EPPO Datasheet: *Cocadviroid rimocitri*

Last updated: 2021-10-18

IDENTITY

Preferred name: *Cocadviroid rimocitri*
Taxonomic position: Viruses and viroids: Viroids: Pospiviroidae
Other scientific names: *CBCVd, Citrus bark cracking cocadviroid, Citrus bark cracking viroid, Citrus viroid IV*
view more common names online...
EPPO Categorization: A2 list
view more categorizations online...
EU Categorization: RNQP (Annex IV)
EPPO Code: CBCVD0

more photos...

Notes on taxonomy and nomenclature

Citrus bark cracking viroid (CBCVd) was initially designated as Citrus viroid IV (CVd- IV) because it was the fourth viroid species discovered in citron (*Citrus medica*) and which were distinguished by electrophoretic mobility. This viroid had the fastest electrophoretic mobility by sequential PAGE. Northern blot hybridization revealed no homology to the CVd-I, CV-II, and CV-III sequences and only weak homology to Citrus exocortis viroid (CEVd) probes (Duranvila *et al*., 1988). Its complete nucleotide sequence of 284 bp was discovered in a sample of dwarf grapefruit (*Citrus paradisi*) from Israel in 1991 and described as a chimeric viroid resembling parts of CEVd and Hop stunt viroid (HSVd)(Puchta *et al*., 1991). It belongs to the family Pospiviroidae and the genus *Cocadviroid* (Flores *et al*., 1998), although its position in the genus was debated (Semancik and Vidalakis, 2005). The presence of CVd-IV was found to be associated with bark cracking symptoms in trifoliate oranges, so it was renamed the more descriptive CBCVd (Vernière *et al*., 2004; Vernière *et al*., 2006). Recent discovery of its natural presence in hops supports its current *Cocadviroid* classification together with Hop latent viroid (HLVd).

HOSTS

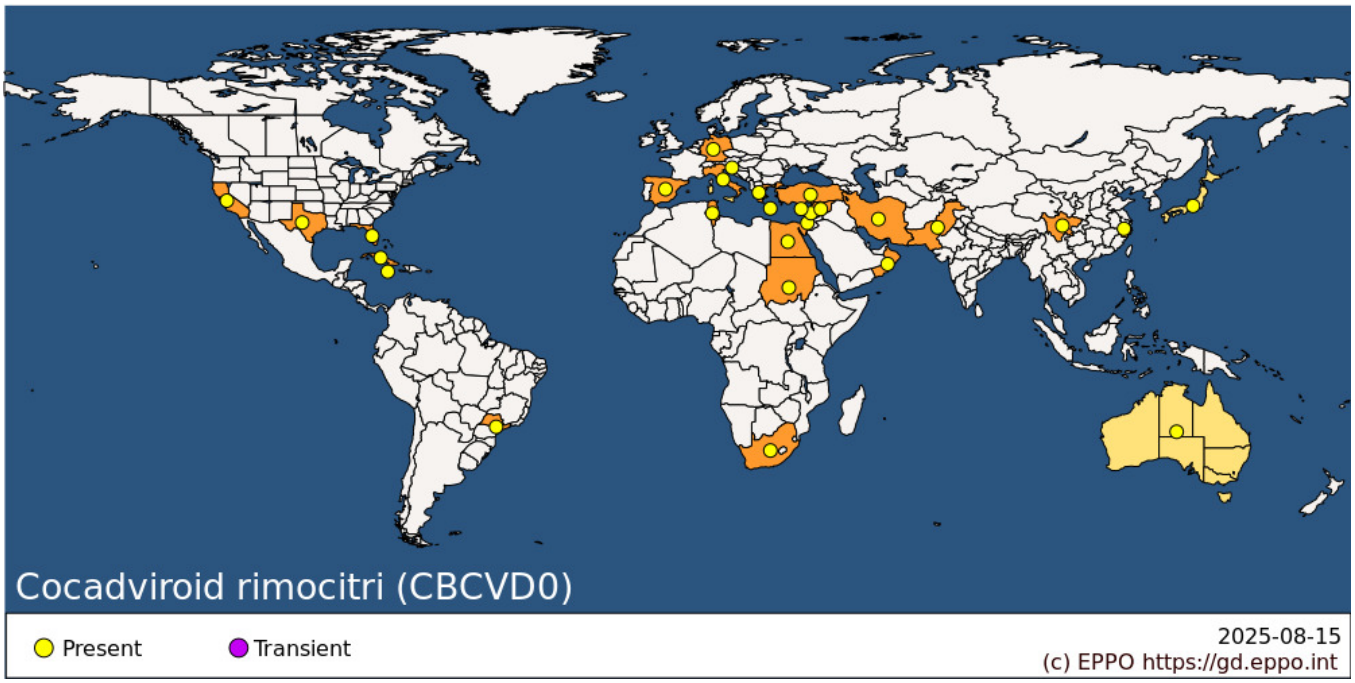
Known natural hosts for CBCVd were until recently restricted to the various species and interspecies of the *Citrus* genus, including lime (*Citrus aurantiifolia*), sour orange (*Citrus aurantium*), nagami kumquat (*Fortunella margarita*), Tahiti lime (*Citrus latifolia*), sweet lime (*Citrus limettioides*), lemon (*Citrus limon*), etrog citron (*Citrus medica*), Meyer lemon (*Citrus meyeri*), grapefruit, tangerine (*Citrus reticulata*), sweet orange (*Citrus sinensis*), trifoliate orange (*Citrus trifoliata*) and various hybrids. CBCVd was confirmed in hop (*Humulus lupulus*) fields in 2015 causing severe stunt disease of hop (Jakse *et al*., 2015) and in pistachio (*Pistacia vera*), where the viroid is known under the name citrus bark cracking viroid-pistachio (CBCVd-pis) due to the lower similarity (87% nucleotide similarity) with other CBCVd variants (Al Rwahnih *et al*., 2018).

