Express PRA[1] for Citrus bark cracking viroid – Occurrence –

Prepared by: Julius Kühn-Institute, Institute for national and international Plant Health; Dr. Anne Wilstermann, Dr. Gritta Schrader, *Dr. Ernst Pfeilstetter, Dr. Bernhard Carl Schäfer, Dr. Heiko Ziebell (JKI-Epidemiology and Pathogen Diagnostics); on:* 26-03-2020 (*replaces version of: 30-07-2019*). (translated by: Elke Vogt-Arndt)

Dr. Sebastjan Radišek (Slovenian Institute for Hop Research and Brewing) provided helpful information in respect to the Slovenian infestation area.

Initiation: Occurrence on hop in the Federal State Bavaria

Initiation for the revision: *New scientific information*

Express PRA	**Citrus bark cracking viroid**		
Phytosanitary risk for Germany	**high** ☒	**medium** ☐	**low** ☐
Phytosanitary risk for EU-Member States	**high** ☒	**medium** ☐	**low** ☐
Certainty of the assessment	**high** ☒	**medium** ☐	**low** ☐
Conclusion	For the first time, the citrus bark cracking viroid (CBCVd) was detected in citrus cultivation in California (USA) in 1988. *The first detection in hop cultivation in Germany was in 2019*. The occurrence of CBCVd is known from Greece, Italy and Slovenia. So far, it is not listed in the Annexes of *Implementing Regulation (EU) 2019/2072* but since 2017, it is included in the EPPO-A2-List. *In 2015,* after the occurrence in hop, Slovenia took emergency measures against the introduction and distribution of the viroid *that were adapted to the rapid infestation development in 2019.* CBCVd is mainly known as a viroid with low damage potential for citrus plants. In 2007, firstly symptoms of CBCVd were observed on hop in Slovenia. Infected plants die after 3 - 5 years. Due to appropriate climate conditions, it is assumed that CBCVd is able to establish outdoors in *the hop cultivation regions in* Germany. The establishment in South European EU Member States (*Italy and Greece*) in *Citrus* spp. did already happen. Due to its high damage potential for hop, CBCVd bears a high phytosanitary risk for Germany and other EU Member States with hop cultivation. Based on this risk analysis, it is assumed that the harmful organism is able to establish in Germany or another Member		

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	State and cause severe damage. Thus, measures on the prevention of the introduction *and spread on hop plants* of this potential quarantine pest should be met according to *Article 29 Regulation (EU) 2016/2031*. An infection has to be notified and eradicated accordingly. *It is recommended to conduct a comprehensive monitoring on hop in the endangered areas in respect to this harmful organism.*
Taxonomy[2)]	Kingdom: Viruses and viroids; Class: viroids; Family: Pospiviroidae; Genus: Cocadviroid; Species: Citrus bark cracking viroid (CBCVd)
Common name	Severe hop stunt disease
Synonyms	Citrus viroid IV; Citrus bark cracking cocadviroid
Does a relevant earlier PRA exist?	An Express-PRA for CBCVd on hop is available from Slovenia. The risk for Slovenia is considered high. *Subsequently, this risk analysis was adapted for the complete EPPO-region. Without phytosanitary measures, severe damage through the introduction and establishment in the hop cultivation areas in the EPPO-Member States has to be expected (EPPO, 2016).*
Biology	Viroids are the smallest known pathogens on plants. They consist of a non-encapsulated single-strand RNA-segment propagated by the host plant cell. In hop, CBCVd leads to massive changes in the activity of more than 2000 genes. Amongst others, the changes relate to the immune defence, signal pheromones, the pigment metabolism, the photosynthesis as well as the sugar and protein metabolism (MISHRA *et al.*, 2018). CBCVd is highly infectious for hop. Possible infection sources are infected plants, cutting devices and soil/plant residues. Infected plants may remain symptomless for 4 months to 1 year, but are already infectious. Apparently, pollen, seed and weeds play no relevant role for survival and distribution of CBCVd (RADIŠEK, 2016). In Slovenia, an infection-distribution of up to 20% was observed in hop cultivations, mainly alongside of the planting rows. Thus, it is assumed that the viroid was distributed in the crop through infected cutting tools (JAKSE *et al.*, 2015). The viroid may remain in the soil for years, being infectious without any living host plant (AFSVSPP, 2018), *until remaining infected plant material is decomposed completely.*

