

EPPO Datasheet: *Orthotospovirus chrysanthinecrocaulis*

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IDENTITY

Preferred name: *Orthotospovirus chrysanthinecrocaulis*

Taxonomic position: Viruses and viroids: Riboviria: Orthornavirae: Negarnaviricota: Polyploviricotina: Bunyaviricetes: Elliovirales: Tospoviridae

Other scientific names: *CSNV*, *Chrysanthemum stem necrosis orthotospovirus*, *Chrysanthemum stem necrosis tospovirus*, *Chrysanthemum stem necrosis virus*

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EPPO Categorization: A1 list

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EU Categorization: A1 Quarantine pest (Annex II A)

EPPO Code: CSNV00



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Notes on taxonomy and nomenclature

Chrysanthemum stem necrosis orthotospovirus (CSNV) is assigned to the American clade 1 of tospoviruses (Hassani Mehraban *et al.*, 2005).

HOSTS

CSNV occurs on florists' chrysanthemum (*Dendranthema × grandiflorum*), lisianthus (*Eustoma russellianum*) and tomato (*Solanum lycopersicum*). CSNV has been isolated from the chrysanthemum cultivars 'Cocarde', 'Fiji', 'Majoor Bosshardt', 'Reagan', 'Spider', 'Tiger', 'Tigerrag' and 'Vyking' (Verhoeven *et al.*, 1996), 'Calabria' (Mumford *et al.*, 2003), 'Seinotama' (Matsuura *et al.*, 2007), 'Ludo', 'Jorca Pink', 'Mirage Yellow', 'Miral White' (De Jonghe *et al.*, 2013), 'Jimba' (Matsuura *et al.*, 2007, Yoon *et al.*, 2017). There is a record of CSNV in one sample of *Gerbera* (Ravnikar *et al.*, 2003) and it has been reported on aster (*Callistephus chinensis*) (Momonoi *et al.*, 2011) and *Capsicum annuum* (Carmo *et al.*, 2023).

In artificial inoculation studies, CSNV induced symptoms on a large number of test plants (Duarte *et al.*, 1995; Verhoeven *et al.*, 1996; Bezerra *et al.*, 1999, Momonoi *et al.*, 2011, Yoon *et al.*, 2017). Other artificial hosts of CSNV identified in experiments are aubergine, cucumber and lettuce, common bean, cowpea, pea and courgette (Bezerra *et al.*, 1999), capsicum (Verhoeven *et al.*, 1996, Momonoi *et al.*, 2011), tobacco (Duarte *et al.*, 1995; Verhoeven *et al.*, 1996), spinach and physalis (Momonoi *et al.*, 2011).

Host list: *Begonia tuberhybrida* hybrids, *Callistephus chinensis*, *Capsicum annuum*, *Chrysanthemum x morifolium*, *Cyclamen persicum*, *Eustoma russellianum*, *Gerbera* sp., *Gomphrena globosa*, *Pericallis cruenta*, *Solanum lycopersicum*, *Zinnia elegans*

GEOGRAPHICAL DISTRIBUTION

CSNV was found in tomato fields in the states of Minas Gerais and Sao Paulo in Brazil in the 1990s (Nagata *et al.*, 1998). In November 2012, plants of *Eustoma russellianum* (= *Eustoma grandiflorum*) showing necrotic spots on leaves and stems, followed by a systemic necrosis were found in a commercial glasshouse at Atibaia (Sao Paulo State). Infection was caused by CSNV (Duarte *et al.*, 2014).

Necrotic streaks on stems, leaf distortions, chlorotic and necrotic spots and rings on leaves were observed on *Dendranthema × grandiflorum* cultivars 'Jimba' and 'Seinotama' in August 2006 in Japan. The disease was observed on the premises of one grower in Hiroshima Prefecture (Honsu) and its incidence reached 70%. The

presence of CSNV was confirmed by laboratory tests (serology, PCR) and pathogenicity tests (Matsuura *et al.*, 2007).

Symptoms of stem necrosis, chlorotic and necrotic ring leaf spots, and leaf distortions were observed on chrysanthemum plants cv. 'Jimba' in a greenhouse in Changwon (Republic of Korea) in September 2013. The presence of CSNV was confirmed by laboratory analysis (electron microscopy, inoculation to indicators, DAS-ELISA, RT-PCR, sequencing) (Yoon *et al.*, 2017).