In addition to natural hosts, it was shown that CBCVd can infect experimentally other hosts with or without symptoms including ornamentals, vegetables and weeds such as purple velvetplant (*Gynura aurantiaca*), tomato (*Solanum lycopersicum*), *Solanum lycopersicum* × *Solanum peruvianum*, cucumber (*Cucumis sativus*), chrysanthemum (*Dendranthema* × *grandiflorum*), eggplant (*Solanum melongena*), jimsonweed (*Datura stramonium*), *Nicotiana benthamiana* and bittersweet nightshade (*Solanum dulcamara*). Interestingly, pure cultures of CBCVd for its sequence characterization were obtained in ash gourd (*Benincasa hispida*) (Puchta *et al*., 1991). The pistachio variant was shown to be graft transmittable to UCB-1 hybrid rootstock (*P. atlantica* × *P. integerrima*) (Al Rwahnih *et al*., 2018).

Host list: *Citroncirus webberi, Citrus hybrids, Citrus medica, Citrus reticulata, Citrus sp., Citrus trifoliata, Citrus x aurantiifolia, Citrus x aurantium var. paradisi, Citrus x aurantium var. sinensis, Citrus x aurantium, Citrus x latifolia, Citrus x limon var. limettioides, Citrus x limon var. meyerii, Citrus x limon, Citrus x tangelo, Fortunella margarita, Humulus lupulus, Pistacia atlantica, Pistacia vera*

GEOGRAPHICAL DISTRIBUTION

CBCVd is believed to have a worldwide distribution. Mainly reported in citrus growing areas, it has also been confirmed in the last decade in the hop growing areas of the Savinja Valley in Slovenia (Jakse *et al*., 2015) and in Bavaria, Germany (EPPO, 2019). Recently, a sequence with 87% nucleotide similarity was reported in pistachio trees in the USA (Al Rwahnih *et al*., 2018).



EPPO Region: Cyprus, Germany, Greece (mainland, Kriti), Israel, Italy (mainland), Slovenia, Spain (mainland), Tunisia, Türkiye
Africa: Egypt, South Africa, Sudan, Tunisia
Asia: China (Sichuan, Zhejiang), Iran, Islamic Republic of, Israel, Japan, Lebanon, Oman, Pakistan, Syrian Arab

Republic
North America: United States of America (California, Florida, Texas)
Central America and Caribbean: Cuba, Jamaica
South America: Brazil (Sao Paulo)
Oceania: Australia

BIOLOGY

CBCVd is one of the less well studied viroids in citruses. All parts of plants can be systemically infected with CBCVd. As is the case for the other members of *Pospiviroidae*, CBCVd replicates in the nucleus. The process is known as an asymmetrical rolling-circle mechanism and involves the host enzymes (Flores *et al*., 2009). Members of this family share a central conserved region (CCR) which is important to import the viroid into the nucleus, and multiple host factors are likely to be involved in this process (Abraitiene *et al*., 2008). Movement of the viroid from the nucleus to other cells is possible via plasmodesmata to neighbouring cells and via the phloem for long-distance transport. This process is probably dependent on the RNA structural features of the viroid and host proteins (Takeda and Ding, 2009). Little is known about the synergistic effects of viroid coinfection. A possible synergistic mechanism of CBCVd with HLVd was investigated at the transcriptome level, where different dynamic changes in the hop transcriptome were observed in single and mixed infections (Štajner *et al*., 2019).

As for other viroids, CBCVd can be spread by vegetative propagation and mechanically. It is one of the virus viroid species detected in the citrus 225-T isolate, which is known to be a graft-transmissible dwarfing agent used to dwarf grapefruit trees (Puchta *et al*., 1991).

There are no other reports of other possible transmission routes in citrus plants. In hops, distribution in fields also suggests mechanical spread within rows by cultivation practices and between fields by exchange of infected planting material and contaminated machinery (Jakse *et al*., 2015) (see Pathways for movement).

A transmission study excluded major hop pests as vectors for CBCVd on hop plants (Radišek *et al*., 2018).

In hops, it has been confirmed that CBCVd is not pollen transmissible. In transgenic tobacco (*Nicotiana tabacum*) carrying the CBCVd sequence in its genome, its transcripts in pollen were dramatically reduced during pollen development, suggesting that CBCVd is also not pollen transmissible (Matoušek *et al*., 2020).

