EPPO Datasheet: *Pospiviroid impedichrysanthemi*

Last updated: 2023-11-10

IDENTITY

Preferred name: *Pospiviroid impedichrysanthemi*
Taxonomic position: Viruses and viroids: Viroids: Pospiviroidae
Other scientific names: *CSVd*, *Chrysanthemum stunt mottle virus*, *Chrysanthemum stunt pospiviroid*, *Chrysanthemum stunt viroid*
Common names: measles of chrysanthemum, stunt of chrysanthemum
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EPPO Categorization: A2 list
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EU Categorization: RNQP ((EU) 2019/2072 Annex IV)
EPPO Code: CSVD00



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Notes on taxonomy and nomenclature

Ratification by the International Committee on Taxonomy of Viruses (ICTV) of the new species name *Pospiviroid impedichrysanthemi* is pending (**ICTV website**, proposal n. 2023.032P) and expected to be adopted early in 2024.

HOSTS

The major natural host of CSVd is the florists' chrysanthemum (*Chrysanthemum x morifolium*). In addition, several ornamental plants mainly belonging to the family Asteraceae, a few plant species of the family Solanaceae, including potato (*S. tuberosum*, Matsushita *et al.*, 2019a; 2021), the ornamentals *Solanum laxum* (syn *Solanum jasminoides*, Verhoeven *et al.*, 2006), and *Petunia x hybrida* (Verhoeven *et al.*, 1998), and a few species in the families Verbenaceae (*Verbena* sp., Bostan *et al.*, 2004) and Apocynaceae (*Vinca major*, Nie *et al.*, 2005) have been reported as natural hosts of CSVd. This viroid has also been detected in several wild chrysanthemum species in Japan (Matsushita *et al.*, 2012) and in wild *Oxalis latifolia* (family Oxalidaceae, Gobatto *et al.*, 2019) and *Solanum nigrum* (Matsushita *et al.*, 2007) in Colombia and Russia, respectively, suggesting the role of weeds as reservoirs of CSVd. While chrysanthemum plants infected by CSVd develop symptoms of variable severity on the cultivars (30% being asymptomatic), most of the other natural hosts remain symptomless.

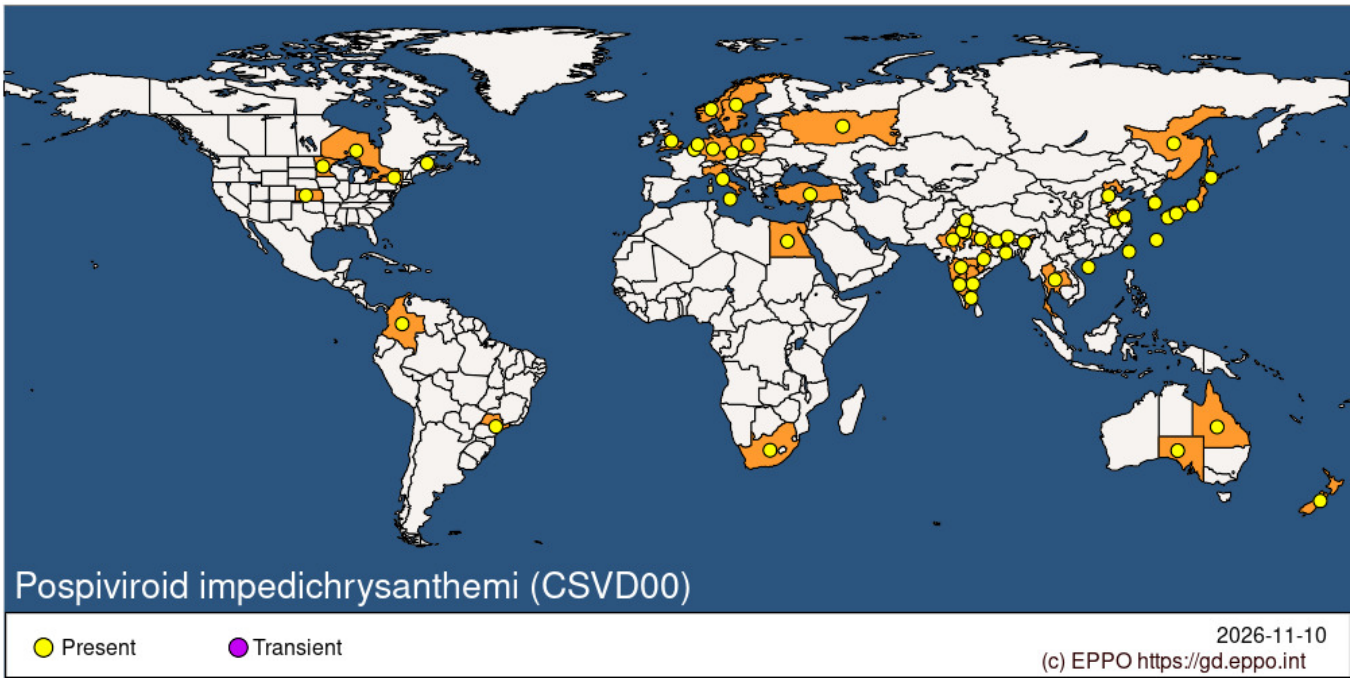
The list of plant species identified as experimental hosts of CSVd includes many other species belonging to the families reported above and a few species belonging to the families Amaranthaceae, Brassicaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Nyctaginaceae, Poaceae and Portulacaceae. However only few of the experimental hosts develop symptoms upon infection.