CSNV was detected in Iran in 2008 in samples from the Mashhad region, Khorasan-e-razavi province (Jafarpour *et al.*, 2010).

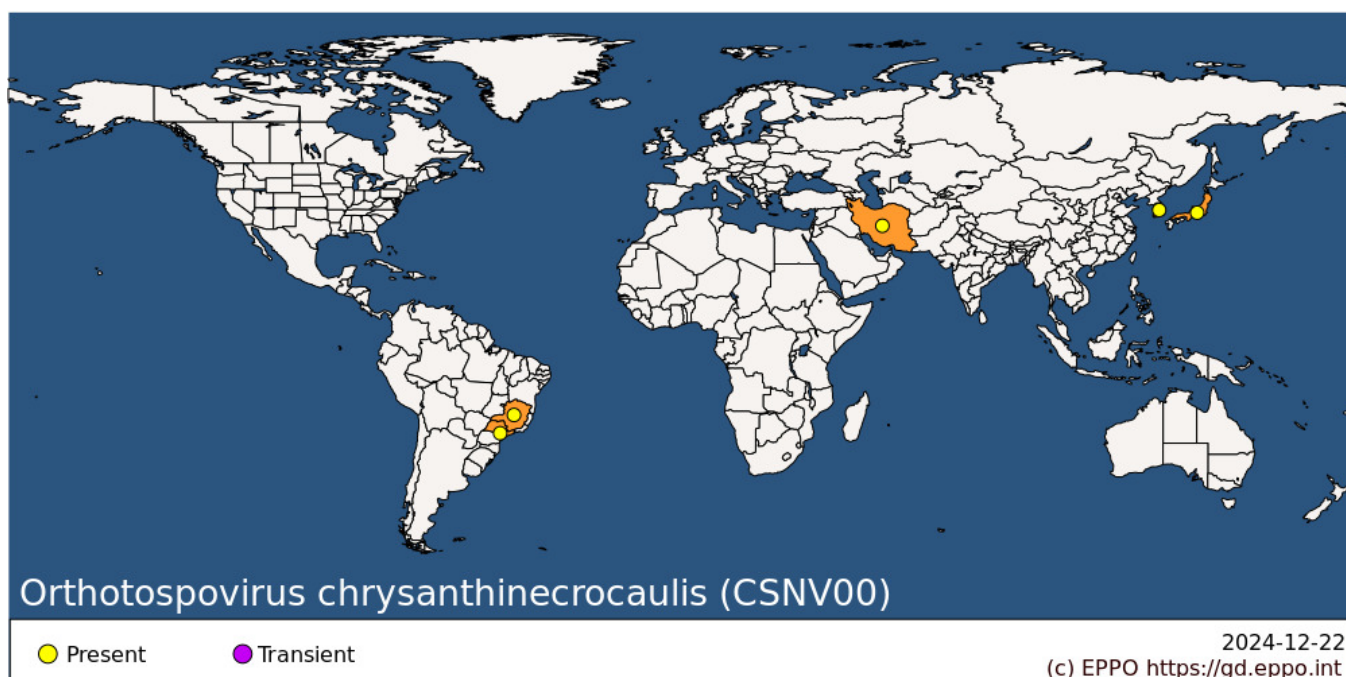
In the Netherlands, CSNV was found in 1994-1995, in four chrysanthemum nurseries that had imported cuttings from Brazil. The disease was believed to be eradicated by 1996 (Verhoeven *et al.*, 1996; Verhoeven & Roenhorst, 1998).

Presence of CSNV was confirmed in chrysanthemum plants (in 2001) and in one sample of *Gerbera* (in 2002) in Slovenia by DAS-ELISA and RT-PCR. All infected plants were destroyed and CSNV was eradicated (Ravnikar *et al.*, 2003).

In the United Kingdom, CSNV was found on chrysanthemum plants imported from Brazil in a nursery in the south-west of England in November 2002. In August 2003, CSNV was considered eradicated as confirmed by laboratory tests (Mumford *et al.*, 2003). A new outbreak was detected in 2010 in the same nursery in chrysanthemum cuttings imported from Brazil. Eradication measures were taken, and plants infected by CSNV and the vectors were destroyed.