DETECTION AND IDENTIFICATION

Symptoms

CBCVd has natural hosts among citrus species and citrus relatives on which it induces mild symptoms or no symptoms at all (Barbosa *et al*., 2002; Duran-Vila *et al*., 1988). CBCVd causes severe symptoms on hops (Jakše *et al*., 2015) and CBCVd-pis variant infections in pistachio are asymptomatic (Al Rwahnih *et al*., 2018). Among experimental herbaceous hosts, symptoms can be observed on tomato, *Gynura* and chrysanthemum (Semancik and Vidalakis, 2005).

On citrus species

CBCVd is a minor pathogen on citrus species, which induces bark cracking on the rootstock trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and Carrizo citrange (*P. trifoliata x Citrus sinensis*) (Murcia *et al*., 2015; Vernière *et al*., 2004; 2006). Under the cracks, green streaks with protuberances can be observed which are located in the depressions on the wood. Studies showed that single CBCVd infections have minor negative effect on growth and yield in citrus plants grafted on trifoliate orange (Verniére *et al*., 2004; 2006), whereas plants grafted on Carrizo citrange showed significant reduction in tree height and canopy volume (Murcia *et al*., 2015). Reduction of yield was also observed in trees co-infected with HSVd, whereas mixed infection with CEVd showed that CBCVd could suppress symptoms in CEVd infected plants (Verniére *et al*., 2006). In addition, CBCVd infection of the susceptible viroid bioindicator host ‘Etrog’ citron (*Citrus medica*) Arizona 861-S-1 induces moderate stunting and random leaf epinasty associated with mid-vein and petiole necrosis (Timmer *et al*., 2000).

On hops

CBCVd causes severe symptoms on hops, which include plant stunting resulting from a shortening of the internodes of main and lateral branches, leaf yellowing and down curling, small cone formation and dry root rot (Jakše *et al.*, 2015). Infected plants normally begin to sprout in spring; so the first symptoms seen as slower growth are observed in end of May or early June (over BBCH 35), depending on weather conditions and variety.

Later, during the vegetative season, the occurrence of disease symptoms intensifies, with a distinctive shortening of internodes of the main bines and of the lateral branches. Main bines also show intensive longitudinal bark cracking, and some smaller cracks can also be found on lateral bines. At the top of the plant, plants often undergo some decline in the parts attached by the strings and their climbing upwards is disturbed.

In most cases, the infected plants fail to reach the level of the trellis and begin to blossom up to 10 days prior to the uninfected plants. The leaves remain smaller, and they turn yellowish with down curling edges. In some varieties leaves also show intensive chlorotic speckling. The cones are distinctly smaller and lighter. The disease severely affects the root system with dry rot development which leads to a complete dieback of the entire root system. Severity of symptoms is also dependent on the susceptibility of the variety and weather conditions, with more severe stunting and early symptom development observed in years with higher temperatures (Radišek, 2017).

The described symptoms are similar to hop stunt viroid (HSVd) infections in terms of effect on individual hop tissues (Sano, 2003; Eastwell and Sano, 2009). However, the incubation period of CBCVd infected plants is significantly shorter and disease progression is much faster. For HSVd the first signs of disease may be expected 3 to 5 years after infection, whilst for CBCVd, the first significant symptoms (stunting) can be seen 1 year after infection. In field conditions CBCVd infected plants (susceptible varieties) die off completely between 3 and 5 years after infection (Jakse *et al.*, 2015), whilst the HSVd-infected plants survive for 10 or more years (Eastwell and Sano, 2009).

Morphology

CBCVd consists of a circular single-stranded RNA molecule, of 284 nucleotides (nt) (NCBI GenBank). The nucleotides form a viroid-specific rod-like secondary structure in which 63 G:C, 32 A:U and 8 G:U pairs are present so that approximately 71% of all its nucleotides are base-paired (Puchta *et al.*, 1991). Like other viroids of the family *Pospiviroidae*, CBCVd contains five structural domains: terminal (left and right), pathogenicity, central and variable (Di Serio *et al.*, 2014). As a member of the genus *Cocadviroids*, CBCVd has a central conserved region (CCR) that differs from other genera of the *Pospiviroidae* family and has a terminal conserved hairpin (TCH) (Di Serio *et al.*, 2021; Wang *et al.*, 2018).

Detection and inspection methods

Plants in the field are asymptomatic in the first year of infection and this asymptomatic period carries over to the following spring. CBCVd infected hop plants can therefore only be recognised by visual inspection in the second year of infection in late spring and in the summer period of vegetation (over BBCH 35). The exception is in artificially infected plants of susceptible varieties on which mild symptoms can be observed on leaves 4 months post inoculation (Jakše *et al.*, 2015). On citrus plants bark cracking might be observed on trifoliate orange rootstock and on hybrid rootstock citrange (Murcia *et al.*, 2015). Since similar symptoms to CBCVd infections may be induced by other biotic and abiotic factors, detection and identification requires testing. Because of the incubation period, asymptomatic testing is also crucial for early detection in plants for planting material.

