**INŠTITUT ZA HMELJARSTVO IN PIVOVARSTVO SLOVENIJE**

**Slovenian Institute of Hop Research and Brewing Slowenisches Institut für Hopfenanbau und Brauereiwesen**

## EXPRESS PEST RISK ANALYSIS FOR HOP STUNT VIROID (HSVd) ON HOP

EPPO PM 5/5(1)

| **Summary1 of the Express Pest Risk Analysis for Hop stunt viroid (HSVd) on hop (*Humulus lupulus*)** |
| --- |
| **PRA area:** Slovenia |
| **Describe the endangered area:** Hop growing areas in Slovenia |
| **Main conclusions:**HSVd has a broad spectrum of host plants, and many of them are non-symptomatic. In Slovenia, HSVd is present on vines and certain stone fruit plants, where it does not cause any significant economic loss. Nevertheless, Slovenia belongs to the major hop growing countries, and due to the specificity of hop production, the transmission from other host plants is assessed as posing a lower risk. Introduction of HSVd with infected hop plants for planting from other countries (Japan, USA, and China) is unlikely. In general, imports of hop planting material from other countries are negligible as the Slovenian hop species are primarily grown in Slovenia. Introduction of HSVd into Slovenia mostly takes place via the import and sales of citrus fruits. The scope of such imports is very high, though most household waste thereof primarily ends up in regulated city dumps. However, transmission to hop or other plants is possible in the case of illegal citrus fruit waste disposal. Imports of citrus plants for planting into Slovenia are relatively low and intended exclusively for the non-commercial use, as ornamentals.At transmission of HSVd to hop, the spread is rapid in particular due to the specific agro-technology in hop growing industry which, at the time of vegetation, provides the ideal conditions for mechanic transmission, and due to a high scope of plant remnants and vegetative hop propagation. As regards the spread it is necessary to emphasize, the high density of hop gardens in all hop growing areas of Slovenia, which are proprietarily intertwined, increasing the risk of disease spread between farms.Impact of HSVd on hop production is very high as the infected plant crop yields are almost halved. Slovenia belongs to the 6 most important hop growing countries in the world, exporting 95 % of hop production per annum, constituting a turnover of around EUR 10 million. If phytosanitary measures for hop would not be implemented high economic losses may be expected which, on account of international interconnectedness of hop production, may extend to other hop growing countries of the European Unionand worldwide. |
| **Phytosanitary risk for the *endangered area*** | High   | Moderate ☐ | Low ☐ |
| **Level of uncertainty of assessment** | High ☐ | Moderate   | Low ☐ |
| ***Other recommendations:***The presence of HSVd on hop was confirmed for the first time in 2011 in Slovenia and from since that time it has officially been monitored with the systematic surveys of plantations, the certification of planting material, and through applicable legislation, aimed at its eradication. Despite finding that HSVd is not the causative agent of hop stunt disease outbreaks in Slovenia, it is recommended to continue the activities in conjunction with those activities linked to the severe viroid hop stunt disease caused by CBCVd so as toconfirm its presence and potential harmfulness. |

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1 The summary should be elaborated once the analysis is completed

Cesta Žalskega tabora 2, SI-3310 Žalec, Slovenia

Phone: 03 71 21 600, Fax: 03 71 21 620, [www.ihps.si,](http://www.ihps.si/) E-mail: tajnistvo@ihps.si

VAT ID No.: (SI) 93987161, Registry No.: 5051762000, Transaction Account No.: 01100-6000006134

# Express Pest Risk Analysis:

Hop stunt viroid (HSVd) on hop (Humulus lupulus)

## Prepared by:

Dr. Sebastjan RADIŠEK Slovenian Institute of Hop Research and Brewing Inštitut za hmeljarstvo in pivovarstvo of Slovenia Plant Health Department/Oddelek za varstvo rastlin, Diagnostic Laboratory/Diagnostični laboratorij, Cesta Žalskega tabora 2,

SI-3310 Žalec Slovenia

**Date:** 5 June 2015

# Stage 1. Initiation

## Reason for performing the PRA:

In 2007, symptoms resembling those of *Hop stunt viroid* (HSVd) were observed in hop gardens in Slovenia. Diagnostic analysis based on screening for a range of all known hop pathogens confirmed the presence of *Hop Stunt viroid* (HSVd) (Radisek *et al*., 2012), the causative agent of hop stunt disease (Eastwell and Sano, 2009; Sano, 2003). Since 2011, when HSVd was detected, official measures have been adopted to prevent further spreading, and systematic surveys have been carried out on the annual basis. Despite the positive identification of HSVd and the development of HSVd representative symptoms, the hop stunt disease in Slovenia showed some characteristics that were not typical of HSVd. The incubation period for the development of symptoms of hop stunt disease is 3–5 years (Sano, 2003), whereas plants in Slovenia have developed severe symptoms in the first year. In addition, RT-PCR detection of HSVd has been unreliable and often limited to hop cones, which is unusual for a systemic pathogen causing such severe symptoms. Further diagnostic research of symptomatic plants using next generation sequencing (NGS) analysis revealed the presence of *Citrus bark cracking viroid* (CBCVd) (Jakse *et al*., 2014) which, until this finding, had been described as a minor pathogen of citrus plants (Semancik and Vidalakis, 2005; Verniere *et al*., 2006). Infection tests using a biolistic inoculation technique proved the high aggressiveness and infectivity of CBCVd on hop and, on the basis of this and the results of NGS and RT-PCR testing of samples from all infected hop gardens, CBCVd was recognised as the causative agent of a new viroid disease on hop, named ‘severe hop stunt disease’ (Jakse *et al*., 2014). Regardless of clarification that HSVd is not the causative agent of the new hop disease in Slovenia, its detection on hop and its pathogenic potential demand risk analysis.

**PRA area:** *Slovenia*

# Stage 2. Pest risk assessment

## Taxonomy:

**Hop stunt viroid (HSVd):**

Kingdom: Viruses and viroids; Class: Viroids; Family: Pospiviroidae; Genus: Hostuviroid

Other scientific names:

* + Cucumber pale fruit viroid
	+ Citrus cachexia viroid
	+ Citrus viroid II
	+ [Dapple plum and peach fruit disease viroid](http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=12886&lvl=3&lin=f&keep=1&srchmode=1&unlock)

English identification: HSVd; EPPO code: HSVD00 Sources of taxonomic classification:

International Committee on Taxonomy of viruses: <http://www.ictvonline.org/>

The National Center for Biotechnology Information (NCBI): <http://www.ncbi.nlm.nih.gov/> EPPO PQR: <http://www.eppo.int/DATABASES/pqr/pqr.htm>