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Is the pest a vector?[3)]	No
Is a vector needed?[4)]	No, so far, there is no evidence that CBCVd can be transmitted via vectors.
Host plants	Main hosts are citrus plants (*Citrus* spp.), trifoliate orange (*Poncirus trifoliata*)) and hop (*Humulus lupulus*). *A viroid with a genetic similarity to CBCVd of 87% was detected on* Pistacia vera *in California. Currently, the viroid is known under the name citrus bark cracking viroid-pistachio (*AL RWAHNIH *et al., 2018).* Plants from the family Rutaceae could be artificially infected: kumquat (*Fortunella margarita*, *F. crassifolia*, *F. obovata*), lime (*Microcitrus warburgiana*; *M. australis* x *M. australasica*), *Pleiosperum* sp., Chinese Box-Orange (*Severinia buxifolia*). Further artificially infected hosts are cucumber (*Cucumis sativus*), wax gourd (*Benincasa hispida*), tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*)*, Gynura (Gynura aurantica), Datura (Datura stramonium), Bittersweet Nightshade (Solanum dulcamara)* and Chrysanthemum (*Chrysanthemum morifolium, Chrysanthemum sp.*) (RADIŠEK, 2016).
Symptoms[5)]	On Citrus spp. local necrosis develops on the mid veins of the leaves as well as accidental malposition of the leaves (DURAN-VILA *et al.*, 1988). *The plants show a dwarfed growth which is to be regarded as advantageous in Citrus-cultivation.* Infected hop show dwarf growth. The internodes of the main shoot and the side branches are distinctly shortened. The adhesive hairs of the vines do not develop sufficiently and thus, the plant cannot anchor and grow upwards normally. Infected plants flower up to ten days too early. The hop leaves are smaller. Vesicles develop on the leaves. The leaves of some hop varieties show yellowing and the leaf edges curl. The hop cones are smaller and lighter, abnormally formed and develop less hop glands that contain lupulin. The root of the plant is severely infected. A dry rot develops that leads to the complete dieback of the root system. Firstly, the symptoms are similar to those of a Hop stunt viroid-infection. However, the symptoms develop distinctly faster, already after 4 months to one year after the infection. 3-5 years after the infection with CBCVd, the hop plants die. *Asymptomatic plants are already infectious. The*

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	complete plant may be systemically infected. Pospiviroids are directly dependent on the biological functions of their host plants. There is no evidence that their environmental requirements differ from those of their host plant (RADIŠEK, 2016*). In a mixed infection with the Hop latent viroid (HLVd), the development of symptoms is accelerated and intensified (*ŠTAJNER*, 2019).* So far, no symptoms were observed on other host plants.
Presence of the host plants in Germany[6)]	In Germany, there are 1,165 companies with totally 18,703 ha hop (as of 2016; DESTATIS, 2019). Furthermore, hop occurs wildly in Germany. *In 2019, hop was cultivated on an area of 20,417 ha in Germany (SCHRAMM, 2019).*
Presence of the host plants in the Member States[7)]	In 2017, totally 27,629 ha hop were cultivated in the EU. Apart from Germany, the Czech Republic (4,945 ha), Poland (1,671 ha) and Slovenia (1,591 ha) are the main producers of hops in the EU (FAOSTAT, 2019). Hops grow naturally in all Member States of the EU except Ireland (RADIŠEK, 2016). Citrus plants *(citrus, lime and orange)* are the main hosts for the virus and mainly are cultivated in Spain (*app. 185,000* ha), Italy (*app. 103,000 ha*), Greece (*app. 36,000 ha*) *and Portugal (app. 18,500 ha)*. *In 2018, citrus fruits were harvested from totally 346,501 ha in the EU* (FAOSTAT, *2020*; Results for *2018*).
Known infested areas[8)]	In the EU, the viroid occurs on *Citrus* sp. in Greece and Italy. In 2007, CBCVd occurred on hop for the first time in Slovenia (the detection of the viroid was only in 2014) where it is under eradication (EPPO GD, 2017; *EPPO, 2019*). In 1988, the viroid was described under the name Citrus viroid IV (CVd IV) for the first time on *Citrus* sp. in the USA (California) (DURAN-VILA *et al.*, 1988). Furthermore, the occurrence in *Citrus* sp. is known from Africa (Egypt, South Africa, Sudan, Tunisia), China, Iran, Israel, Japan, Lebanon, Oman, Syria, and the European part of Turkey (EPPO GD, 2017).
Pathways[9)]	Infected plant material (fruits and plants of Citrus; plants / propagation material of hop; *plant parts*) and non-disinfected tools and machines.