Presence of CSNV was confirmed by laboratory analysis (RT-PCR, sequencing) in symptomatic chrysanthemum plants in September 2012 in Belgium. Later during the official inspection several chrysanthemum cultivars affected by CSNV were found. These cultivars had been grown from cuttings (rooted and unrooted) imported from Brazil. All infected chrysanthemum plants have been destroyed (De Jonghe *et al.*, 2013). The official surveys for CSNV were carried out in all Belgian breeding companies of chrysanthemums in 2013 and 2014. The absence and eradication of CSNV were confirmed by laboratory tests.

In Italy, CSNV was detected by ELISA and RT-PCR on *Dendranthema × grandiflorum* in February 2014 in a nursery located in the province of Savona, Liguria region, during official survey activities. Infected plants had necrosis and leaf malformations. Eradication measures were taken, and all infected plants were destroyed. Laboratory tests confirmed that CSNV was eradicated.



Asia:

Iran, Japan (Honshu), Korea, Republic
South America: Brazil (Minas Gerais, Sao Paulo)

BIOLOGY

CSNV is transmitted and spread in nature by insects of the family *Thripidae* (*Thysanoptera*) in a persistent manner. *Frankliniella occidentalis*, *F. schultzei* (Bezerra *et al.*, 1999, Nagata & de Ávila, 2000), *F. gemina* (Whitehouse *et al.*, 2015) and *F. intonsa* (Okuda, 2013) are vectors of CSNV. *Thrips tabaci* was not found as vector of CSNV (Bezerra *et al.*, 1999, Nagata & de Ávila, 2000). *F. occidentalis* and *F. schultzei* have been used experimentally to transmit CSNV from *Datura stramonium* to leaf discs of petunia. *F. schultzei* transmits tospoviruses with high efficiency and has been proposed as an important vector of CSNV in Brazil (Bezerra *et al.*, 1999). These results have been confirmed in more recent studies where CSNV was found to be efficiently transmitted by *F. occidentalis* (65.1% of insects transmit CSNV to plants) and *F. schultzei* (78.1%) (Nagata & de Ávila, 2000)

In Europe, the major vector of CSNV would most probably be *F. occidentalis* which is a pest of major economic importance in glasshouses and some field crops in southern Europe, causing significant damage to its many host plants by feeding and oviposition, as well as being a vector of virus diseases (EPPO/CABI, 1997). This EPPO A2 quarantine pest now present in many countries of the EPPO region and can cause the rapid spread of CSNV. CSNV was detected in thrips from the affected glasshouse using RT- real-time PCR.

The other known vector, *F. schultzei*, has a pantropical distribution. It is less common in the subtropics and in temperate regions where the insect is restricted to heated places, such as glasshouses and storehouses. *F. schultzei* is commonly found on plants in international trade (Vierbergen & Mantel, 1991). Its establishment on flowers of *Cactaceae* has been reported in glasshouses in the Netherlands (Vierbergen & Mantel, 1991) and it has more recently been found on other plant species in Dutch glasshouses (Vierbergen, 1995). *F. schultzei* has also been recorded as a glasshouse pest in Belgium and is present in Israel, Egypt, Morocco and mainland Spain (CABI/EPPO, 1999). Italy and the Canary Islands (Spain) have also been reported as locations where the thrips is found (Nakahara, 1997), but the Italian record is now regarded as incorrect (CABI/EPPO, 1999). A record for the United Kingdom (CABI/EPPO, 1999) is based on a single female found on *Pinus* in Berkshire (Mound *et al.*, 1976), but the thrips is not known to be established.

The results of Okuda *et al.* (2013) in the study of insect vectors of CSNV showed that *F. intonsa* is not a major vector for CSNV but the acquisition efficiency of CSNV by *F. intonsa* may vary among strains. To characterize the translocation and replication of CSNV virions during the infection of *F. intonsa* additional studies are required.

DETECTION AND IDENTIFICATION

Symptoms

The disease symptoms caused by CSNV on different host plants may vary and can be quite severe (Boben *et al.*, 2007). On chrysanthemum, CSNV causes symptoms which are similar to those of Tomato spotted wilt virus (TSWV). In the Netherlands and South Korea, they were described as mild or severe necrotic streaks on the stem, wilting of leaves and stems, and chlorotic or necrotic spots and rings on some leaves. However, symptoms of CSNV are more severe and can result in complete necrosis of the stem resulting in wilting of sections of plants (Verhoeven *et al.*, 1996, Yoon *et al.*, 2017). Disease caused by CSNV often develops faster and is more destructive than TSWV-induced disease (De Jonghe *et al.*, 2013). In Brazil, symptoms were described as necrotic lesions surrounded by yellow areas on leaves followed by necrosis on stems, peduncles and floral receptacles (Duarte *et al.*, 1995). In the British outbreak, symptoms included distinct dark stem lesions with some leaf necrosis (Mumford *et al.*, 2003).