## Pest overview

*Hop stunt viroid* (HSVd) consists of a circular RNA molecule, sized between 294-303bp (NCBI GenBank). The viroid is named after hop as this is the first host plant on which the viroid has been identified and where it causes the viroid hop stunt disease (Sasaki and Shikata, 1977). The disease was detected for the first time already in 1940 in Japan within the areas of the Nagano and Fukushima prefectures where due to the unawareness of the causative agent the disease spread rapidly and in the period 1950-1960 caused epiphytocy in hop plantations of the northern Japan (Yamamoto et al., 1973; Sano, 2003c). In 1977, the causative agent was identified as a viroid-infective RNA and called the *Hop stunt viroid* (HSVd) (Sasaki and Shikata, 1977). The disease was restricted to the area of Japan up to 1988, when it was detected also in Southern Korea (Lee et al., 1988). In 2004, HSVd was confirmed also in the USA (Eastwell and Nelson, 2007), in 2007 in China (Guo et al., 2008) and in 2011 also for the first time in Europe, in Slovenia (Radisek et al., 2012). *Hop stunt viroid* (HSVd) is a viroid with a broad spectrum of host plants as, in addition to hop, it infects also the vines, certain stone fruit plants, citrus trees, and cucumbers. On most of these plants it is restricted to latent infections, which are difficult to detect, whilst the distinctive signs of disease and ensuing economic losses are, in addition to hop, observed only on apricot trees, plum trees, cucumbers, and citrus trees (Astruc et al., 1996; Elbeaino et al., 2012; Shikata 1990; Sano 2003a,b,c; Pallás et al., 2003; Zhang et al., 2009). Taxonomically, HSVd belongs to an extensive viroid family of *Pospiviroidae* where, on account of its specific structure, it singly constitutes the genus of *Hostuviroid.* Characteristic of this viroid is a high level of variability as, to date, more than 90 HSVd variants have been identified, which are phylogenetically arranged into five groups: plum type, hop type tip, citrus type, plum- citrus type, and plum-hop-citrus type (Amari et al., 2001). Most recent research of the HSVd variants from Greece and China has confirmed the existence of novel phylogenetic groups (Elbeaino et al., 2012; Zhang at al., 2012). The rapid adaptation of this viroid is possible through recombination, creating new types and variants which, in turn, differently impact the occurrence of disease in host plants (Sano, 2003abc). In the case of stunted growth of plants occurring in Slovenia, we identified several HSVd variants, and among these, also a novel variant that constitutes a recombination between the vine and citrus variants, and may be classified into the plum-hop-citrus type group (Radišek et al., 2012)

## Signs of Viroid Hop Stunt Disease Caused by HSVd

HSVd causes a serious disease on hop recognized as viroid hop stunt disease. Symptoms on hop include plant stunting resulting from a shortening of the internodes of main and lateral branches, leaf yellowing and down curling, and small cone formation. The first visual symptoms appeared 3-5 years after infection, although reduction of alpha acid content in cones could be detected in latent period. In plants produced from already diseased plants symptoms are evident in the first growing season (Eastwell and Sano, 2009; Sano, 2003).

## Diagnostic analyses of HSVd

In viroid detection, different methods are used, including the biological testing, molecular hybridisation, polyacrylamide gel electrophoresis (PAGE), reverse transcription polymerase chain reaction (RT-PCR), RT-PCR for the simultaneous detection of viroids (mRT-PCR), RT- PCR linked with hybridisation, reverse transcription loop-mediated isothermal amplification (RT-LAMP), and naturally, the quantitative real time RT-PCR (RT-qPCR). Within the recent decade, the routine viroid diagnostics most frequently applies the RT-PCR using the viroid- specific oligonucleotide primers. For the RT-PCR detection of HSVd, more than 10 different oligonucleotide primers (PCR primers) have been developed by scientists in conjunction with the HSVd phylogenetic groups. Among the most frequently used primers, which are appropriate also for HSVd detection on hop, are the PCR primers developed by Astruc et al., 1996; Bernad and Duran-Vila, 2006; Farkas et al., 1999; Hadidi et al., 1992; Matoušek et al., 2003; and Sano et al., 2001. An important part of diagnostic analysis that may impact the detection is also the RNA isolation from the infected plant tissue. The use of commercially available plant RNA isolation kits is advisable.

**3. Is the pest a vector? Yes** ☐ **No**

HSVd and other viroids do not belong among vectors.

**4. Is a vector needed for pest entry or spread? Yes** ☐ **No** 

HSVd has no known vectors. It is transmitted mechanically, by infected plants and infected plant residues.

## Regulatory status of the pest

HSVd is neither listed as harmful organism in Council Directive 2000/29/EC, nor is it on the EPPO list. HSVd is listed in Council Directive 93/48/EC as one of the causal agents of disease (citrus cachexia), which shall be absent in certified citrus planting material production.

## Slovenia (HSVd)

HSVd is regulated within the Slovenian legislation:

* Decision on emergency measures against the introduction and spread of viroid hop stunt diseases (UL RS, 21/2015, 17.03.2015).
* Rules on the marketing of hop propagating material and of hop plants (UL RS, [45/13](http://www.uradni-list.si/1/objava.jsp?urlurid=20131726), 24/15).

**Other (HSVd):** IAPCS A2 list French legislation:

In the Decree of 1990, supplemented by that of 3 December 1991 (JORF 16/02/1992, Annex IIB),

HSVd, identified as “citrus cachexia”, is listed among organisms that may not be introduced into Réunion. Permanent mandatory control was adopted for this viroid in the Decree of 31 July 2000 (JORF 31/08/2000).

## Distribution

Viroid hop stunt disease caused by HSVd is present in the following hop growing areas:

* Japan, 1940 (Yamamoto et al., 1973)
* South Korea, 1988 (Lee et al., 1988)
* USA, 2004 (Eastwell and Nelson, 2007)

- China, 2007 (Guo et al., 2008)

* Slovenia, 2007 (Radisek et al., 2012)

In addition to hop, HSVd may infest certain other plants and has thus been spread outside the hop growing areas.

Table 1: Data on global prevalence of HSVd

| ***Continent*** | ***Distribution*** | ***Provide comments on the pest status in the different countries where it occurs*** | ***Reference*** |
| --- | --- | --- | --- |
| *Africa* | *Algeria* | *present* | Rouag et al., 2008 |
| *Egypt* | *present* | El-Dougdoug et al., 2010 |
| *Libya* | *present* | Nour-Eldin and Fudl-Allah,1976 |
| *Morocco* | *present* | Amari et al., 2000 |
| *Sudan* | *present* | Mohamed et al., 2009 |
| *Tunisia* | *present* | Mahfoudhi et al., 2010 |
| *America and the Caribbean* | *Canada* | *present* | Michelutti et al., 2005 |
| *USA* | *present; outbreaks on hop* | Rezaian et al., 1992; Kunta et al., 2007;Eastwell and Nelson, 2007 |
| *Jamaica* | *present* | Bennett et al., 2010 |
| *Brazil* | *present* | Eiras et al., 2006 |
| *Columbia* | *present* | Murcia et al., 2010 |
| *Uruguay* | *present* | Pagliano et al., 2000 |
| *Asia and the Middle East* | *China* | *present* | Wang et al., 2010;Zhang et al., 2012 |
| *India* | *present* | Roy and Ramachandran, 2003 |
| *Iran* | *present* | Bagherian and Izadpanah, 2010 |
| *Israel* | *present* | Puchta et al., 1989 |
| *Japan* | *spread* | Sasaki and Shikata, 1977 |
| *South Korea* | *present* | Lee et al., 1988 |
| *Lebanon* | *present* | Elbeaino et al., 2012 |
| *Syria* | *present* | Kubaa et al., 2011 |
| *Europe* | *Cyprus* | *present* | Amari et al., 2000 |
| *Czech Republic* | *present* | Matoušek et al., 2003 |
| *Finland* | *present* | Lemmetty et al., 2011 |
| *France* | *present* | Pallás et al., 2003 |
| *Germany* | *present* | Puchta et al., 1989 |
| *Hungary* | *spread on vines* | Farkas et al., 1999 |
| *Greece* | *present* | Amari et al., 2000 |
| *Italy* | *present* | Elbeaino et al., 2012 |
| *Serbia* | *present* | Mandic et al., 2008 |
| *Slovenia* | *present; outbreaks on hop* | Viršček Marn et al., 2006Radisek et al., 2012 |
| *Turkey* | *present* | Amari et al., 2000 |
| *Spain* | *present* | Cañizares et al., 1999 |
| *Oceania* | *Australia* | *present* | Gillings et al., 1991 |
| *New Zealand* | *present* | Ward et al., 2011 |

## Host plants /habitats\* and their distribution in the PRA area

HSVd has a broad spectrum of host plants, including citrus trees, pear trees, peach trees, apricot trees, plum trees, almond trees, jujube trees, vines, and cucumbers. On most of these plants, HSVd is maintained in the form of latent infections that are difficult to notice, whilst the distinctive signs of disease with subsequent economic losses are in addition to hop found only on citrus trees, peach trees, apricot trees, plum trees and cucumbers (Sano, 2003a,b,c).