For CBCVd detection different methods have been developed including biological indexing (Duran-Vila and Semacik, 2003), molecular hybridisation (Malfitano *et al.*, 2005, Murcia *et al.*, 2009), polyacrylamide gel electrophoresis (PAGE) (Duran-Vila *et al.*, 1988), PCR methods (RT-PCR, RT-real-time PCR) (Bernad and Duran-Vila, 2006; Ito *et al.*, 2002, 2003; Osman *et al.*, 2017; Wang *et al.*, 2009, 2013) and next generation sequencing (NGS) (Al Rwahnih *et al.*, 2018; Jakše *et al.*, 2015).

For routine detection the preferred testing methods are RT-PCR and RT- real-time PCR. Specificity testing of different RT-PCR primers showed that primers developed by Bernad and Duran-Vila (2006) and Ito *et al.* (2002) are

the most reliable for CBCVd detection in hops as well in citrus hosts (Gu?ek *et al.*, 2019). Vidalakis and Wang (2014) developed RT- real-time PCR test for the universal detection of citrus viroids, including CBCVd, which is routinely used in Citrus Nursery Stock Pest Cleanliness Program in California, and Seigner *et al.* (2020) developed RT-real-time PCR for CBCVd detection in hops.

PATHWAYS FOR MOVEMENT

CBCVd can be transmitted by vegetative propagation or mechanically (by contact between neighbouring plants, by contact with plant sap contaminated tools (e.g. during grafting, pruning), via clothing, and via machinery) (Barbosa *et al*., 2005; Radišek *et al*., 2019). The spread of CBCVd in hop gardens is extremely rapid since hop cultivation practices often cause mechanical damage on plants. The most damaging practices include pruning, other spring operations (e.g. the training of shoots), and harvesting (e.g. when the majority of green parts are cut down). The disease was reported to infect up to 20% of new hop plants per year, mainly along plant rows, as a result of intensive cultivation practices during the vegetative period (Jakše *et al*., 2015). Cultivation practices such as returning fresh hop waste back to hop gardens is also responsible for local spread. In contrast to hop, surveys on citruses have demonstrated a relative low incidence and progression in commercial orchards (Duran-Vila and Semancik, 2003; Semancik and Vidalakis, 2005). Citrus plants for planting are generally not grown in hop growing areas (they may be present as ornamentals, but not in production, and consequently the likelihood of transfer to hop crops is low). There are no reports of seed, pollen or vector transmission; however, additional studies should be done in the future to confirm that such pathways do not present any risk.

The main pathways for long distance spreading are plants for planting and other plant material (e.g. parts of plants). In particular, infected citrus fruits might present a risk if the host plants are exposed to infected citrus waste (peels), however such infections are extremely rare since most household waste ends up in regulated disposal facilities (Radišek *et al*., 2019). Hop cones are not considered as a pathway since they are directly used in brewing processes. CBCVd on machinery is not considered able to survive long distance transport. Long distance spread via tools and persons (clothing and footwear) is considered unlikely.

PEST SIGNIFICANCE

Economic impact

CBCVd on citruses is considered as a disease agent of minor importance (Semancik & Vidalakis, 2005). Single CBCVd infections are directly associated with bark cracking on trifoliate orange rootstock and on hybrid rootstock citrange, whereas co-infections with other citrus viroids can cause economical important diseases such as exocortis and cachexia (Vernière *et al*., 2004; 2006).

In contrast to the situation in citruses, CBCVd causes high economic damage on hop. Hop is a deciduous climbing herbaceous perennial plant cultivated for the production of female inflorescences, termed cones, which are primarily used in beer production. In the EPPO region, hops are grown on approximately 30 000 ha in 13 countries, of which Germany, Czech Republic, England, Poland and Slovenia provide the majority of European hop production (IHGC, 2021). Hop production requires high investments for mechanization and the extensive and long-term field support system (trellis). The plants are often cultivated for more than 20 years, therefore fast CBCVd spread and aggressive disease development causes major losses. To reestablish production after an outbreak, crop rotation (for a minimum of 2 years) is a prerequisite and farmers need to invest to obtain new healthy (certified) planting material (Radišek *et al*., 2017). In Slovenia, since the first finding of the stunted plants in 2007, almost 500 ha of hop gardens have been affected of which approximately 300 ha were removed and destroyed in order to eradicate the viroid. The majority of the plants (235 ha) were removed and destroyed in 2019 and 2020 when the new (stricter) official state CBCVd eradication program was established, and reached costs of approximately 4 million EUR (MKGP, 2019, 2021). This estimation does not include human costs and material costs for disinfection and for adapted cultivation practices by the farmers. Therefore, the final economic impact was evaluated at more than 4.2 million EUR (EPPO, 2021).

Economic losses in hop production areas are comparable to eradication of HSVd outbreaks in Japan in the period 1977-1987, where more than 120 ha were removed and replanted with HSVd-free planting material (Sano, 2013).

Due to CBCVd being a more severe disease compared to HSVd, and the fact that there is more extensive hop production in Europe compared to Japan, even higher economic losses may result from a wider spread and from the non-implementation of control measures.

Control

As with other viroids, CBCVd infections cannot be directly cured by chemical or biological treatments (Barba *et al*., 2017). Therefore, disease management relies first on prevention, and in the case of outbreaks eradication measures should be taken based on a risk analysis (Singh *et al*., 2003).