In Slovenia, infected chrysanthemum plants showed TSWV-like symptoms, i.e. necrotic lesions surrounded by yellow areas, and occasionally rings and line patterns on some leaves, followed by necrosis on stems, peduncles, and floral receptacles. Gerbera plant showed no such characteristic symptoms of tospoviral infection as was seen on chrysanthemum plants. Slight yellowing and necrosis on leaves can be observed on gerbera plants infected by CSNV

(Boben *et al.*, 2007).

In naturally infected tomato in Brazil, plants showed stem necrosis with necrotic spots and rings on leaves (Nagata *et al.*, 1998). On inoculated tomato cultivars 'Moneymaker', 'Pronto' and 'Trust', systemic symptoms have been described as chlorotic and necrotic lesions, chlorosis, rugosity and severe growth reduction, although not all inoculated plants developed symptoms (Verhoeven *et al.*, 1996); On comparing the reaction of these cultivars to CSNV and TSWV, Verhoeven *et al.* (1996) considered that tomato may be less susceptible to CSNV than to TSWV.

Infected plants of aster (*Callistephus chinensis*) and lisianthus (*Eustoma grandiflorum*) in a greenhouse in Toyama Prefecture (Japan) developed systemic necrosis with necrotic spots on leaves, followed by necrosis on stems and petioles (Momono *et al.*, 2011). In Belgium, infected plants showed wilting and had numerous necrotic lesions surrounded by yellow areas. On the infected chrysanthemum the necrotic zones quickly extended to stems, ultimately killing most of the plants.

The detection of CSNV based on the observation of symptoms is of important diagnostic value, however, the identification of the virus is only possible using serological and molecular methods.

Morphology

The virion of tospoviruses is quasi-spherical in shape and 80–110 nm in diameter and has three filamentous nucleocapsids per envelope, each 200–3000 nm long (depending on arrangement) and 2–2.5 nm in diameter, with helical symmetry. It has a characteristic lipid envelope and possesses a tripartite genome with large (L), medium (M) and small (S) ssRNAs. All three RNAs have a panhandle structure formed by base pairing of complementary nucleotides (nt) at the 5' and 3' ends. The L RNA is of negative sense and encodes an RNA-dependent RNA polymerase (RdRp) for replication and transcription. Both M and S RNAs are ambisense and contain two open reading frames (ORFs), oriented in opposite directions, that are flanked by an AU-rich intergenic region (IGR). In the viral (v) sense, the M RNA encodes a movement protein named NSm (King *et al.*, 2012, Wu *et al.*, 2015). Virions of tospoviruses are composed of 1–2 % nucleic acid, over 50 % protein and 20–30 % lipid.

Duarte *et al.* (1995) reported that leaf extracts of chrysanthemum negatively stained with 2% uranyl acetate consistently contained tospovirus-like particles 90–120 nm in diameter. Using the same stain, Verhoeven *et al.* (1996) found tospovirus-like particles 75–95 nm in diameter in dip preparations from chrysanthemum leaves. These particles were readily detected in extracts of infected *N. benthamiana* plants. In the UK, electron microscopy of affected chrysanthemum tissue from the British outbreak revealed spherical, tospovirus-like virus particles 90–110 nm in diameter (Mumford *et al.*, 2003).

Detection and inspection methods

Mechanical inoculation of herbaceous test plants (*Datura stramonium*, *Nicotiana benthamiana* and *Nicotiana occidentalis*-P1) can be used for tospoviruses detection with subsequent identification by other methods. CSNV as with some other tospoviruses leads to systemic symptoms on *Nicotiana benthamiana* and *Nicotiana occidentalis*-P1. Inoculated test plants *N. clevelandii*, *N. tabacum* cv. White Burley, and *Physalis floridana* by CSNV caused only local disease symptoms and the presence of the virus was not systemically confirmed (Boben *et al.*, 2007).

