributed through a certification scheme. Details on certification schemes are described in PM 4/18 *Pathogen-tested material of Vaccinium* (EPPO, 1997), PM 4/8 *Pathogen-tested material of grapevine varieties and rootstocks* (EPPO, 2008) and other Standards with the PM4 series. Since TRSV is covered by North American fruit certification schemes, similar requirements apply to other fruit crops. For crops propagated via seeds, such as soybeans, the absence of the virus should be ensured by testing. Furthermore, in areas where TRSV and vectors are present, the use of cultivars and/or rootstocks that have resistance to the virus and/or its vectors can be used (Maliogka *et al.*, 2015; Oliver & Fuchs, 2011; Rowhani *et al.*, 2017).

In areas, such as the EPPO region, where vector transmission is not known to occur, establishment of TRSV can be prevented by removal and destruction of infected plants. The existence of many symptomless hosts, however, may hamper detection and full elimination of the virus. In areas where vectors are present, such as North America, it is recommended to determine whether vector nematodes are present in a field before growing host species (Maliogka *et al.*, 2015; Martin *et al.*, 2012; Rowhani *et al.*, 2017). If vector nematodes are present, treatment with nematicides can be applied.

Phytosanitary risk

In the EPPO region, so far blueberries and grapevines have been considered the main hosts at risk. In addition, soybeans may be at risk given the increase in cultivation in the southeastern part of the region (Krön & Bittner, 2015). The presence of TRSV in vegetatively propagated ornamentals reported from many EPPO countries does not pose a risk to these major hosts as long as the known nematode vectors remain absent, provided that other nematode species present in the EPPO region, will not be found to be vectors.

EFSA PLH (2019) considered that no eco-climatic constraints exist for TRSV, except for those affecting the host.

Therefore, it is expected that TRSV would be able to establish wherever host plants are cultivated in the EPPO region, but with higher impacts if a vector is present (which would make eradication more challenging and diseases in blueberries and grapevines difficult to control).

PHYTOSANITARY MEASURES

Appropriate phytosanitary measures to import host plants of TRSV for planting into the EPPO region could require that these plants are produced in a pest-free area or in a pest-free place/site of production established according to EPPO Standard PM 5/8 *Guidelines on the phytosanitary measure 'Plants grown under physical isolation'* (EPPO, 2021). The physical isolation should allow prevention of both the virus and the vector from entering the place/site of production.

Planting material of *Vaccinium* and *Vitis* from North America could be derived from certification schemes guaranteeing freedom from TRSV. EPPO recommends certification schemes for blueberries PM 4/18 (EPPO, 1997) and grapevines PM 4/8 (EPPO, 2008). Additionally, soil tests to guarantee the absence of nematode vectors can be applied to imported plant material with adherent soil or growing media, or on soil used for planting. Details are described in EPPO Standard PM 4/35 *Soil test for virus-vector nematodes in the framework of EPPO standard PM 4 Schemes for the production of healthy plants for planting of fruit crops, grapevine, Populus and Salix* (EPPO, 2009).

For soybean, in which TRSV has been reported at least from Australia, Brazil, Canada, China, Cuba, India, Iran, Nepal, Russia and the USA (Golnaraghi *et al.*, 2004; Hill & Whitham, 2014; Khanal & Poudel, 2018), seed testing can be applied to ensure the absence of this virus in seeds for cultivation.

In regard to ornamental crops, which concern the majority of TRSV findings in the EPPO region, the lack of impact raises the question whether certification schemes should cover this risk. Certification schemes are already available for numerous herbaceous ornamental crops and are described in PM 4/34 *Production of pathogen-tested herbaceous ornamentals* (EPPO, 2008). This Standard could be expanded with the ornamentals in which TRSV has been found in the EPPO region, including *Aeonium*, *Ajuga reptans*, *Bacopa*, *Celosia*, *Coleus*, *Hemerocallis*, *Impatiens*, *Iris*, *Pelargonium*, *Phlox* and *Portulaca*. Additionally, testing these ornamentals on a more systematic basis when moved within and between countries would help to trace the origins of (symptomless) infections.

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