Host list: *Argyranthemum frutescens*, *Argyranthemum maderense*, *Argyranthemum sp.*, *Chrysanthemum crassum*, *Chrysanthemum indicum*, *Chrysanthemum japonense*, *Chrysanthemum lavandulifolium*, *Chrysanthemum makinoi*, *Chrysanthemum x morifolium*, *Chrysanthemum yoshinaganthum*, *Chrysanthemum zawadskii*, *Chrysanthemum zawadzkii subsp. zawadzkii*, *Chrysanthemum*, *Dahlia*, *Oxalis latifolia*, *Pericallis x hybrida*, *Petunia hybrids*, *Portulaca oleracea*, *Solanum laxum*, *Solanum nigrum*, *Solanum tuberosum*, *Tanacetum parthenium*, *Verbena hybrids* , *Verbena sp.*, *Verbena x hybrida*, *Vinca major*

GEOGRAPHICAL DISTRIBUTION

CSVd has been reported in several countries worldwide with recent reports in South America. Several accessions (MT995842-MT995845) in **GenBank** also suggest its presence in Malaysia. Over the years, CSVd has been reported in several other countries from which it has now been eradicated. However, since CSVd infections may remain

symptomless in several hosts, there is some uncertainty concerning the current distribution.



EPPO Region: Belgium, Czechia, Germany, Italy (mainland, Sicilia), Netherlands, Norway, Poland, Russian Federation (Central Russia, Far East), Sweden, Türkiye, United Kingdom (England)
Africa: Egypt, South Africa
Asia: China (Anhui, Hainan, Hebei, Jiangsu), India (Andhra Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Delhi, Haryana, Himachal Pradesh, Karnataka, Maharashtra, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal), Japan (Hokkaido, Honshu, Kyushu, Ryukyu Archipelago, Shikoku), Korea, Republic of, Taiwan, Thailand
North America: Canada (New Brunswick, Ontario), United States of America (Kansas, Minnesota, New York)
South America: Brazil (Sao Paulo), Colombia
Oceania: Australia (Queensland, South Australia), New Zealand

BIOLOGY

Chrysanthemum stunt disease, first reported in 1947 (Dimock, 1947), was shown to be caused by a viroid twenty-five years later (Diener and Lawson, 1973; Hollings and Stone, 1973). Viroids are non-protein coding, single-stranded circular RNA particles infecting plants (Navarro *et al.*, 2021). The complete genome of CSVd was first determined at the beginning of the 1980s (Haseloff and Symons, 1981; Gross *et al.*, 1982) and nowadays several hundred CSVd variants of different geographic or host origin have been completely sequenced (**NCBI**, consulted on 16/10/2023). The genome of most CSVd variants ranges in size between 354 and 356 nt (Palukaitis, 2017). CSVd is classified in a species belonging to the genus *Pospiviroid* of the family Pospiviroidae. The family Pospiviroidae includes viroids that, as is the case for the reference viroid of the family potato spindle tuber viroid PSTVd, replicate in the nucleus and contain typical conserved structural motifs (Di Serio *et al.*, 2021). As for the other members of this family, CSVd circular RNA assumes a rod-like conformation that contains i) the central conserved region (CCR) conserved in all members of the genus *Pospiviroid* and which is thought likely to be involved in replication (as shown for PSTVd); and ii) the terminal conserved hairpin (TCH), a structural motif also contained in the other members of the genus *Pospiviroid* and for which the role remains unknown.

CSVd can be spread by vegetative propagation and transmission via contact, and grafting. Pollen and seeds may also play a role.