**HSVd host plants:**

Hop (*Humulus lupulus*)

Pear trees (*Pyrus communis*) Peach trees (*Prunus persica*) Apricot trees (*Prunus armeniaca*) Plum trees (*Prunus domestica*) Almond trees (*Prunus dulcis*) Jujube trees(*Zizypus jujuba*) Vines (*Vitis vinifera*)

Cucumbers (*Cucumis sativus*)

**Citrus trees and related plants of the family of rutaceous plants (Rutaceae):** (*Citrus* spp., *Fortunela* spp., *Poncirus trifoliata*, *Microcitrus* sp*.*; *Pleiosperum sp.*; *Severinia buxifolia*)

**Artificial infections:**

 Fringed quickweed (*Galinsoga ciliata.*)

Prior to detection on hop, and within the systematic survey, HSVd was to a lesser extent confirmed in Slovenia on the peach and apricot trees (Viršček Marn et al., 2006), and in a minor number of samples of vines of the Žalec area, all of which tested positive (Matoušek et al., 2003). In the light of the HSVd prevalence research study on vines, conducted in the neighbouring country of Hungary, which identified more than 90 % prevalence of HSVd in samples taken (Farkas et al., 1999), and of other similar research study results (Sano, 2003b), a similar situation in Slovenia may be inferred therefrom. HSVd infection of vines is latent only.

*Hop distribution in Slovenia:*

**Hop:** In 2014, 1783 ha (1349 ha of active hop gardens and 434 ha in crop rotation (Source: Hop Yield Certification Body of SIHRB). In nature, hop is present in the lowlands of the entire Slovenia. Growing areas: the Savinja Valley with the Celje Basin; the Carinthia Region (Radlje on Drava, and Slovenj Gradec); the surroundings of Ptuj and Ormož (Annex 2, Figure 2).

## Pathways for entry

HSVd may at greater distances be transmitted by way of infected planting material, infected plant remnants (parts of plants), non-disinfected machinery or tools. Most trading in hop planting material takes place within Slovenia, as the Slovenian hop growing has been based on Slovenian hop varieties. Imports of plants for planting from other countries do exist, but at a lesser level, or restricted to plant breeding material only. Critical in this context are all the countries with the HSV infection present in their hop growing areas. Transmission of HSVd from other host plants to hop is possible and has been proven. Such transmission occurred in the past in Japan, where scientists demonstrated that the cause of occurrence of stunted plants was HSVd transmission from vines to hop plants. In these areas, the farmers used to grow vines along the sides of hop growing areas, which most probably contributed to HSVd transmission from vines to hop plants

(Sano, 2003c). In Slovenia, HSVd is also present on vines and on certain stone fruit trees, which constitutes a potential risk of transmission to hop, but only where hop growing is resumed on grubbed-up land that had previously been cultivated with the permanent crops of HSVd host plants, or where non-composted remnants of infected plants had been dumped in hop growing areas which, however, is not a good agricultural practice. There exists a possibility of transmission between host plants growing in a farm, for instance, hops and vines and stone fruit plants, but it is low in particular due to the specificity of cultivation and tools specific for each type of agricultural production. Introduction of HSVd into Slovenia is possible also via the citrus fruits, citrus plants for planting (ornamental) or other ornamental plants. The largest portion thereof constitutes the import of fresh citrus fruits that, according to data of the Ministry of the Republic of Slovenia for Agriculture, Forestry and Food (MAFF), amounts to approximately 40,000 tons/annum (AIS, 2014). Based on a research study that comprised the main food shopping centres in Slovenia, the presence of HSVd was identified in more than 60 % of citrus fruit tested. Testing of ornamental citrus fruits was conducted in a lesser extent, and did not identify the presence of HSVd. Considering these facts, the risk of transmission of HSVd from other areas and from host plants to hop is relatively low, provided that all the good agriculture practice are implemented and appropriate handling of organic waste is implemented. In case of non-compliance with these measures, the risk may substantially increase.

| **Possible pathways***(in order of importance)* | **Short description explaining why it is considered as a pathway** | Pathway prohibited in the PRA area?Yes/No | Pest already intercepted on the pathway? Yes/No |
| --- | --- | --- | --- |
| Hop plants for planting | Systemic infection of plants or plant parts | No | No |
| Plants for planting of other host plants | Systemic infection of plants or plant parts | No | No |
| Citrus fruits | Systemic infection of plants or plant parts | No | Yes |
| Parts of plants (hop remnants) | Systemic infection of plants or plant parts | No | Yes |
| Machinery and tools | Infected plant remnants | No | Yes |

Rating of the likelihood of entry Low ☐ Moderate  High ☐

Rating of uncertainty Low ☐ Moderate  High ☐

## Likelihood of establishment outdoors in the PRA area

Similarly as other viroids, HSVd may survive in infected host plants or on remains of infected plants for a limited period of time. HSVd may settle, in particular at infection of permanent plants, on which it subsequently spreads by vegetative propagation and mechanic transmission. In case of latent infections and absence of monitoring of plants, such establishment is all the more probable. Such a case is the high prevalence of HSVd on vines, or of the hop latent viroid (HLVd) in hop plantations. HSVd on hop has a relatively long incubation period (3 to 5 years) that constitutes the time in which the plants are infectious, though showing no signs of disease. Given the high infectivity and intensity of agricultural production, the viroid may quickly spread within an infected plantation and later, with an increased potential, to other plantations. HSVd transmission via seeds has been proven on vines and plum trees, though in a very low range.

Rating of the likelihood of establishment outdoors Low ☐ Moderate ☐ High 

Rating of uncertainty Low  Moderate ☐ High ☐

## Likelihood of establishment in protected conditions in the PRA area

The risk of establishment or spread of HSVd in closed areas is low, considering the available set of host plants. An exception is the growing of planting material of host plants in greenhouses where, at non-verification of health status of parent plants, the propagation and spread of infected material are possible. In Slovenia, such a case is the growing of certified hop plants (certification level A) that takes place in greenhouses and enclosed areas only. Propagation of hop plants for planting in greenhouses takes place most intensively and therefore, a system of control needs to be guaranteed, which prevents the introduction of HSVd infected plants into the greenhouse and ensures the verification of the state of health prior to propagation. The occurrence and spread of HSVd in greenhouses was perceived on cucumbers in the Netherlands (Van Dorts and Peters; 1974) and most recently in Finland (Lemmetty and Soukainen, 2011). Outbreaks affected several greenhouses, but the prevalence of infected plants was relatively low, and the further spread was effectively prevented by destroying the infected plants. However, the method of perseverance of HSVd in cucumber production greenhouses still remains unexplored (Sano, 2003a).

Rating of the likelihood of establishment in protected conditions

 Low ☐ Moderate  High ☐

Rating of uncertainty

 Low  Moderate High ☐

## Spread in the PRA area

On longer distances, HSVd may spread in particular by the infected plants for planting and infected plant remnants. Hop plants produced from infected plants begin to express the signs/symptoms of disease already during the year of planting. In the case of hop plants infected during the vegetation, the incubation period extends between 3 and 5 years. During the incubation period, plants do not develop visual symptoms, though being infective, and may spread infection within the hop garden. In hop gardens, the disease may be transmitted mainly mechanically, by infected plant sap that remains deposited on machinery and tools during the different agro-technical activities. The spread is most intensive at the time of cutting and at other spring operations, including the training of offshoots, and at harvesting, which are most damaging to the plants.

Significant sources of spread are the infected plant remnants, in which HSVd may survive all until the tissues are fully decomposed. Thus returning fresh hop waste from an infected hop garden to non-infected hop gardens can cause spread of HSVd.