Planting of CBCVd-free material is the basic step to prevent infections and to stop spread of the viroid into new regions. Production of planting material should be done under certification programs or schemes which include regular testing and inspections of mother plants and nuclear stock to provide guarantees that the propagative material is free from CBCVd. Since CBCVd is mechanically transmissible, it is important that propagation facilities regularly clean and disinfect tools and equipment, and that staff use separate clothes from those worn in the fields, to prevent CBCVd introduction via human activities. Disinfection and cleaning are also important for farms which share equipment or seasonal workers. In the case of infected germplasm, CBCVd can be eliminated from citrus with shoot-tip-grafting (Navarro *et al.,* 1975). In hops, the hop latent viroid (HLVd) elimination technique consisting of growing '*in vitro*' meristematic tissue is well documented (Morton *et al*., 1993) and could potentially be used also for CBCVd.

In the case of hop, measures are needed to prevent further spread. Symptomatic plants should be removed and destroyed as soon as possible. Because of the one-year incubation period, it is possible that some neighbouring plants are already infected but symptomless. For this reason, it is important that plants in an adequate buffer zone are removed and destroyed around infected plants. Infected plants and plants from this buffer zone should be destroyed on appropriate disposal sites by composting, burning or they can be buried. Changes in cultivation practices are also important to prevent or slow down spread of this pest, for example by reducing the number of cultivation operations and by the disinfection/cleaning of equipment and tools when used in different fields. Eradication measures should include the removal of as many remaining roots as possible, a crop rotation with non-host plants and if necessary an herbicide treatment on the total surface of the field.

Monitoring for CBCVd symptoms and testing is important in hop gardens, especially in areas where CBCVd is present, to detect outbreaks in the initial stage of infection. CBCVd testing of 32 hop varieties and genotypes from different areas worldwide revealed differences in susceptibility. Most genotypes were classified as highly sensitive, whereas few genotypes showed a moderately sensitive response and tolerance (Radišek *et al*., 2018). Genetic mechanisms of tolerance/resistance are still unexplored.

Phytosanitary risk

Infections on hop were, until 2021, only reported in Slovenia and Germany (Jakše *et al*., 2015; EPPO, 2019). Based on pest risk analyses (PRAs), CBCVd was assessed as presenting a significant risk of further spreading in the hop growing countries of the EPPO region (Radišek & Benko-Beloglavec, 2016; Wilstermann *et al*., 2020). In terms of spread, it is necessary to emphasize the high density of hop gardens in all EU hop growing regions, which increases the risk of disease spreading among farms. Vegetative propagation and the one-year incubation period present a high risk for the spread of CBCVd in propagation facilities as well as for long distance spreading by plants for planting material. CBCVd would be expected to cause similar impacts in other hop production areas to those reported in Slovenia, thus causing the death of hop plants and therefore considerably affecting the brewing industry.

PHYTOSANITARY MEASURES

Slovenia, as the first country with CBCVd hop infections, established national quarantine regulations on this viroid in 2015. Based on a PRA performed by Slovenia in 2016 (Radišek & Benko-Beloglavec, 2016) and an express PRA revised by Germany in 2020 (Wilstermann *et al*., 2020), EPPO recommends the following management measures on hop plants (EPPO, 2021): Hop plants for planting (except seeds) should be produced in a pest free area or in a pest free place/site of production. When produced in a pest free place/site of production, personnel working in such

facilities should apply hygiene protocols appropriate for CBCVd. An alternative to the production in a pest free area, or in a pest free place/site of production, is the testing of all plants, or a post-entry quarantine with visual checks and testing (in the framework of a bilateral agreement).

In addition to the measures to be implemented by the exporting countries, EPPO encourages importing countries to clean and disinfect used machinery and tools/equipment to prevent entry of CBCVd in places/sites of production that also grow host plants. Citrus fruit waste should be disposed of safely, preferably not on agricultural land.

Eradication may be possible if CBCVd is confirmed in a small area but requires stringent measures that are mostly based on the systematic controls, eradication of infected plants and plants in a buffer zone, hygiene measures and production of certified planting material (see Control).

When not regulated as a quarantine pest, CBCVd could be regulated as a regulated non-quarantine pest (RNQP): In 2021, CBCVd was added to the draft revised EU list of RNQP for hop plants for planting other than pollen and seeds (draft revised Commission Implementing Regulation (*EU*) 2019/2072).

REFERENCES

Abraitiene A, Zhao Y & Hammond R (2008) Nuclear targeting by fragmentation of the potato spindle tuber viroid genome. *Biochemical and Biophysical Research Communications* **368**(3), 470-475.

Al Rwahnih M, Rowhani A, Westrick N, Stevens K, Diaz-Lara A, Trouillas FP, Preece J, Kallsen C, Farrar K & Golino D (2018) Discovery of viruses and virus-like pathogens in pistachio using High-Throughput Sequencing. *Plant Disease* **102**, 1419-1425

Barba M, Hosakawa M, Wang QC, Taglienti A, & Zhang Z (2017) Viroid elimination by thermotherapy, cold therapy, tissue culture, in vitro micrografting, or cryotherapy. In *Viroids and Satellites* (eds. Hadidi A, Flores R, Randles JW, Palukaitis P), pp. 425-435. Academic Press, London (UK).