Vegetative propagation

The most efficient way of spreading of CSVd is through vegetative propagation. Once an infection is established in ‘mother plants’, the viroid will be present in all progeny derived from these infected plants, including cuttings.

Contact (mechanical transmission)

CSVd is easily transmitted by grafting, mechanical means, such as rubbing sap onto leaves or stem slashing or needle inoculation of sap into the stem; transmission can also happen via contaminated hands or cutting and grafting tools (Brierley and Smith, 1949; Brierley, 1953; Hollings and Stone, 1973; Runia and Peters, 1980, Palukaitis and Symons, 1980; Palukaitis, 2017). Transmission through plant-to-plant contact is considered possible, but erratic (Hollings and Stone, 1973; EFSA plant Health Panel, 2012). Data on transmission through dodder are contradictory (Keller, 1949 quoted by Bowen and van Zaayen, 2003; Hollings & Stone, 1973).

Pollen and seeds

Seed transmission in chrysanthemum is subject to some uncertainty since it was reported under certain experimental conditions (Moison *et al.*, 1973), but it was not under others (Hollings and Stone, 1973). Chung *et al.* (2008) observed very different seed transmission rates in chrysanthemum, ranging from 6.7% to 96%, depending on whether two or only one of the parental plants were infected. The temperature under which crosses were made probably also influenced the observed seed transmission rate. CSVd pollen and seed-transmission in tomato also has been documented (Kryczynski *et al.*, 1988).

The intensity of symptoms in chrysanthemum plants varies depending on the variety and on the growth condition, including light intensity, photoperiod and temperature (Bachelier *et al.*, 1976; Hollings and Stone, 1973; Handley and Horst, 1988; Chung *et al.*, 2005). The accumulation of CSVd in infected chrysanthemum plants is temperature dependent: the higher the temperature (from 10°C up to 25-35°C), the higher the viroid concentration (Chung *et al.*, 2006).

Movement of the viroid in its host is also largely dependent on temperature, as shown by low temperature treatments (6 months at 2°C) strongly interfering with the distribution of CSVd in chrysanthemum shoot tips, which is likely to be due to a reduction in viroid replication and/or the inhibition of viroid intra- and intercellular movement (Matsushita and Shima, 2015). Changes in the accumulation and distribution of CSVd in *Argyranthemum* shoot apical meristems were also caused by low temperature treatments, although the host cultivar also played a role (Zhang *et al.*, 2016).

DETECTION AND IDENTIFICATION

Symptoms

In florists' chrysanthemums, about 30% of plants are symptomless carriers. However, even when symptoms are manifest, comparison with a healthy plant cannot always confirm diagnosis which, in general, can only be established by molecular tests or indexing on differential hosts. In addition, the incubation period of the viroid in chrysanthemum is relatively long, from 2 to 3 months depending on the cultivar and on growth conditions. Such a long latent period limits the reliability of monitoring or surveillance when they are exclusively based on visual inspection.

The most common symptoms include stunting, poor root development, reduction in the numbers and size of flowers, which may show colour-breaking, malformations and bleaching. Leaf chlorosis with the development of chlorotic spot of different sizes can also be observed and sometimes stems have a tendency to break (Bachelier *et al.*, 1976; Hollings and Stone, 1973; Chung *et al.*, 2005; Horst *et al.*, 1977; Palukaitis 2017). Severe symptoms on roots (significantly shorter roots and higher number of roots) were observed in CSVd-infected *C. x morifolium* and *Chrysanthemum seticuspe* (Osaka *et al.*, 2021).

Infected plants bloom earlier than normal plants of the same cultivar and this effect increases with time; on plants propagated from infected mother plants, the difference in blooming time is usually shorter in the first year of infection (a few days) than in the following year (up to more than 3 weeks). CSVd has been reported to alter the photoperiodic response of chrysanthemum plants, with infected plants flowering under long-day conditions (Hosokawa *et al.*, 2004a).

Natural hosts other than chrysanthemum are generally symptomless and some infected cultivated chrysanthemum cultivar may also remain asymptomatic (Bachelier *et al*., 1976; Hollings and Stone 1973). However, symptoms of stunting, small leaves and small flowers were reported in some cultivars of dahlia naturally infected in Japan and similar symptoms were observed in dahlia plants of two cultivars experimentally inoculated (Asano *et al*., 2020). Moreover, while most *Argyranthemum* cultivars remain symptomless, leaf yellowing and necrosis and flower distortion have been observed on CSVd-infected *Argyranthemum* cultivar 'Butterfly' in France, although the role of co-infecting viruses could not be excluded (Marais *et al.*, 2011). In conclusion, since symptom expression depends on multiple factors, visual inspection cannot be considered a reliable detection method.