In case of eradication of hop gardens, the plant remnants shall fully be ploughed through/grubbed-up and at least a two-year crop rotation with non-host plants should be done, to ensure that all the hop remnants have fully been decomposed, and thereby also the HSVd. Pollen, seeds and weeded vegetation have according to analyses conducted to date no relevant role in maintaining and spreading HSVd. Likewise, HSVd has no known vectors. Notwithstanding the extensive imports of citrus fruits, which may be HSVd infected, this factor does not impact its spread within hop growing areas, as most household waste does not end up in plantations.

HSVd has a broad spectrum of host plants, and many thereof are non-symptomatic. In Slovenia, HSVd is present also on vines and certain stone fruit trees in which, however, it does not cause any economic losses. Though Slovenia belongs to the major hop growing countries, the

transmission of HSVd from other host plants is assessed as posing a lesser risk on account of the specificity of hop growing practice.

Rating of the magnitude of spread Low ☐ Moderate  High ☐

Rating of uncertainty Low  Moderate ☐ High ☐

## Impact in the current area of distribution

The occurrence of viroid hop stunt disease in Japan belongs to the viroid diseases, which have to date caused the highest economic losses. After the initial latency, the progressive signs/symptoms of disease intensify from year to year. Severely affected plants develop only up to 40 % of the height of healthy plants, the number of cones decreases by 30 to 50 %, they are much smaller and the content of alpha acids is considerably reduced. From the initial occurrence in 1940, despite certain prevention measures, the level of infection of hop growing areas amounted to 19 % in 1977 (Sano, 2003c). On detection of HSVd as causative agent in 1977 (Sasaki and Skikata, 1977), the emergency measures were immediately adopted, including the systematic monitoring, destruction of affected plants and plantations, and certification of planting material. Within a decade, they succeeded in controlling the disease, though the full eradication was unsuccessful, and thus, Japan still has in place the measures of prevention intended for controlling the disease (Sano, 2003c). Outbreaks of viroid hop stunt disease and economic losses have also been reported from the USA, where the disease had first been detected in 2004 (Eastwell and Nelson, 2007), and from China as from 2007 (Guo et al., 2008).

In addition to hop, HSVd has been showing symptoms of disease on certain plants of the genus *Prunus*. In Japan, the dapple fruit disease of plum and peach has been described in detail, causing the deformity and impaired quality of the plum and peach fruits (Sano, 2003b). HSVd infection of apricot fruits is mostly latent; however, a novel disease has been reported from Spain as from 2007, showing the new variant HSVd symptoms on fruits (Amari et al., 2007). HSVd has also been causing the deformities of cucumbers, with major outbreaks registered only in the Netherlands (Van Dorts and Peters; 1974). HSVd infection of citrus fruits frequently occurs in conjunction with the simultaneous infections by other viroids present on citrus trees and linked to the complex diseases, as cachexia and exocortis (Duran Vila and Semancik, 2003).

Measures of prevention are adapted to the particular plant variety and disease. They are mostly based on destruction of affected plants, systematic controls, production of certified planting material, control of latent infections, breeding more resistant or tolerant plant varieties and, in certain cases, cross-resistance.

Rating of the magnitude of impact in the current area of distribution Low ☐ Moderate ☐ High 

Rating of uncertainty Low  Moderate ☐ High ☐

## Potential impact in the PRA area

In Slovenia, prior to detection on hop, the presence of HSVd was within the systematic survey to a lesser extent confirmed in the peach and apricot trees (Viršček Marn et al., 2006), and on a small number of vine samples from the Žalec region, all of which tested positive (Matoušek et al., 2003). Infection on such hosts is mostly latent and, to date, no economic losses have been reported. The presence of HSVd was confirmed on hop for the first time in the severely stunted plants detected in the Savinja Valley area, but the subsequent investigations showed that the real causative agent of the new disease was the CBCVd.

However, the presence of HSVd was confirmed on certain plants of hop that belongs to the principal host plants with the resulting economic losses. In case of hop infection with HSVd, the spread of disease may seriously jeopardise the Slovenian hop production (1783 hectares; 139 holdings), and in case of spread to the other EU Member States, also the European hop production (approximately 30,000 hectares). Effective prevention is based on destruction of infected plants, the hygienic measures, planting healthy planting material, and involvement of all the competent services. At annual level, the value of hop production in Slovenia on the average amounts to approximately 10 million Euros. Table 4 shows the collective data on hop production in Slovenia in the period 2000-2014.

In Slovenia, in the period 2011-2014, the viroid hop stunt diseases were detected in 13 hop farms. Most infected farms (10) and hop gardens are situated in the Žalec Municipality, or in the vicinity of the primary outbreak location in Šempeter within the Savinjska Valley. Infections in Carinthia are limited to 2 farms, where the disease after eradication did not reappear in one (1) holding in 2014. In the case of Polskava which is a hop farm near Slovenska Bistrica four infected hop gardens were detected. The size of individual outbreaks, with individual exceptions, is relatively small, but in the case of infection of a new hop garden, the disease may successfully be eradicated through destruction of infected plants only, and at strict observation of the hygiene measures, or the eradication of an entire hop garden or a major part which includes all plant rows where the infected plants have been detected.

Table 3: Data on occurrence of viroid hop stunt diseases in Slovenia in the period 2011 - 2014.

| **Year** | **Confirmed infections in current****year (ha)\*** | **Total number of infected plants in****current year** | **Eradicated areas (ha)** | **Number of infected farms** | **Number of all hop farms in Slovenia** |
| --- | --- | --- | --- | --- | --- |
| *2011* | 50,1 | 3210 | 10,80 | 10 | 129 |
| *2012* | 28,89 | 868 | 8,30 | 12 | 115 |
| *2013* | 44,82 | 1201 | 4,43 | 13 | 115 |
| *2014* | 64,5 | 1918 | 1,5 | 12 | 136 |

\*Graphic presentation of infected hop gardens is shown in Annex 2, Figure 3.

 Will impacts be largely the same as in the current area of distribution? Yes /No Economic loss in hop growing areas may be compared to the outbreaks in Japan and in the USA, where the infection affected a major number of plantations and required the setting-up of a long- term eradication strategy.

**If No**

Rating of the magnitude of impact in the area of potential establishment

Low ☐ Moderate ☐ High 

Rating of uncertainty

Low  Moderate ☐ High ☐

## Identification of the endangered area

Primarily, HSVd may jeopardise the entire hop production in Slovenia. The scope of production in the recent years has involved more than 100 hop production farms, covering approximately 1700 hectares of hop gardens. In the recent decade, the number of farms has decreased and, at the same time, the production of the existing farms increased. At annual level, the value of hop production in Slovenia on the average amounts to approximately 10 million Euros. Table 4 shows the collective data on hop production in Slovenia in the period 2000  2014. In nature, hop is present in the lowlands of the entire Slovenia and in particular along the watercourses. The risk of spread of HSVd to natural vegetation is relatively low. The transmission is possible only in the case of disposal of affected plant remainders in the vicinity of natural hop habitats. Further spread within the natural hop population would be possible only, where regular floods occur, washing

away the plants. Transmission of HSVd through seeds has not been proven in the case of hop, so this factor does not impact the spread of HSVd in natural hop habitats.