D. stramonium was identified by Verhoeven *et al.* (1996) as a suitable indicator host to differentiate CSNV from other tospoviruses as only CSNV causes stem necrosis after mechanical inoculation. However, considering information about a new tospovirus, Alstroemeria yellow spot virus (AYSV), which also leads to local symptoms on *D. stramonium* and does not have systemic symptoms (NPPO-NL and/or the Laboratory of Virology of Wageningen University, the Netherlands) reaction on *D. stramonium* plants can no longer be used for confirmation of detection CSNV.

On chrysanthemum, it is difficult to distinguish symptoms caused by CSNV and TSWV, though CSNV symptoms are more severe to the trained eye. *P. floridana* can be used to differentiate CSNV from TSWV as only the latter causes systemic symptoms (chlorotic and necrotic lesions or rings, wilting).

With closely related viruses, serological cross-reactivity may lead to cross reactions and false positive results.

Commercial ELISA kits to CSNV have been produced by DSMZ (Germany) with two options: DAS-ELISA and B-Fast ELISA. Antisera do not react to other tospoviruses except slight cross-reaction with Alstroemeria necrotic streak virus (ANSV), Tomato chlorotic spot virus (TCSV) and TSWV (Data provided by N. Mehle (NIB, SI) and W. Menzel (DSMZ, DE) 2019). For screening for CSNV it is also possible to use a universal kit for tospoviruses: Tosposcreen (Loewe Biochemica GmbH.) that also detects Groundnut ringspot virus (GRSV), Impatiens necrotic spot virus (INSV), TCSV and TSWV.

CSNV is assigned to the American clade 1 and for the generic detection of CSNV and other viruses that are included to this clade, it is possible to use the conventional RT-PCR from Hassani-Mehraban *et al.* (2016). This RT-PCR can be used for identification when all specific amplicons should be analysed by sequencing and comparison with the target sequences that are located in the nucleocapsid gene and its 5' upstream untranslated region of the S-RNA. For the specific identification is recommended to use the real-time RT-PCR test for CSNV (Boben *et al.*, 2007) with primers and probe for the nucleocapsid (N) gene.

PATHWAYS FOR MOVEMENT

CSNV moves only in its thrips vectors which can spread it between plants, fields or glasshouses in the infested areas. In international trade, the virus could be carried long distances in cuttings and other vegetative plants for planting. CSNV is known to have spread to the Netherlands in chrysanthemum cuttings imported from Brazil (Verhoeven *et al.*, 1996) as well as to the United Kingdom by the same route (Mumford *et al.*, 2003). These plants may not have shown symptoms at the time of dispatch. As a tospovirus, CSNV is unlikely to be seed-transmitted.

PEST SIGNIFICANCE

Economic impact

Tospoviruses in general cause significant losses in yield and quality of economically important crops worldwide (Pappu *et al.*, 2009).

In Brazil, CSNV is growing in economic importance as it continues to spread to new geographical areas since 1997 (Bezerra *et al.*, 1999). Losses due to CSNV are difficult to assess as damage to chrysanthemum and tomato in Brazil due to CSNV has not been quantified. CSNV is now frequently detected on tomato, but has not reached epidemic proportions. No specific data is available on losses in tomato from first report until now. In Brazil at least 4 tospovirus species (CSNV, GRSV, TCSV, TSWV) can infect tomato plants and their effects differ from region to region. However, experimental work suggests that CSNV can kill tomato plants in a few days.

In September 2009 in a greenhouse in Toyama Prefecture (Japan), CSNV was observed on ca. 6000 aster plants and several lisianthus plants in each greenhouse. Infected plants were unfit for market, resulting in serious economic losses. (Momonoi *et al.*, 2011).

The potential impact in Europe is difficult to estimate. Tomato and chrysanthemum are crops of major economic importance. The impact that CSNV would have on either of them is difficult to assess, as statistics on crop losses from Brazil are not available. It could be expected to be substantial. In addition, it can be noted that the eradication campaign has already cost the Netherlands 25–30 000 EUR. The cost of the British campaign against CSNV has not yet been calculated.

De Jonghe *et al.* (2013) reported that in 2012, plants suspected to be virus-infected (they were showing wilting and having numerous necrotic lesions surrounded by yellow areas) were received from a seasonal chrysanthemum grower, growing under glass in western Belgium. On the infected plants the necrotic zones quickly extended to stems, ultimately killing most of the crop in the case of cultivars Ludo and Jorca Pink. Losses for the cv. Mirage Yellow also grew to more than 50%.

