

**Data sheets on quarantine pests**  
**Fiches informatives sur les organismes de quarantaine**

## ***Chrysanthemum stem necrosis tospovirus***

### **Identity**

**Name:** *Chrysanthemum stem necrosis virus*

**Taxonomic position:** Viruses: *Bunyaviridae*: *Tospovirus*

**Common names:** CSNV (acronym)

**Special notes on taxonomy or nomenclature:** CSNV was originally described as a new serogroup of *Tomato spotted wilt virus* (TSWV), tentatively designated Chr 1 (Duarte *et al.*, 1995). Verhoeven *et al.* (1996) concluded that a new virus isolated from *Dendranthema x grandiflorum*, which they tentatively designated Ch-1, was a distinct *Tospovirus*. The name *Chrysanthemum stem necrosis tospovirus* was applied by Nagata *et al.* (1998) when describing an occurrence of CSNV on tomato. However, the first formal recognition of a new virus species was made by Bezerra *et al.* (1996) who proposed that a virus isolated from chrysanthemums (Chry 1) be designated *Chrysanthemum stem necrosis virus*

**EPPO code:** CSNV00

**Phytosanitary categorization:** EPPO A1 action list no. 313

### **Hosts**

CSNV occurs on florists' chrysanthemum *Dendranthema x grandiflorum* and tomato (*Lycopersicon esculentum*). In the Netherlands, CSNV has been isolated from the chrysanthemum cultivars 'Cocarde', 'Fiji', 'Majoor Bosshardt', 'Reagan', 'Spider', 'Tiger', 'Tigerrag' and 'Vyking' (Verhoeven *et al.*, 1996). In the UK, CSNV has been isolated from the chrysanthemum cultivars 'Fiji' and 'Calabria' (Mumford *et al.*, 2003). There is a record of CSNV in one sample of *Gerbera* (Ravinkar *et al.*, 2004).

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). Other artificial hosts of CSNV identified in experiments are aubergine, cucumber and lettuce, bean, cowpea, pea and courgette (Bezerra *et al.*, 1999), capsicum (Verhoeven *et al.*, 1996) and tobacco (Duarte *et al.*, 1995; Verhoeven *et al.*, 1996).

### **Geographical distribution**

**EPPO region:** outbreaks have occurred in Netherlands (1994/1995), Slovenia (2001/2002), and UK (2002), but the virus is no longer found in those countries

**South America:** CSNV (as Chr 1) was first recognized on chrysanthemum in São Paulo State in Brazil (Duarte *et al.*, 1995). More recently, it has been found on tomato in Minas Gerais State in Brazil (Nagata *et al.*, 1998). It may also occur in other South American countries (Verhoeven *et al.*, 1996)

**EU:** absent

In the Netherlands, CSNV (as Ch-1) was found in 1994/1995, in four chrysanthemum nurseries that had imported cuttings from Brazil. The disease was considered serious enough to warrant eradication. All chrysanthemums at one nursery were destroyed and selective roguing was carried out at the other three in attempts to eradicate the disease. Plants with symptoms continued to be found at the three nurseries where only roguing was undertaken in 1995, but CSNV was believed eradicated by 1996 (Verhoeven *et al.*, 1996; Verhoeven & Roenhorst, 1998).

In the UK, CSNV was found on chrysanthemum plants on a nursery in the south-west of England in November 2002. As in the Netherlands, the plants had been grown from cuttings originally imported from Brazil, on this occasion during August 2002. The affected crop was subjected to containment and eradication procedures. Nine other nurseries which had received imported chrysanthemum cuttings from Brazil during the same period were inspected and no CSNV was detected. (Mumford *et al.*, 2003). CSNV was declared eradicated in the UK in August 2003.

In Slovenia, CSNV was detected in 23 samples of chrysanthemum in 2001, and again in 2002 in 1 sample of *Gerbera*. The identity of CSNV was confirmed by ELISA, different test plants and PCR. All infected plants were destroyed by incineration. In 2003, CSNV was not detected. The virus has remained subject to official control (Ravnikar *et al.*, 2003).

### **Biology**

CSNV is transmitted and spread in nature by insects of the family *Thripidae* (*Thysanoptera*) in a persistent manner. *Frankliniella occidentalis* and *Frankliniella schultzei*, but not *Thrips tabaci*, are vectors of CSNV. *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 great efficiency and has been proposed as an important vector of CSNV in Brazil. *T. tabaci* was not found to be a vector in these experiments (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%) and *F. schultzei* (78.1%), but not at all by *T. tabaci* (0.0%) (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)<sup>1</sup>. *F. occidentalis* has been implicated in the spread of CSNV in glasshouses in the Netherlands (Verhoeven *et al.*, 1996) and has also been found at low levels on the UK nursery affected in 2002. CSNV was detected in thrips from the affected glasshouse using TaqMan.