Table 4: Slovenian hop growing industry: Data on surfaces, number of growers, and produce in the period 2000  2014 (Source: SIHRB, 2015).

| **Year** | **Crop rotation (ha)** | **Area under hop gardens (**first yearlings and fertileplantations) **(ha)** | **Certified production in tons** | **Kg/ha of fertile plantation** | **Number of hop growers** |
| --- | --- | --- | --- | --- | --- |
| **2014** | 488.98 | 1296.16 | 2319 | 2034 | 136 |
| **2013** | 626.55 | 1165.84 | 1297 | 1222 | 136 |
| **2012** | 636.31 | 1159.49 | 1559 | 1457 | 115 |
| **2011** | 453.26 | 1376.14 | 2470 | 1878 | 129 |
| **2010** | 374 | 1488 | 2461 | 1825 | 134 |
| **2009** | 207 | 1517 | 2500 | 1795 | 139 |
| **2008** | 254 | 1618 | 2359 | 1539 | 140 |
| **2007** | 302 | 1573 | 1987 | 1355 | 157 |
| **2006** | 316 | 1468 | 1899 | 1341 | 158 |
| **2005** | 416 | 1462 | 2544 | 1801 | 159 |
| **2004** | 419 | 1520 | 2608 | 1772 | 169 |
| **2003** | 434 | 1625 | 1313 | 835 | 186 |
| **2002** | 273 | 1817 | 2194 | 1274 | 192 |
| **2001** | 251 | 1807 | 2158 | 1317 | 201 |
| **2000** | 323 | 1774 | 1805 | 1095 | 231 |

## Overall assessment of risk

*Summary of ratings and uncertainty*

|  | **Rating level** | ***Uncertainty*** |
| --- | --- | --- |
| Entry | moderate | *moderate* |
| Establishment (Slovenia, EU hop countries) | high | *low* |
| Establishment (glasshouses/protected conditions in the rest of the EPPOregion) | moderate | *low* |
| Spread (PRA region) | moderate | *low* |
| Impact (in current region Japan, USA) | high | *low* |
| Impact in PRA region | high | *low* |

HSVd has a broad spectrum of host plants, and many thereof are non-symptomatic. In Slovenia, HSVd is present on vines and certain stone fruit plants, where it does not cause any significant economic loss. Nevertheless, Slovenia belongs to the major hop growing countries, and due to the specificity of hop production, the transmission from other host plants is assessed as posing a lower risk.

Introduction of HSVd by way of infected hop plants for planting from other countries (Japan, USA, and China) is unlikely. In general, imports of hop planting material from other countries are negligible as the Slovenian hop variety are primarily grown in Slovenia. Plants for planting are imported only for breeding. Introduction of HSVd into Slovenia mostly takes place via the import and sales of citrus fruits. The scope of such imports is very high, though most household waste thereof primarily ends up in regulated city dumps. However, transmission to hop or other plants is possible in the case of illegal citrus fruit waste disposal. Imports of citrus plants for planting into Slovenia are relatively low and intended for non-commercial use and ornamental purposes only.

In case of transmission to hop, HSVd may spread rapidly, though intensive and specific hop cultivation that, at the time of vegetation, provides the ideal conditions for mechanic transmission, produce a high extent of plant residues, and through vegetative hop propagation. As regards the spread it is necessary to emphasize, the high density of hop gardens in all hop growing areas of Slovenia, which are proprietarily intertwined, what increase the risk of disease spread among farms.

Impact of HSVd on produce and hop production is very high as the infected plant crop yields are almost halved. Slovenia belongs to the 6 most important hop growing countries worldwide, with a 95 % export per annum and a turnover of around EUR 10 million. As hop is grown as a permanent crop, requiring high investments, the level of economic loss due to HSVd infection would be high. In the case of non-implementation of phytosanitary measures, high economic losses may be expected and, given the international interconnectedness of hop growing industry, spread to other hop growing countries of the European Union.

# Stage 3. Pest risk management

## Phytosanitary measures

* + In preventing the introduction of HSVd into Slovenia through imports from other hop growing countries, the testing of hop planting and hop breeding material shall be required.
	+ Hop planting material bred in Slovenia shall be based on certified planting material that ensures the appropriate plant health status for growing, and the freedom from causal agents of viroid hop stunt diseases.
	+ An important measure implemented in the areas of HSVd outbreaks and in other endangered areas is the systematic survey that facilitates an overview of the situation, the early detection of disease, the monitoring of dynamics of spread of disease, and expert support to hop growers and to other professional services.
	+ Prevention of spreading and eradication within infected areas are based on the uprooting of infected plants and hop plants growing in the same row in a two metre distance and with infection rate above 20% the eradication of the infected hop garden or its part.
	+ Important factors of spread of infection is hop waste, and hop plant remnants from infected hop plantations. For this reason, any removal of hop waste or other hop plant remnants from the infected area back to uninfected hop gardens shall be prohibited. Hop waste from the infected area shall be removed to the grassland, agricultural land or authorised disposal site only.
	+ In infected areas, at least a two-annual crop rotation period shall follow after uprooting of infected plants, with non-host plants (cereals, corn..) only, so as to let the remnants of old hop plants to degrade, and to prepare the land for new hop planting. Any new or reappearing hop offshoots shall be removed.
	+ HSVd mostly spreads to new hop gardens within the already infected farms. It is therefore very important to disinfect the machinery and equipment, and to observe the sequence of tillage of plantations, where the infected ones shall be tilled last.
	+ To prevent spreading of CBCVd from infected farms there is a need to prohibit the movement of plants for planting from the infected place of production. An exception may only be major agricultural farms that comprise several farms with the separate agricultural machinery and tools.
	+ To prevent the spreading within infected farms, the production of planting material in infected hop gardens shall be prohibited.
	+ Professional support in hop growing and other professional services shall be at hand.
	+ Hop growers shall be notified of the disease and familiarised with the measures of prevention of its spread.
	+ As from 2011, measures have been implemented in Slovenia in compliance with the provisions of the Decision on emergency measures against the introduction and spread of viroid hop stunt disease */* [Odločba o nujnih ukrepih za preprečevanje vnosa in širjenja](http://www.uradni-list.si/1/content?id=104893) [viroidne zakrnelosti hmelja](http://www.uradni-list.si/1/content?id=104893) (UL RS 64/2011, 12.08.2011).

## Uncertainty

Since the discovery that HSVd is not the causative agent of a new disease on hop in Slovenia, a question arose about its presence in certain symptomatic plants. It is assumed that during first transmission to hop, HSVd and CBCVd were transmitted together from remains of imported citrus fruits or plants on which these two viroids could be found together. Primary outbreak of the new disease occurred in a hop garden established on the site of a former waste dump, where such transmission probably happened. The possible antagonistic relationship between HSVd and CBCVd on hop leads to the dominance of CBCVd and reduction or elimination of HSVd from symptomatic plants during the subsequent years. This could be the reason that HSVd was

detected only on cones which, as known, contain highest concentrations of viroids like *Hop latent viroid* (HLVd) among all tissues (Matoušek et al., 1995). However, the hypothesis of possible transmission of CBCVd and HSVd from infected citrus fruits or plants to hop and interaction of these two viroids in hop has not been proven yet and should be examined further in the future.

## Remarks

At non-implementation or inappropriate implementation of appropriate measures it may be expected that the eradication of HSVd in hop growing areas will not be successful. Further spread of the disease may seriously jeopardise the Slovenian hop production industry.

## REFERENCES

Amari K, Cañizares MC, Myrta A, sead Sabanadzovic S, Srhiri M, Gavriel I, Çaglayan K, Varveri C, Gatt M, Terlizzi B and Pallás V, 2000. First report on hop stunt viroid (HSVd) from some Mediterranean countries. Phytopathologia Mediterranea, 39: 271-276.

Amari K, Gomes G, Myrta A, Di Terlizzi B, Pallás V, 2007. An important new apricot disease in Spain is associated with Hop stunt viroid infection. European Journal of Plant Pathology, 118: 173-181.

Amari K, Gomez G, Myrta A, Di Terlizzi B, Pallás V, 2001.The molecular characterization of 16 new sequence variants of Hop stunt viroid reveals the existence of invariable regions and a conserved hammerhead-like structure on the viroid molecule. Journal of General Virolology, 82: 953-962.