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	The risk for the introduction via hop plants into so far non-infected areas is high for plants that were not produced in the frame of a certification system. The possibility of a local distribution of the infection via machines to other companies or production sites is high. *Presumably,* the outbreak in Slovenia *can be traced back to the cultivation of hop on a former extensive illegal disposal site of fruit residues* (RADIŠEK, 2016). *The introduction risk via citrus residues is estimated as low if citrus residues (mainly substrate / compost) are not apllied in hop cultivation areas.* *The commercial cultivation areas of citrus plants and hop do not overlap in the EPPO-region. According to current knowledge, pollen, seed and weeds are not relevant in the distribution and preservation of the infection (RADIŠEK, 2016).* *The origin of the outbreak in Germany unknown.*
Natural distribution[10)]	Unknown
Establishment and distribution to be expected in Germany[11)]	The viroid could establish in all hop cultivation areas in Germany. *The infected area in Slovenia corresponds to the climatic conditions in the hop cultivation areas in Germany.*
Establishment and distribution to be expected in the Member States[12)]	CBCVd did already establish in *Citrus* cultivation in Italy and Greece. The distribution and establishment in the hop cultivation in Czech Republic, Poland and other hop-producing Member States is possible. *Hop is cultivated in Central Europe. The infected area in Slovenia corresponds to the climatic conditions in the important hop cultivation areas in the Member States.* In Slovenia, comprehensive eradication measures against CBCVd are taken (JAKSE *et al.*, 2015; EPPO GD, 2017*; AFSVSPP, 2019*).
Known damage in infested areas[13)]	No severe damage on *Citrus* sp. through the viroid is known. In Slovenia, CBCVd distributed rapidly in infected hop plantings. Infected plants die within 3-5 years. Until 2013, 13 companies in Slovenia were infected and more than 20 ha hop had to be destroyed to prevent further spread of the viroid (JAKSE et al., 2015). Despite the implementation of emergency measures against CBCVd in Slovenia, the viroid spread further in the hop crops *because initially no systematic surveys were carried out and asymptomatic plants remained in the hop gardens (RADIŠEK, pers comment)*. In 2016, 17 companies with an area of 92.3 ha were concerned and even 101.9 ha, in 2017 (AFSVSPP, 2018). *Thus, the emergency*

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	measures in Slovenia against the viroid were intensified in 2019 (AFSVSPP, 2019).
Limitation of the endangered area in Germany	The main cultivation area for hop in Germany (as of: *2019)* is Bavaria *with 16,995 ha in the Hallertau and 415 ha in Spalt. In 2019, 1,547 ha hop were cultivated in the Elbe-Saale-Region, 1,438 ha in Tettnang in Baden-Württemberg and 22 ha in Bitburg in Rhineland Palatinate (SCHRAMM, 2019).*
Damage to be expected in endangered area in Germany[14)]	Hop as a crop has a high economic value. The cultivation of hop is perennial and requires a high cultivation expenditure. The expected economic damage on hop in Germany is very high. *It is assumed that the damage potential of the viroid for hop in Germany is equivalent to those in Slovenia. So far, potentially damage-decreasing factors for Germany are not known.*
Damage to be expected in endangered area in Member States[15)]	Severe damage is expected in countries with significant cultivation of hop *(like Czech Republic, Poland and Slovenia)*. *In the EU, hop is cultivated in 14 countries. In 2018, the total yield was app. 60,000 tons (EUROPEAN COMMISSION, 2019).*
Control feasibility and measures[16)]	An important measure to prevent an outbreak is the use of healthy plant material. The following control measures are based on the *updated* Slovenian emergency measures *(AFSVSPP, 2019) because the former measures in Slovenia could not sufficiently limit the distribution to further hop crops and within the infected companies (AFSVSPP, 2018)*: Infected hop plants *must be* removed from the crop and be destroyed (burning, digging). *Besides the plant proven to be infected all plants in the same row are to be regarded as potentially infected. The two rows in front of and behind the infected plant are regarded as potentially infected, too. That are 5 rows in total (regardless of their length) (AFSVSPP, 2019). The destruction of these potentially infected plants should serve to preserve the remaining hop garden.* The root system must be destroyed with herbicides to prevent new shooting of the plant. The root system has to be removed after at least 14 days to a maximum of 4 months after the chemical treatment and be destroyed, too. Working in crops, tools have to be disinfected in regular intervals to prevent a further spread in the plant stock.