*F. occidentalis* is known to attack a wide range of plants (over 220 recorded hosts) and is an efficient vector of *Tomato spotted wilt tospovirus*, another destructive tospovirus. Crop hosts of *F. occidentalis* include ornamentals and cut flowers (chrysanthemum, rose, carnation, impatiens, gloxinia, gerbera, aster, primula, cineraria), vegetables (cucumber, lettuce, tomato, beans, aubergine, capsicum) and fruits (strawberries, stone fruits) (Cook *et al.*, 1996). Some of these hosts have also been systemically infected by CSNV in artificial inoculation studies.

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, 1999a). 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 UK (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.

## Detection and identification

### Symptoms

On chrysanthemum, CSNV causes symptoms which are similar to those of TSWV. In the Netherlands, 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). 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). At the British outbreak, symptoms included distinct dark stem lesions with some leaf necrosis (Mumford *et al.*, 2003).

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.

### Morphology

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

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

Antisera to CSNV have been produced in the Netherlands (Verhoeven *et al.*, 1996) and Brazil (Bezerra *et al.*, 1999) for use in ELISA testing. CSNV did not react in DAS-ELISA to antisera of other tospoviruses. Those tested by Verhoeven *et al.* (1996) were TSWV, *Impatiens necrotic spot tospovirus* (INSV), *Iris yellow spot tospovirus* (IYSV) and Tospo-to (an isolate resembling *Watermelon silver mottle tospovirus*, WSMoV). TSWV, INSV, IYSV, *Tomato chlorotic spot tospovirus* (TCSV), *Groundnut ring spot tospovirus* (GRSV) and WSMoV were tested by Bezerra *et al.* (1999). Western immuno-blot analysis also distinguishes CSNV from other tospoviruses, although a slight cross-reaction with antibodies against TSWV, TCSV and GRSV was observed (Bezerra *et al.*, 1999).

Samples of infected plants from the British outbreak were tested for a range of tospoviruses using ELISA (Mumford *et al.*, 2003). CSNV was detected using a polyclonal DAS-ELISA kit. Sap inoculation from symptomatic stem material onto a range

<sup>1</sup>This EPPO A2 quarantine pest does not occur in many countries of the eastern part of the EPPO region.

of indicator species led to typical CSNV symptoms; the affected plants gave a positive result for CSNV when tested by ELISA. RT-PCR performed on extracts of symptomatic stem tissue produced a single product which, when sequenced, shared 98% identity with the 5' end of the capsid protein gene of a published CSNV sequence.

### Pathways for movement

CSNV moves only in its thrips vectors which can spread it between plants, fields or glasshouses in 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 UK 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

In Brazil, CSNV is growing in economic importance as it continues to spread to new geographical areas since 1997. It is expected to become widespread and important (Bezerra *et al.*, 1999). Losses due to CSNV are difficult to determine as damage to chrysanthemum and tomato in Brazil due to CSNV has not been quantified. CSNV is now frequently detected on tomato, but had not reached epidemic proportions. No specific data is available on losses in tomato as there are apparently 3 tospovirus species present on this host in Brazil and their effects differ from region to region. However, experimental work suggests that CSNV could kill tomato plants in a few days.

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 estimate, 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.

#### Control

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. Resistance to the major classes of insecticides has also been reported in thrips. Resistance to insecticides is persistent (Lewis, 1997).

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. It may be possible to use hymenopteran parasitoids and parasitic nematodes in the future (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 predators of biological control agents is a future 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.

#### Phytosanitary risk

CSNV poses a significant threat to chrysanthemum and tomato crops in those parts of the EPPO region where its known vector *F. occidentalis* is present and widespread. CSNV causes a more severe disease on chrysanthemum than TSWV. 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. The two geographically isolated outbreaks of CSNV in the Netherlands and the UK were both related to imported chrysanthemum cuttings from Brazil, which shows that there is a pathway for introduction (though no such direct pathway exists at the moment for tomato). 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 (this has been achieved successfully in the Netherlands and in UK), it is desirable to avoid any further introductions.

#### Phytosanitary measures

CSNV was added in 2003 to the EPPO A1 action list, and EPPO member countries are thus recommended to regulate it as a quarantine pest. So far, there are no specific phytosanitary measures to protect against the introduction of CSNV into the EPPO region. Tomato plants from outside the European and Mediterranean area are prohibited entry, as *Solanaceae*, into the EU (EU, 2000a). Imported plants for planting of chrysanthemum are subject only to phytosanitary certification, and consideration could be given to requiring origin from tested mother plants, place of production freedom, or freedom from the vectors *F. occidentalis* and *F. schultzei*. 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), but this is costly and difficult to implement in practice.

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