Asai M, Ohara T, Takahashi T, Saito S, Tanaka K, 1998. Detection of viroids in fruit trees by return gel electrophoresis.

Research Bulletin of the Plant Protection Service, Japan, 34:99-102.

Astruc N, Marcos JF, Macquaire G, Candresse T, Pallás V, 1996. Studies on the diagnosis of [Hop stunt viroid](http://wiki.pestinfo.org/wiki/Hop_stunt_viroid) in fruit trees: Identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. [European Journal of Plant Pathology, 102: 837-846.](http://wiki.pestinfo.org/wiki/European_Journal_of_Plant_Pathology_%281996%29_102%2C_837-846)

Bagherian SAA, Izadpanah K, 2010. Two novel variants of Hop stunt viroid associated with yellow corky vein disease of sweet orange and split bark disorder of sweet lime. 21st International Conference on virus and other graft transmissible diseases of fruit crops, Julius-Kühn-Archiv no. 427, 105-113.

Bennett S, Tennant P, McLaughlin W, 2010. First report of Hop stunt viroid infecting citrus orchards in Jamaica. Plant Pathology, 59 (2): 393.

Bernard L, Duran-Vila N, 2006. A novel RT-PCR approach for detection and characterization of citrus viroids.

Molecular and Cellular Probes, 20: 105-113.

[Cañizares MC,](http://waesearch.kobv.de/authorSearch.do%3Bjsessionid%3D7A13061B4FA0F18BA4FE32A1CC4A6C26?query=Can%cc%83izares%2c%2BM.%2BCarmen&pageid=1356466008852-30324878006461875) [Marcos JF,](http://waesearch.kobv.de/authorSearch.do%3Bjsessionid%3D7A13061B4FA0F18BA4FE32A1CC4A6C26?query=Marcos%2c%2BJose%2BF.&pageid=1356466008852-3695852848877481) [Pallás Vicente,](http://waesearch.kobv.de/authorSearch.do%3Bjsessionid%3D7A13061B4FA0F18BA4FE32A1CC4A6C26?query=Palla%cc%81s%2c%2BVicente&pageid=1356466008852-4765273972569368) 1999. Molecular Characterization of an Almond Isolate of Hop Stunt Viroid (HSVd) and Conditions for Eliminating Spurious Hybridization in its Diagnosis in Almond Samples. [European](http://link.springer.com/journal/10658) [Journal of Plant Pathology,](http://link.springer.com/journal/10658) 105 (6): 553-558.

Diener T, Smith D, Hammond R, Albanese G, La Rosa R, Davino M, 1988. Citrus B viroid identified as a strain of hop stunt viroid. Plant Disease, 72:691-693.

Diener TO, 1987. Biological properties. In: Diener TO, ed. The viroids. Plenum Press, New York, USA, 9-35.

Duran-Vila N, Roistacher CN, Rivera-Bustamante R, Semancik JS, 1988. A definition of citrus viroid groups and their relationship to the exocortis disease. Journal of General Virology, 69:3069–3080.

Duran-Vila N, Semancik JS, 2003. Citrus viroids. In: Hadidi A, Flores R, Randles JW, Semancik JS, eds. Viroids.

CSIRO Publishing, Collingwood, Australia, 178-194.

Duran-Villa N, Pina JA., Ballester JF., Juarez J., Roistacher CN., Rivera –Bustamante R., Semancik JS (1988). The citrus exocortis disease: a complex of viroid RNAs. Str. 152-164. Proc 10th Conf. Int. Org. Citrus Virol. (Ur). Timmer LW., Garnsey SM., Navaro L., IOCV, Riverside, Kalifornija.

Eastwell KC, Nelson ME, 2007. Occurrence of viroids in commercial hop (*Humulus lupulus* L.) production areas of Washington State. Plant Management Network, 7 pp. <https://sharepoint.cahnrs.wsu.edu/hops/Shared%20Documents/Scientific%20Articles/Hop%20Stunt/hop.pdf>

Eastwell KC, Sano T, 2009. Hop Stunt. In: MahaffeeWF, Pethybridge SJ, Gent DH, eds. Compendium of Hop Diseases and Pests. The American Phytopathological Society, St. Paul, MN, 48-50.

Eiras M, Targon MLPN, Fajardo TVM, Flores R, Kitajima EW, 2006. Citrus exocortis viroid and Hop Stunt viroid Doubly infecting grapevines in Brazil. Fitopatologia Brasilera, 31(5):440-446.

Elbeaino T, Abou Kubaa R, Choueiri E, Digiaro M, Navarro B, 2012. Occurrence of Hop stunt viroid in mulberry (Morus alba) in Lebanon and Italy. Journal of Phytopathology, 160(1): 48-51.

El-Dougdoug, KA, Osman, ME, Hayam SA, Rehab AD, and Reham ME, 2010. Biological and Molecular Detection of HSVd - Infecting Peach and Pear Trees in EGYPT. Australian Journal of Basic and Applied Sciences, 4(1):19-26.

Farkas E, Palkovics L, Mikulás J, Balázs E, 1999. High incidence of hop stunt viroid in Hungarian grapevines. [Acta](http://www.ingentaconnect.com/content/akiado/apeh%3Bjsessionid%3Dab1f4hor6qp37.alice) [Phytopathologica et Entomologica Hungarica,](http://www.ingentaconnect.com/content/akiado/apeh%3Bjsessionid%3Dab1f4hor6qp37.alice) 34(1-2): 7-12.

Farkas E., Palkovics L., Mikulas J., Balazs E. (1999). High incidence of hop stunt viroid in Hungarian grapevines. Acta Phytopathologica et Entomologica Hungarica Vol.34, No.1/2, 7-11

Flores R, Di Serio F, and Hernández C, 1997. Viroids: the non coding genomes. Seminars in Virology, 8, 65-73.

Flores R, Hernández C, García S, Llácer G, 1990. Is apricot »viruela«(pseudopox) induced by a viroid ? In: Proceedings of the 8th Congress of the Mediterranean Phytopathological Union, Agadir.

Flores R, Hernandez C, Martinez de Alba AE, Daros JA, and Di Serio F, 2005. Viroids and viroid-host interactions.

Annual Review of Phytopathology, 43:117-139.

Flores R, Randles JW, Bar-Joseph M, Diener TO, 1998. A proposed scheme for viroid classification and nomenclature.

Archives of Virology 143: 623-629.

[G. Cook,](http://apsjournals.apsnet.org/action/doSearch?Contrib=Cook%2C%2BG) [S. P. van Vuuren,](http://apsjournals.apsnet.org/action/doSearch?Contrib=van%2BVuuren%2C%2BS%2BP) [J. H. J. Breytenbach,](http://apsjournals.apsnet.org/action/doSearch?Contrib=Breytenbach%2C%2BJ%2BH%2BJ) [B. Q. Manicom](http://apsjournals.apsnet.org/action/doSearch?Contrib=Manicom%2C%2BB%2BQ) (2012). *Citrus Viroid IV* Detected in *Citrus sinensis*

and *C. reticulata* in South Africa, Plant Disease, Vol. 96, Number 5, 772

Gillings MR, Broadbent P, Gollnow BI, 1991. Viroids in Australian Citrus: Relationship to Exocortis, Cachexia and Citrus Dwarfing. Australian Journal of Plant Physiology, 18(5): 559 – 570.

Guo L, Liu S, Wu Z, Mu L, Xiang B, Li S, 2008. Hop stunt viroid (HSVd) newly reported from hop in Xinjiang, China. Plant Pathology, 57(4), 764.

Hadidi A, Flores R, Randles JW, Semancik JS, 2003. Viroids. CSIRO Publishing, Collingwood, Australia, 370.