Detection and inspection method

Several laboratory tests are available for rapid and reliable detection of CSVd, including EPPO-recommended methods such as RT-PCR tests targeting either several pospiviroids or specifically CSVd (EPPO, 2021). When generic primers amplifying all or almost all known pospiviroids are used, sequencing of amplicons is needed for a conclusive identification of CSVd. Alternatively, CSVd identification can be achieved directly using the specific real-time RT-PCR test developed by Mumford *et al.* (2000). Other molecular detection tests include dot-blot (Candresse *et al.*, 1988), tissue blot (Hooftman *et al.*, 1996) and Northern blot hybridization (Torchetti *et al*., 2012), which have the advantage of being less prone to generate false positives caused in PCR by contamination, but are time demanding and generally less sensitive than RT-PCR and real-time RT-PCR. A more sensitive test than RT-PCR, consisting of a loop-mediated isothermal amplification technique (LAMP) test, has also been developed for the specific detection of CSVd (Supakitthanakorn *et al.*, 2022a).

Recently, a quantitative RT-PCR detection test for the simultaneous detection of CSVd and chrysanthemum chlorotic mottle viroid (ChCMVd, a viroid of the family *Avsunviroidae* infecting chrysanthemum) (Supakitthanakorn *et al.*, 2023) or a nested multiplex assay to detect CSVd, CChMVd and chrysanthemum virus B (Supakitthanakorn *et al.*, 2022b) have been developed.

Bioassays based on grafting onto chrysanthemum indicator plants or mechanical inoculation onto *Pericallis cruenta* (syn. *Senecio cruentus*) were used in the past to detect CSVd infection, but they have been replaced by the more reliable and cost-effective molecular tests.

PATHWAYS FOR MOVEMENT

International spread of CSVd is most likely to result from the movement of infected chrysanthemum plants for planting (incl. cuttings) in trade. The trade of infected plants for planting of other host species also represents a pathway although it is more difficult to assess as many species are asymptomatic. Seed and pollen transmission has been demonstrated in chrysanthemum and tomato therefore seed and pollen of these species may also be a pathway for international spread and local spread of the viroid. Taking into consideration the mechanical transmission of CSVd, its local human-assisted spread within crops is likely, especially in the absence of appropriate hygiene control measures.

PEST SIGNIFICANCE

Economic impact

Chrysanthemum stunt was first recognized in the USA, in a major epidemic in 1947. One year after the first observation, 30 to 60% of the plants were seriously affected and unmarketable (Dimock, 1947; Brierley and Smith, 1949; Diener 1979). CSVd causes a serious disease in chrysanthemum and is reported to reduce plant height. Reductions in plant height (from 32 to 50%), leaf size (from 25 to 35%), flower number and size (from 14 to 36% and from 14 to 75%, respectively) were recorded in several infected chrysanthemum varieties in South Korea (Chung *et al.*, 2005). If cuttings from infected plants are used, reductions in plant height of over 90% may result the following year. Moreover, within 2 years, the number of cuttings which can be produced from infected mother plants may be reduced by 90%. The induction of early flowering has serious consequences for producers of potted plants. In 1987, a CSVd outbreak in Australia (Victoria) required control measures with a cost quoted at 3 million AUD

(Moran and Bate, 1988 quoted by Hill *et al.*, 1996). With yield reduction of up to 65%, pompon-type chrysanthemum varieties have been reported to be more sensitive to CSVd infection than standard cultivars, with a clear seasonal effect leading to higher losses in summer flower crops in both cases (Horst *et al.*, 1977). Epidemic events can be associated with very high incidence, as reported in South Korea (100%, Chung *et al.*, 2005), Japan (90%, Matsushita *et al.*, 2007) and India (70%, Singh *et al.*, 2010).

Flowering time alteration induced by CSVd (Hollings and Stone 1973; Sugiura and Hanada, 1998; Hosokawa *et al.*, 2004a; Chung *et al.*, 2005), may also have important economic relevance if it interferes with the expected commercialization period.

In addition to the cost to implement eradication efforts imposed by legislation in some countries, no direct important economic losses have been recorded on CSVd-infected ornamental hosts other than chrysanthemum. However, the incidence of CSVd in ornamentals may reach very high levels as shown during outbreaks in dahlia in Japan (Nakashima *et al.*, 2007) and in *Argyranthemum frutescens* in Italy (Torchetti *et al.*, 2012).