Control

Control of CSNV is difficult. To prevent spread of the virus infected plants should be immediately removed from

neighboring plants.

Control of the disease is essentially targeted at eliminating or excluding the thrips vectors. Thrips are generally difficult to control with chemicals. They are very small and hard to detect; the prepupal and pupal stages survive in the soil and are difficult to treat. They have a high fecundity, which would hamper eradication in glasshouses. Spinosyn based insecticides have been found to be some of the most effective chemicals, but local overuse of spinosyns has led to resistance development in *F. occidentalis* populations (Herron and James, 2005; Bielza *et al.*, 2007). Similar results have been observed for other classes of insecticides. Resistance to the major classes of insecticides has been reported in thrips. Resistance to insecticides is persistent (Lewis, 1997). A number of experts recommend the use of cultural and biological methods of control before turning to the use of pesticides (Stavisky *et al.*, 2002; Momol *et al.*, 2004).

However, it must be borne in mind that at the moment no entirely reliable method of biological control of thrips has been identified. Predatory mites and insects and to a lesser degree pathogenic fungi have been used in glasshouses with varying degrees of success. Using of predators such as *Orius insidiosus* can significantly reduce *F. occidentalis* populations. (Demirozer *et al.*, 2012). It may be possible to use hymenopteran parasitoids and parasitic nematodes (Jackson, 1997).

Glasshouse hygiene, such as the eradication of weeds which may serve as thrips hosts, used in conjunction with chemical control measures, may reduce populations of the thrips vectors. Manipulation of the glasshouse environment to suit biological control agents is an alternative possibility. Some growers report success in controlling *F. occidentalis* by heating the glasshouse to 30°C for 4–5 days and then washing down the structure with disinfectant (Lewis, 1997). Screens to prevent the entry of thrips into glasshouses and sticky traps within glasshouses have been used as control measures.

There is no published information regarding resistance of chrysanthemum or tomato to CSNV. But it is known that earlier attempts have been made to develop CSNV-resistant tomato genotypes both by conventional breeding techniques (Lourencao *et al.*, 2001; Lima *et al.*, 2003) and by molecular genetic transfer (Rudolph *et al.*, 2003).

Phytosanitary risk

As is the case for other tospoviruses, CSNV will have the highest economic importance in countries of the EPPO region where its vectors are present and widespread, and where the climatic conditions are favorable for overwintering of vectors outdoors. However, due to the widespread of *F. occidentalis* in glasshouses CSNV will also pose a significant threat to chrysanthemum and tomato crops in countries with relatively cool climates. In addition, *F. schultzei*, which is the major vector in Brazil (Bezerra *et al.*, 1999), is present in glasshouses in the Netherlands and Belgium and has been reported in various southern countries of the EPPO region. CSNV causes a more severe disease on chrysanthemum than TSWV.

The outbreaks of CSNV have been repeatedly reported in the EPPO region (the Netherlands, the United Kingdom, Slovenia, Belgium, Italy). These outbreaks (excluding Italy) were related to the import of cuttings of chrysanthemum from Brazil. All detected outbreaks of virus have been eradicated and CSNV is currently considered as absent in the EPPO region. However, taking into account the import of planting material of flower crops from Brazil to several countries in the EPPO region possibility of re-introduction of CSNV is still assessed as high.

CSNV also poses a threat to tomato cultivation under glass. Another danger is that, because symptoms of CSNV closely resemble those caused by TSWV, they could easily be mistaken by the grower for this virus in nurseries that already have a TSWV problem. Although isolated outbreaks of CSNV can be eradicated (as mentioned above), it is desirable to avoid any further introductions.

PHYTOSANITARY MEASURES

Tomato plants from outside the European and Mediterranean area are prohibited entry, as *Solanaceae*, into the EU (EU, 2000).

Imported plants for planting of chrysanthemum and other host-plants of CSNV, from countries where Chrysanthemum stem necrosis virus occurs, are subject to phytosanitary certification, and should originate from

tested mother plants, from an area or place of production freedom from CSNV, or have been grown under protected conditions for the vectors *F. occidentalis*, *F. schultzei*, *F. gemina* and *F. intonsa*. In fact, since these thrips are polyphagous, freedom from them could be required for plants for planting of any herbaceous plant species from countries where CSNV occurs. Eradication of isolated outbreaks can be achieved by destruction of affected hosts and of the vector(s).

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CABI resources used when preparing this datasheet

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