Hadidi A, Terai Y, Powell CA, Scott SW, Desvignes JC, Ibrahim LM, Levy L, 1992. Enzymatic cDNA amplification of hop stunt viroid variants from naturaly infected fruit crops. Acta Horticulturae, 309: 339-344.

Hirashima K, Noguchi Y, Ushijima K, Kusano N, 1994. Diagnosis of plum dapple fruit disease by polyacrylamide gel electrophoresis and mechanical transmission. Bulletin of the Fukuoka Agricultural Research Center, B-13: 65-68.

Jakše J. Radisek S., Pokorn T., Moatoušek J., Javornik B. (2014) Deep-sequencing revealed a CBCVd viroid as a new and highly aggressive pathogen on hop. Plant Pathology; doi: 10.1111/ppa.12325

Kofalvi SA, Marcos JF, Caňizares MC, Pallás V, Candresse T, 1997. Hop stunt viroid (HSVd) sequence variants from Prunus species: evidence for recombination between HSVd isolates. Journal of General Virology, 78: 3177-3186.

Koltunow AM, Rezaian MA, 1988. Grapevine yellow speckle viroid. Structural features of a new viroid group. Nucleid Acids Research. 16: 849-864.

Kubaa AR, El-Khateeb A, D'Onghia AM, Djelouah K, 2011. First record of Hop stunt viroid infecting citrus orchards in Syria. Journal of Plant Pathology, 93(4S): S4.67.

Kunta M, Gracxa JV, Skaria M, 2007. Molecular Detection and Prevalence of Citrus Viroids in Texas. Hortscience, 42(3):600–604.

Lee JY, Puchta H, Ramm K, Sänger HL, 1988. Nucleotide sequence of the Korean strain of hop stunt viroid (HSVd).

Nucleic Acids Research, 16: 8708.

Lemmetty A, Werkman AW, Soukainen M, 2011. First report of Hop stunt viroid in greenhouse cucumber in Finland.

Plant Disease, 95(5), 615.

Lemmetty A, Werkman AW, Soukainen M, 2011. First report of Hop stunt viroid in greenhouse cucumber in Finland.

Plant Disease, 95(5), 615.

Li S, Onodera S, Sano T, Yoshida K, Wang G, Shikata E, 1995. Gene diagnosis of viroids. Comparison of return – PAGE and hybridisation using DIG labeled DNA and RNA probes for practical diagnosis of hop stunt, ctrus exocortis and apple scar skin viroids in their natural host plants. Annals of the Phytopathological Society of Japan. 61: 93-102.

Li SF, Guo R, Tsuji M, Sano T, 2006. Two grapevine viroids in China and the possible detection of a third. Plant Pathology, 55(4): 564.

M. J. Cao, Y. Q. Liu, X. F. Wang, F. Y. Yang, C. Y. Zhou (2010). First Report of *Citrus bark cracking viroid* and

*Citrus viroid V* Infecting Citrus in China. Plant Disease Volume 94, Number 7, Page 922

Mahfoudhi N, Salleh W, Djelouah K, 2010. First report of Hop stunt viroid in apricot in Tunisia. Journal of Plant Pathology, 92(S4):116.

Mandic B, Al Rwahnih M, Myrta A, Gomez G, Pallas V, 2008. Incidence and genetic diversity of Peach latent mosaic viroid and Hop stunt viroid in stone fruits in Serbia. European Journal of Plant Pathology, 120(2): 167-176.

Matoušek J., Orctová L, Patzak J, Svoboda P, Ludvíková I, 2003. Molecular sampling of hop stunt viroid (HSVd) from grapevines in hop production area in the Czech Republic and hop protection. Plant Soil Environment, 49:168-175.

Matoušek J., Orctová L, Patzak J, Svoboda P, Ludvíková I, 2003. Molecular sampling of hop stunt viroid (HSVd) from grapevines in hop production area in the Czech Republic and hop protection. Plant Soil Environment, 49:168-175.

Matoušek J., Orctová L, Ptáček J, Patzak J, Dědič P, Stegor G, Reisner D, 2007. Experimental transmission of Pospiviroid populations to weed species charavteristics of potato and hop fields. Journal of Virology, 81(21) 11891- 11899.

Michelutti R, Myrta A, Pallás V, 2005. A preliminary account on the sanitary status of stone fruits at the Clonal Genebank in Harrow, Canada. Phytopathologia Mediterranea, 44: 71–74.

Mohamed ME, Bani Hashemian SM, Dafalla G, Bové JM, Duran-Vila N, 2009. Occurrence and identification of citrus viroids from Sudan. Journal of Plant Pathology, 91(1): 185-190.

Mu LX, Wu YH, Li SF, 2009. First report of hop stunt viroid from almond tree in China. Journal of Plant Pathology, 91(S4):112.

Murcia N, Bernad L, Caicedo A, and Duran-Vila N. Citrus Viroids in Colombia. Proceedings of the, 17th Conference, IOCV, 2010 – Viroids, 158-166.

Nour Eldin F, Fudl Allah AESA, 1976. Citrus virus and virus-like diseases in Libya. Libyan Journal of Agriculture 5:101-110.

Ohno T, Takamatsu N, Meshi T, Okada Y, 1983. Hop stunt viroid: Molecular cloning and nucleotide sequence of the complete cDNA copy. Nucleic Acids Research, 11:6185-6197.

Önelge N., Kersting U., Guang Y., Bar-Joseph M., Bozan O. (2000). Nucleotide sequence of citrus viroids CVd IIIa and CVd IV obtained from dwarfed Meyer lemon trees grafted on sour orange. Journal of Plant Disease and Protection, 107, 387-391.

Pagliano G, Peyrou M, Del Campo R, Orlando L, Gravina A, Wettstein R, Francis M, 2000. Detection and characterizationof citrus viroids in Uruguay.. In: J Gracxa JV, Lee RF,. Yokomi RK, eds. Proceedingsof the. 14th Conference of the. International Organisation Citrus Virologits, IOCV, Riverside, California, 282–288.

Pallás V, Gómez G, Duran-Vila N, 2003. Viroids in Europe in: Viroids Hadidi A, Flores R, Randles JW, Semancik JS, eds. CSIRO Publishing, Collingwood, Australia. 268-275.

[Puchta](http://www.ncbi.nlm.nih.gov/pubmed/?term=Puchta%20H%5Bauth%5D) H, [Ramm](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ramm%20K%5Bauth%5D) K, [Hadas](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hadas%20R%5Bauth%5D) R, [Bar-Joseph](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bar-Joseph%20M%5Bauth%5D) M, [Luckinger](http://www.ncbi.nlm.nih.gov/pubmed/?term=Luckinger%20R%5Bauth%5D) R[, Freimüller](http://www.ncbi.nlm.nih.gov/pubmed/?term=Freim%26%23x000fc%3Bller%20K%5Bauth%5D) K[, Sänger](http://www.ncbi.nlm.nih.gov/pubmed/?term=S%26%23x000e4%3Bnger%20HL%5Bauth%5D) HL, 1989. Nucleotide sequence of a hop stunt viroid (HSVd) isolate from grapefruit in Israel. Nucleic Acids Research, 17(3): 1247.

[Puchta](http://www.ncbi.nlm.nih.gov/pubmed/?term=Puchta%20H%5Bauth%5D) H, [Ramm](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ramm%20K%5Bauth%5D) K, [Luckinger](http://www.ncbi.nlm.nih.gov/pubmed/?term=Luckinger%20R%5Bauth%5D) R, [Freimüller](http://www.ncbi.nlm.nih.gov/pubmed/?term=Freim%26%23x000fc%3Bller%20K%5Bauth%5D) K, [Sänger](http://www.ncbi.nlm.nih.gov/pubmed/?term=S%26%23x000e4%3Bnger%20HL%5Bauth%5D) HL, 1989. Nucleotide sequence of a hop stunt viroid (HSVd) isolate from the German grapevine rootstock 5BB as determined by PCR-mediated sequence analysis. Nucleic Acids Research, 17(14): 5841.