Control

There are no chemical or biological methods available to control CSVd in infected plants. Therefore, prevention is necessary to avoid infection. Limiting the impact of the disease in an area where the viroid is established is mainly based on the use of CSVd-free certified propagation material (EPPO, 2000; EPPO, 2008). Hygiene best practice, including the use of disposable outer clothes, gloves, overshoes and the treatment of cutting tools and machineries with disinfectants (e.g. sodium hypochlorite and commercial agricultural disinfectants) will also contribute to limit CSVd spread.

Viroid-free chrysanthemum may be obtained, with variable results, by meristem-tip culture, which may be combined with heat or cold therapy treatments (Hollings & Stone, 1970; Bachelier *et al.*, 1976; Paduch-Cichal and Kryczynski, 1987; Hosokawa *et al.*, 2004b; Savitri *et al.*, 2013). Low temperature treatments associated with meristem tip culture was also effective to produce viroid free Argyranthemum 'Border Dark Red', but not 'Yellow Empire' (Zhang *et al.*, 2016). Cryopreservation, a technique mostly used for germplasm conservation but shown to efficiently eliminate pathogens, has been proposed as a possible alternative method for the treatment of CSVd-infected chrysanthemum plants, with efficacy largely depending on the genotype and the initial viroid concentration (Jeon *et al.*, 2016).

There are so far no commercial chrysanthemum cultivars showing total resistance to CSVd, but the existence of resistance in commercial chrysanthemum cultivars has been reported by several authors (reviewed by Nabeshima *et al.*, 2018). However, segregation of the resistance in the progeny and genetic analyses are complex due to the high polyploidy of cultivated chrysanthemum varieties. The recent identification of a resistant accession of the wild diploid *C. seticuspe* is expected to facilitate the analyses of the inheritance of CSVd-resistance (Matsushita *et al.*, 2019b). Transgenic resistance to CSVd could provide an alternative as it has been achieved by expressing various constructs in transformed chrysanthemum plants, such as an RNA nuclease targeting double-stranded RNA molecules (pac1, Ogawa *et al.*, 2005), a catalytic single-chain antibody (3D8 scFv, Tran *et al.*, 2016) or sense and antisense CSVd RNA sequences (Jo *et al.*, 2015).

Phytosanitary risk

There are no known ecoclimatic constraints for the establishment of CSVd in new areas, except those affecting its hosts. Due to the severity of the disease induced in commercial chrysanthemum cultivars, CSVd is included in the EPPO A2 list of pests recommended for regulation as a quarantine pest. CSVd is considered as a quarantine pest in several countries worldwide (**EPPO global database**). In the EU, CSVd is currently included in the list of regulated non-quarantine pests for plants for planting (other than seeds) of *Argyranthemum* and *Chrysanthemum*. The phytosanitary risk associated with CSVd in the EU, and possibly elsewhere, is largely reduced by the certification of chrysanthemum propagation material (EFSA PLH Panel, 2012).

PHYTOSANITARY MEASURES

To prevent the introduction and spread of CSVd, plants for planting of chrysanthemum and other hosts should be produced in a pest free area, in a pest free place/site of production, or shown to be free from CSVd by appropriate

test methods and surveys. A number of EPPO countries already have such regulations in place. Seeds and pollen are also potential entry pathways in the case of chrysanthemum and tomato, but they have been considered by EFSA (EFSA PLH Panel, 2012) as being of minor significance due to the inconsistent volumes of traded chrysanthemum seeds and pollen and the rarity of natural CSVd infections in tomato. This pathway may also be relevant in the case of the other natural hosts for which seed and pollen transmission may exist but has not been demonstrated.

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CABI resources used when preparing this datasheet

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ACKNOWLEDGEMENTS

This datasheet was extensively revised in 2023 by Francesco di Serio (CNR, Italy) and Thierry Candresse (INRAE, France). Their valuable contribution is gratefully acknowledged.

How to cite this datasheet?

EPPO (2026) *Pospiviroid impedichrysanthemi*. EPPO datasheets on pests recommended for regulation. Available online. https://gd.eppo.int

Datasheet history

This datasheet was first published in the EPPO Bulletin in 1978 and revised in the two editions of 'Quarantine Pests for Europe' in 1992 and 1997, as well as in 2023. It is now maintained in an electronic format in the EPPO Global

Database. The sections on 'Identity', ‘Hosts’, and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

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Co-funded by the
European Union