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	In general, hop shoots should not reach the lane to avoid the distribution of the viroid via plant sap on the tyres of the agricultural machines. It is recommended to shorten the hop shoot accordingly, mainly immediately after the harvest. The pruning wounds should be disinfected immediately (RADIŠEK, pers. comment, 2019). Plant material may not be moved from infected to healthy hop gardens. Machines, shoes, cutting tools have to be disinfected *before the use in other hop gardens*. For several years, the surveillance of the crops after the removal of infected plants is necessary (AFSVSPP, 2015). In Germany and Europe, the single approved pesticide against phytopathogenic viroids for the disinfection is MENNO Florades®. After the removal of the infested plants the crop-free interval *of at least two vegetation periods has to follow in which neither hop nor other potential host plants for the viroid (see above) are cultivated (AFSVSPP, 2019)*. *The survivability of the viroid depends on the absence of host plant material. Thus, the survival in the soil corresponds to the decomposition time of the plant residues in the soil, dependant on the climatic conditions, soil type and microbial activity.*
Detection and diagnosis[17)]	*Initially, the symptoms are similar to an infection with the „hop stunt viroid". Nevertheless, the symptoms develop distinctly faster.* The viroid can be detected through single-stage parallel high throughput sequencing and RT-PCR (Reverse-Transkriptase-Polymerase-Chain Reaction) (JAKSE *et al.*, 2015).
Remarks	*The origin of the outbreak in Germany is unknown.*
Literature	AFSVSPP, 2015: Decision on emergency measures against the introduction and spread of viroid hop stunt diseases. Director Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection, 7S. AFSVSPP, 2018: Update on Emergency measures against CBCVd and HSVd on hop (*Humulus lupulus*). Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection. https://www.ippc.int/en/countries/slovenia/eventreporting/201

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Explanations

1) Compilation of the most important directly available information allowing a first preliminary estimation of the phytosanitary risk. This short assessment is necessary for the decision on a notification to EU and EPPO as well as the preparation of a complete risk analysis, for the information of the countries and as a basis for the possible initiation of eradication measures. Regarding the phytosanitary risk especially the possibility of the introduction into and spread in Germany and the Member States as well as possible damage are taken into account.

2) Taxonomic classification – also subspecies; in case that the taxonomical classification is uncertain the JKI-scientist initiates the taxonomic classification, as far as possible.

3) If so, which organism (which organisms) is (are) transmitted and does it (do they) occur in Germany / the MS?

4) If so, which organism serves as a vector and does it occur in Germany / the MS?

5) Description of the pattern of damage and the severity of the symptoms/damage on the different host plants

6) Presence of the host plants in protected cultivation, open field, amenity plantings, forest.
Where, in which regions are the host plants present and to which extent?
How important are the host plants (economical, ecological,..)? Possible origin

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Where, in which regions are the host plants present and to which extent?
How important are the host plants (economical, ecological,..)? Possible origin

8) f. e. acc. to CABI, EPPO, PQR, EPPO Datasheets

9) Which pathways are known for the pest and how important are they for the possibility of introduction? Primarily the transport of the pest over long distances is meant, normally with infested traded plants, plant products or other contaminated articles. This does not comprise the natural spread resulting from introduction.

10) Which pathways are known for the pest and of which relevance are they in respect of the possibility of spread? In this case the natural spread resulting from introduction is meant.

11) under the given prevalent environmental conditions

12) under the given prevalent environmental conditions (native areas and areas of introduction)

13) Description of the economic, ecological/environmental relevant and social damage in the area of origin resp. areas of occurrence up to now

14) Description of the economic, ecological/environmental relevant and social damage to be expected in Germany, as far as possible and required, differentiated between regions

15) Description of the economic, ecological/environmental relevant and social damage to be expected in the EU/other Member States, as far as possible and required, differentiated between regions

16) Can the pest be controlled? Which possibilities of control are given? Are plant health measures conducted in respect to this pest (in the areas of current distribution resp. by third countries)?

17) Description of possibilities and methods for detection. Detection by visual inspections? Latency? Uneven distribution in the plant (sampling)?