Radisek S, Majer A, Jakse J, Javornik B, Matoušek J, 2012. First report of Hop stunt viroid infecting hop in Slovenia.

Plant Disease, 96(4): 592.

Reanwarakor K, Semancik JS, 1999. Correlation of hop stunt viroid variants to cachexia and xyloporosis diseases of citrus. Phytopathology, 89: 568-574.

Rezaian MA, Krake LR, Golino DA, 1992. Common identity of grapevine viroids from USA and Australia revealed by PCR analysis. Intervirology, 34:38-43.

Roistacher CN, Blue RL, Calavan EC, 1973. A new test for citrus cachexia. Citrograph, 58: 261-262.

Rouag N, Guechi A, Matic S, Myrta A, 2008. Viruses and viroids of stone fruits in Algeria. Journal of Plant Pathology, 90(2): 393-395.

Roy A, Ramachandran P, 2003. Occurrence of a Hop stunt viroid (HSVd) variant in yellow corky vein disease of citrus in India. Current Science, 85: 1608–12.

Sano T, 2003a. Hop stunt viroid in cucumber. In: Hadidi A, Flores R, Randles JW, Semancik JS, eds. Viroids. CSIRO Publishing, Collingwood, Australia, 134-136.

Sano T, 2003b. Hop stunt viroid in plum and peach. In: Viroids. Hadidi A, Flores R, Randles JW, Semancik JS, eds.

Viroids. CSIRO Publishing, Collingwood, Australia, 165-167.

Sano T, 2003c. Hop stunt viroid. In: Hadidi A, Flores R, Randles JW, Semancik JS, eds. Viroids. CSIRO Publishing, Collingwood, Australia, 207-212.

Sano T, Hataya T, Shikata E, 1988. Complete nucleotide sequence of a viroid isolated from Etrog citron, a new member of hop stunt viroid group. Nucleid Acids Research, 16:347.

Sano T, Hataya T, Terai Y, Shikata E, 1989. Hop stunt viroid strains from dapple fruit disease of plum peach in Japan.

Journal of General Virology, 70:1311-1319.

Sano T, Mimura R, Ohshima K, 2001. Phylogenetic analysis of hop stunt viroid supports a grapevine origin for hop stunt disease. Virus Genes, 22:53-59.

Sano T, Ohsima K, Hataya T, Uyeda I, Shikata E, Chou T, Meshi T, Okada Y, 1985. A viroid-like RNA isolated from grapevine has high sequence homology with hop stunt viroid. Journal of General Virology, 66:333-338.

Sano T, Sasaki M, Shikata E, 1981. Comparative studies on hop stunt viroid, cucumber pale fruit viroid and potato spindle tuber viroid. Annals of the Phytopathological Society of Japan, 47: 599-605.

Sano T, Uyeda I, ShikataE, Ohno T, Okada Y, 1984. Nucleotide sequence of cucumber pale fruit viroid : homology to hop stunt viroid. Nucleid Acids Research, 12:3427-3434.

Sasaki M, Shikata E, 1977a. Studies on the host range of hop stunt disease in Japan. Proceedings of the Japan Academy.

Series B, 53:103-108.

Sasaki M, Shikata E, 1977b. On some properties of hop stunt disease agent, a viroid. Proceedings of the Japan Academy. Series B, 53:109-112.

Semancik JS, 2003. Considerations for the introduction of viroids for economic advantage. In: Hadidi A, Flores R, Randles JW, Semancik JS, eds. Viroids. CSIRO Publishing, Collingwood, Australia, 357-362.

Semancik JS, Vidalakis G, 2005. The question of Citrus viroid IV as a Cocadviroid. Archives of Virology 150, 1059– 67.

Serraa P, Gagob S, Duran-Vilaa N, 2008. A single nucleotide change in Hop stunt viroid modulates citrus cachexia symptoms. Virus Research, 138:130–134.

Shamloul AM, Hadidi A, 1999. Sensitive detection of potato spindle tuber and temperature fruit free viroids by reverse transcription –polymerase chain reaction-probe capture hybridization. Journal of Virological Methods, 80: 145-155.

Shikata E, 1990. New viroids from Japan. Seminars in Virology, 1: 107-115.

Singh RP, Randles JW, Hadidi A, 2003. Strategies for the control of viroid diseases. In: Hadidi A, Flores R, Randles JW, Semancik JS, eds. Viroids. CSIRO Publishing, Collingwood, Australia 295-302.

Van Dorst HJM, Peters D, 1974. Some biological observations on pale fruit, a viroid-incited disease of cucumber.

Netherlands Journal of Plant Pathology, 80:85-96.

Van Dorst HJM, Peters D, 1974. Some biological observations on pale fruit, a viroid-incited disease of cucumber.

Netherlands Journal of Plant Pathology, 80:85-96.

Viršček Marn M., Mavrič Pleško I., Urek G., Myrta A., Žežlina I. (2006). First report of Peach Latent Mosaic Viroid and Hop Stunt Viroid in Prunus in Slovenia. *Journal of plant pathology*, ISSN 1125-4653, 2006, letn. 88, št. 3, Supplement, str. S67.

Wang XF, Zhou Y, Li ZA, Tang K, Liu YQ, Cao M, Zhou C, 2010. Molecular, biological and phylogenetic analysis of Chinese isolates of Hop stunt viroid associated with citrus cachexia disease. Journal of Phytopathology, 158(5): 372- 377.

Ward LI, Burnip GM, Liefting LW, 2011. First report of Grapevine yellow speckle viroid and Hop stunt viroid in grapevine (Vitis vinifera) in New Zealand. Plant Disease, 95(5): 617.

Yamamoto H, Kagami Y, Kurukawa M, Nishimura S, Kubo S, 1973. Studies on hop stunt disease in Japan. Report of the Research Laboratories of Kirin Brewery Co., Ltd, 16, 49-62.

Zhang B, Liu GY, Liu C, Wu Z, Jiang D, Li S, 2009. Characterisation of Hop stunt viroid (HSVd) isolates from jujube trees (*Ziziphus jujuba*). European Journal of Plant Pathology, 125: 665-669.

Zhang Z, Shou Y, Guo R, Mu L, Yang Y, Li S, Wang H, 2012. Molecular characterization of Chinese Hop stunt viroid isolates reveals a new phylogenetic group and possible cross transmission between grapevine and stone fruits. European Journal of Plant Pathology, 134(2): 217-225.

## Annex 1: Symptoms of HSVd infection of hop





Plants of the hop cultivar Glacier in the center of the photo showing reductions in vigour due to infection by Hop Stunt viroid, compared to the vigorous plant on the left. Photo by Ken Eastwell, 2006.



'Perle' leaves infected with HpSVd showing speckling concentrated along leaf veins. Photo by Ocamb lab, 2007

Lime-green leaves symptomatic of Hop Stunt viroid infection during early June in a 'Glacier' hop plant. Photo by Ken Eastwell, 2007



'Willamette' leaves that were infected with HpSVd showing light green coloration. Photo by Ocamb lab, 2007

**Annex 2: Geographic distribution of HSVd and overview of hop growing areas in Slovenia.**



Figure 1: Geographic distribution of HSVd (Source: EPPO PQR, 2014)



Figure 2: Hop growing areas in Slovenia in 2014 (Source: MAFF Public Graphic Data Viewer (Web Application) / Javni pregledovalnik grafičnih podatkov MKGP, 2014).



Figure 3: Geographic distribution of viroid hop stunt diseases on hop in Savinjska dolina in a period 2011-2014 (Source: FURS APL, by mag. M. Knapič, J. Persolja, 2014).