Barbosa C, Pina JA, Perez-Panades J, Bernad L, Serra P, Navarro L & Duran-Vila N (2005). Mechanical transmission of citrus viroids. *Plant Disease* **89**, 749-754.

Barbosa CJ, Pina JA, Navarro L & Duran-Vila N (2002) Replication/accumulation and symptom expression of citrus viroids on some species of citrus and related genera. *Fifteenth IOCV Conference* **15**, 264–271.

Bernad L & Duran-Vila N (2006). A novel RT-PCR approach for detection and characterization of citrus viroids. *Molecular and Cellular Probes* **20**, 105-113.

Di Serio F, Flores R, Verhoeven JTJ, Li S-F, Palla´s V, Randles JW, Sano T, Vidalakis G & Owens RA (2014) Current status of viroid taxonomy. *Archives of Virology* **159**, 3467-3478.

Di Serio F, Owens RA, Li SF, Matoušek J, Pallás V, Randles JW, Sano T, Verhoeven JTJ, Vidalakis G & Flores R (2021) ICTV Report Consortium. ICTV Virus Taxonomy Profile: Pospiviroidae. *Journal of General Virology* **102**, 2.

Duran-Vila N & Semancik J (2003) Citrus viroids. In *Viroids* (eds. Hadidi, A, Flores R, Randles JW, Semancik, JS) pp. 178-194. CSIRO Publishing, Collingwood (AU).

Duran-Vila N, Roistacher CN, Riverabustamante R & Semancik JS (1988) A definition of citrus viroid groups and their relationship to the exocortis cisease. *Journal of General Virology* **69**, 3069-3080.

Eastwell KC & Sano T (2009) Hop Stunt. In: *Compendium of Hop Diseases and Pests* (eds Mahaffee WF, Pethybridge SJ, Gent DH) pp. 48-50 The American Phytopathological Society, St. Paul, (USA).

EPPO (2015) EPPO Reporting Service no. 06 - 2015 Num. article: 2015/112, Citrus bark cracking viroid is causing ‘severe hop stunt disease’ in Slovenia: addition to the EPPO Alert List.

EPPO (2017) EPPO Reporting Service no. 09 - 2017 Num. article: 2017/158, New additions to the EPPO A1 and A2

Lists.

EPPO (2019). EPPO Reporting Service no. 08 - 2019 Num. article: 2019/165, First finding of Citrus bark cracking viroid (CBCVd) in Germany.

EPPO (2021) Report of a pest risk analysis for Citrus bark cracking viroid. EPPO, Paris. Available at https://gd.eppo.int/taxon/CBCVD0/documents

Fisher L, Bennett S, Tennant P & Mc Laughlin W (2010) Detection of Citrus tristeza virus and citrus viroid species in Jamaica. In *International Symposium on Tropical Horticulture* **894**, 117-122.

Flores R, Gas ME, Molina-Serrano D, Nohales MÁ, Carbonell A, Gago S, De la Peña M & Daròs JA (2009) Viroid replication: rolling-circles, enzymes and ribozymes. *Viruses* **1**(2), 317-34.

Flores R, Randles JW, Bar-Joseph M & Diener TO (1998) A proposed scheme for viroid classification and nomenclature. *Archives of Virology* **143**, 623-629.

Garnsey S, Zies D, Irey M, Sieburth P, Semancik J, Levy L & Hilf M (2002) Practical field detection of citrus viroids in Florida by RT-PCR. In *International Organization of Citrus Virologists Conference Proceedings* (1957-2010).

Gu?ek T, Jakše J, Matoušek J & Radišek S (2019) One-Step multiplex RT-PCR for simultaneous detection of four viroids from hop (*Humulus lupulus* L.). *European Journal of Plant Pathology* **154,** 273-286.

IHGC (2021) International Hop Growers' Convention Country Reports. IHGC - Economic Commission Summary Reports. June 21, 2021. Available at http://www.hmeljgiz.si/ihgc/doc/2021_JUN_IHGC_%20EC_SummaryTable.pdf

Ito T, Ieki H, Ozaki K, Iwanami T, Nakahara K, Hataya T, Ito T, Isaka M & Kano T (2002) Multiple citrus viroids in citrus from Japan and their ability to produce exocortis-like symptoms in citron. *Phytopathology* **92**, 542-547.

Jakse J, Radisek S, Pokorn T, Matousek J & Javornik B (2015) Deep?sequencing revealed Citrus bark cracking viroid (CBCVd) as a highly aggressive pathogen on hop. *Plant Pathology* **64**, 831-842.

Kunta M, da Graca JV & Skaria M (2007) Molecular detection and prevalence of citrus viroids in Texas. *Hortscience* **42**, 600-604.

Lavagi I, Matoušek J, Vidalakis G, Hadidi A, Flores R, Randles JW & Palukaitis P. In *Viroids and Satellites* (eds. Hadidi A, Flores R, Randles JW, Palukaitis P), pp. 275-287. Academic Press, London (UK).

Malfitano M, Barone M, Duran-Vila N & Alioto D (2005) Indexing of viroids in citrus orchards of Campania, southern Italy. *Journal of Plant Pathology* **87**, 115-121.

Matoušek J, Siglová K, Jakše J, Radišek S, Brass JRJ, Tsushima T, Gu?ek T, Duraisamy GS, Sano T, Steger G (2017). Propagation and some physiological effects of Citrus bark cracking viroid and Apple fruit crinkle viroid in multiple infected hop (*Humulus lupulus* L.). *Journal of Plant Physiology* **213**, 166-177.

Matoušek J, Steinbachová L, Drábková LZ, Kocábek T, Pot?šil D, Mishra AK, Honys D & Steger G (2020) Elimination of viroids from tobacco pollen involves a decrease in propagation rate and an increase of the degradation processes. *International Journal of Molecular Sciences* **21**, 3029.

Mishra A, Kumar A, Mishra D, Nath V, Jakše J, Kocábek T, Killi U, Morina F & Matoušek J (2018) Genome-wide transcriptomic analysis reveals insights into the response to Citrus bark cracking viroid (CBCVd) in hop (*Humulus lupulus* L.). *Viruses* **10**, 570.

Mishra AK, Duraisamy GS, Matoušek J, Radisek S, Javornik B & Jakse J (2016) Identification and characterization of microRNAs in Humulus lupulus using high-throughput sequencing and their response to Citrus bark cracking viroid (CBCVd) infection. *BMC genomics* **17**, 919.

MKGP (2019) Program izvedbe ukrepov za prepre?evanje širjenja in zatiranja viroidnih zakrnelosti hmelja za obdobje 2019-2021/Program for implementation of measures for prevention and control of hop stunt diseases for the period 2019-2021. Republic of Slovenia, Ministry of Agriculture, Forestry and Food, no. 007-236/2019/14.

MKGP (2021) Spremememba programa izvedbe ukrepov za prepre?evanje širjenja in zatiranja viroidnih zakrnelosti hmelja za obdobje 2019-2021/Modification of the program for the implementation of measures to prevent the spread and control of hop stunt diseases for the period 2019-2021. Republic of Slovenia, Ministry of Agriculture, Forestry and Food, no. 34300-1/2021/3.

Mohamed ME, Hashemian SMB, Dafalla G, Bové JM & Duran-Vila N (2009) Occurrence and identification of citrus viroids from Sudan. *Journal of Plant Pathology* **91**, 185-190.

Morton A, Barbara DJ & Adams AN (1993). The distribution of hop latent viroid within plants of Humulus lupulus and attempts to obtain viroid-free plants. *Annals of Applied Biology* **123**, 47-53.

Murcia N, Hashemian SMB, Serra P, Pina JA & Duran-Vila N (2015) Citrus viroids: Symptom expression and performance of Washington navel sweet orange trees grafted on Carrizo citrange. *Plant Disease* **99**, 125-136.

Murcia N, Serra P, Olmos A & Duran-Vila N (2009) A novel hybridization approach for detection of citrus viroids. *Molecular and Cellular Probes* **23**, 95-102.

Osman F, Dang T, Bodaghi S & Vidalakis G (2017) One-step multiplex RT-qPCR detects three citrus viroids from different genera in a wide range of hosts. *Journal of Virological Methods* **245**, 40-52.

Navarro L, Roistacher CN & Murashige T (1975) Improvement of shoot tip grafting in vitro for virus-free citrus. *Journal of the American Society for Horticultural Science* **100**, 471479.

Nath VS, Mishra AK, Awasthi P, Shrestha A, Matoušek J, Jakse J, Kocábek T & Khan A (2021) Identification and characterization of long non-coding RNA and their response against citrus bark cracking viroid infection in *Humulus lupulus*. *Genomics* **113**, 2350-2364.

Nath VS, Shrestha A, Awasthi P, Mishra AK, Kocábek T, Matoušek J, Se?nik A, Jakše J, Radišek S & Hallan V (2020) Mapping the gene expression spectrum of mediator subunits in response to viroid Infection in plants. *International Journal of Molecular Sciences* **21**, 2498.

Pokorn T, Radišek S, Javornik B, Štajner N & Jakše J (2017) Development of hop transcriptome to support research into host-viroid interactions. *PLOS ONE* **12**, e0184528.

Puchta, H, Ramm K, Luckinger R, Hadas R, Barjoseph M & Sanger HL (1991) Primary and secondary structure of citrus viroid-iv (CVd-IV), a new chimeric viroid present in dwarfed grapefruit in Israel. *Nucleic Acids Research* **19**, 6640-6640.

Radišek S & Benko-Beloglavec A (2016) Pest risk analysis for Citrus bark cracking viroid (CBCVd). Slovenian Institute of Hop Research and Brewing. Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection. Available at https://gd.eppo.int/taxon/CBCVD0/documents

Radišek S, Gu?ek T, Leskošek G, Benko Beloglavec A, Jakše J & Javornik B (2017) Huda viroidna zakrnelost hmelja/Severe hop stunt disease. Žalec: Inštitut za hmeljarstvo in pivovarstvo Slovenije, Žalec (SI), ISBN 978-961-93322-2-1. http://www.ihps.si/wp-content/uploads/2016/08/Huda-viroidna-zakrnelost-hmelja-2017.compressed.pdf

Radišek S, Gu?ek T, Matoušek J & Jakše J (2018) Citrus bark cracking viroid: biological properties and impact on Slovene hop production. In: *Viroid-2018: book of abstracts, International Conference on Viroids and Viroid-Like RNAs*, 5-7 July 2018, Valencia, Spain.

Radišek S, Gu?ek T, Dobrajc M, ?erenak A, Matoušek J & Jakše J (2019) Transmission pathway studies of citrus bark cracking viroid (CBCVd) on hop. V: Proceedings of the Scientific-Technical Commission [of the] International Hop Growers' Convention I.H.G.C., Bischoffsheim, France, 7-11 July 2019, ISSN 2512-3785). Wolnzach: Scientific-

Technical Commission, I.H.G.C. 2019, 39.

Sano T (2003) Hop stunt viroid. In *Viroids* (eds. Hadidi, A, Flores R, Randles JW, Semancik, JS) pp. 207-212. CSIRO Publishing, Collingwood (AU).

Sano T (2013). History, origin, and diversity of hop stunt disease and Hop stunt viroid. *Acta Horticulturae* **1010**, 87-96.

Seigner L, Liebrecht M, Keckel L, Einberger K & Absmeier C (2020) Real-time RT-PCR detection of Citrus bark cracking viroid (CBCVd) in hops including an mRNA-based internal positive control. *Journal of Plant Diseases and Protection* **127**, 763–767.

Semancik JS & Vidalakis G (2005) The question of Citrus viroid IV as a Cocadviroid. *Archives of Virology* **150**, 1059-1067.

Singh RP, Randles JW & Hadidi (2003) Strategies for the control of viroid diseases, In *Viroids* (eds. Hadidi, A, Flores R, Randles JW, Semancik, JS) pp. 295-302. CSIRO Publishing, Collingwood (AU).

Sukumari Nath V, Kumar Mishra A, Kumar A, Matoušek J & Jakše J (2019) Revisiting the Role of Transcription Factors in Coordinating the Defense Response Against Citrus Bark Cracking Viroid Infection in Commercial Hop (Humulus Lupulus L.). *Viruses* **11**, 419.

Vernière C, Perrier X, Dubois C, Dubois A, Botella L, Chabrier C, Bové JM & Vila ND (2004) Citrus viroids: Symptom expression and effect on vegetative growth and yield of clementine trees grafted on trifoliate orange. *Plant Disease* **88**, 1189-1197

Vernière C, Perrier X, Dubois C, Dubois A, Botella L, Chabrier C, Bové JM & Vila ND (2006) Interactions between citrus viroids affect symptom expression and field performance of clementine trees grafted on trifoliate orange. *Phytopathology* **96**, 356-368.

Vidalakis G & Wang J (2014) Molecular method for universal detection of citrus viroids. p. 19 *United States Patent* US 8815547 B2 (USA).

Wang JI, Boubourakas N, Voloudakis AE, Agorastou T, Magripis G, Rucker TL, Kyriakopoulou PE & Vidalakis G (2013) Identification and characterization of known and novel viroid variants in the Greek national citrus germplasm collection: threats to the industry. *European Journal of Plant Pathology* **137**, 17-27.

Wang X, Zhou C, Tang K, Zhou Y & Li Z (2009) A rapid one-step multiplex RT-PCR assay for the simultaneous detection of five citrus viroids in China. *European Journal of Plant Pathology* **124**, 175-180.

Wang YS, Atta X, Wang F, Yang C, Zhou & Cao M (2018) Transcriptome sequencing reveals novel Citrus bark cracking viroid (CBCVd) variants from citrus and their molecular characterization. *PLOS ONE* **13**, e0198022.

Wilstermann A, Schrader G, Pfeilstetter E, Schäfer BC & Ziebell H (2020) Express PRA for Citrus bark cracking viroid. Julius Kühn-Institute, Institute for national and international Plant Health. Replaces version of 2019-07-30. Available at https://pra.eppo.int/pra/0a52821b-0965-4d92-9af1-9c4e932a091a

Takeda R & Ding B (2009). Viroid intercellular trafficking: RNA motifs, cellular factors and broad impacts. *Viruses* **1**(2), 210-221.

Timmer LW, Garnsey SM & Graham JH (2000) Compendium of citrus diseases. APS Press, St. Paul, MN.

Štajner, Radišek NS, Mishra AK, Nath VS, Matoušek J & Jakše J (2019) Evaluation of disease severity and global transcriptome response induced by Citrus bark cracking viroid, Hop latent viroid, and their co-infection in hop (*Humulus lupulus* L.). *International Journal of Molecular Sciences* **20**, 31-54.

ACKNOWLEDGEMENTS

This datasheet was prepared in 2021 by Dr Sebastjan Radišek, Slovenian Institute for Hop Research and Brewing, Plant Protection Department, Žalec (SI); Prof Dr Jernej Jakše, University of Ljubljana, Biotechnical Faculty (SI) and Anita Benko Beloglavec, Ministry of Agriculture, Forestry and Food, Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection. Their valuable contributions are gratefully acknowledged.

How to cite this datasheet?

EPPO (2025) *Cocadviroid rimocitri*. EPPO datasheets on pests recommended for regulation. Available online. https://gd.eppo.int

Datasheet history

This datasheet was first published online in 2021. It is 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.

Co-funded by the European Union