

The Food & Environment Research Agency

Rapid Pest Risk Analysis for

Hop stunt viroid

This document provides a rapid assessment of the risks posed by the pest to the UK in order to assist Risk Managers decide on a response to a new or revised pest threat. It does not constitute a detailed Pest Risk Analysis (PRA) but includes advice on whether it would be helpful to develop such a PRA and, if so, whether the PRA area should be the UK or the EU and whether to use the UK or the EPPO PRA scheme.

STAGE 1: INITIATION

1. What is the name of the pest?

Hop stunt viroid (HSVd) is the sole species within the *Hostuviroid* genus which, along with four other genera constitute the *Pospiviroidae*. This monophyletic family comprises sequence variants of a small (246-400 nucleotides) non-coding RNA molecule (Elena *et al.*, 2001).

Sequence comparison has identified HSVd sub-species level clades referred to as Plum, Hop, and Citrus groups together with two other groups, P-H/Cit3 and P_C clades (Amari *et al.*, 2001). Recent studies have differentiated further HSVd phylogenetic taxa (Zhang *et al.*, 2012; Elbeaino *et al.*, 2012).

HSVd is best known as the cause of hop stunt disease in *Humulus lupulus* L. (Sasaki and Shikata, 1978) however, the viroid also causes cucumber pale fruit disease (Sano *et al.*, 1981), citrus xyloporosis (Diener *et al.*, 1988), cachexia disease of citrus (Reanwarakorn and Semacik, 1999), dapple fruit disease of plum and peach (Sano *et al* 1989), 'degeneracion' of apricot (Amari *et al.*, 2007; Garcia-Ibarra *et al.*, 2012). HSVd has also been associated with citrus gummy bark disease of sweet orange (Onelge *et al.*, 2004), yellow corky vein disease of citrus (Roy and Ramachandran, 2003) and split bark disorder of sweet lime (Bagherian *et al.*, 2009).

2. What is the pest's status in the EC Plant Health Directive (Council Directive 2000/29/EC¹) and in the lists of EPPO²?

HSVd is not listed in the EC Plant Health Directive and is not listed for recommendation for regulation as a quarantine pest by EPPO, nor is it on the EPPO Alert List.

3. What is the reason for the rapid assessment?

The PRA was initiated following increasing reports of HSVd infections in grape, hop, *Prunus* and cucumber in recent years. It has also been suggested that the viroid could be considered for additional regulation.

STAGE 2: RISK ASSESSMENT

4. What is the pest's present geographical distribution?

(EPPO PQR data). Europe: Corsica (France), Cyprus, Czech Republic, Greece, Italy, Portugal, Serbia, Slovenia, Spain, Turkey. Asia: China, India, Iran, Israel, Japan, Jordan, Korea (Republic), Lebanon, Philippines, Saudi Arabia, Syria, Thailand and Yemen.

¹ http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2000L0029:20100113:EN:PDF

² http://archives.eppo.int/EPPOStandards/PM1_GENERAL/pm1-02(21)_A1A2_2012.pdf

Africa: Algeria, Egypt, Morocco, South Africa, Sudan, Tunisia. Oceania: New Zealand. North America: USA: Arizona, California, Florida, Texas, Washington. South America: Argentina, Brazil, Columbia, Ecuador, Jamaica, Surinam, Trinidad and Tobago, Venezuela.

There are also reports of its presence in grapevines in Australia and Germany and of outbreaks in protected cucumber crops in Finland and the Netherlands (Lemmetty and Soukainen, 2001; Dorst *et al.*, 1974).

5. Is the pest established or transient, or suspected to be established/ transient in the UK? *(Include summary information on interceptions and outbreaks here).*

HSVd has not been reported from the UK. An extensive survey of HSVd in hops using a nucleic acid hybridisation assay from 476 commercial premises, which represented approximately 50% of UK production sites, did not detect HSVd, though hop latent viroid was prevalent (Barbara *et al.*, 1990). It is understood all new hop breeding material and PHPS nuclear stock is screened bi-annually against HSVd and other pests listed in the PHPS rules. All hop plants produced under the official PHPS certification scheme are derived from this tested material and are regularly inspected and any suspect plants would be sent for diagnosis. Similarly, any non-certified hop material produced under the plant passporting system is also officially inspected, although not specifically for HSVd, but any suspect plants would be sampled.

Symptoms of HSVd infection are not necessarily expressed and surveys using diagnostic tests are required to confirm absence of the viroid.

Since no surveys have been reported of commercial grapevine or *Prunus* production, the HSVd status in the UK is unknown, as is the infection status of potential environmental hosts. However no findings have been made in any plants sent in for identification of suspect diseases in these crops, and all *Prunus* propagating material is officially inspected every year for plant passporting and other purposes and any unusual symptoms would be sent for diagnosis. Likewise, the grape production industry regularly sends in commercial samples for diagnosis of unknown symptoms and no HSVd has been detected to date.

6. What are the pest's natural and experimental host plants; of these, which are of economic and/or environmental importance in the UK?

HSVd has the widest host range among the *Pospiviroidae* and infection has been reported in many cultivated woody perennial species, especially those that are managed by cutting or that are propagated vegetatively. Symptomless HSVd infections are common. The best known host is hop, however the viroid has also been detected in Europe and elsewhere in several *Prunus* spp. including: *P. salicina* (Chinese plum), plum, almond, apricot and peach. Other recorded hosts are: *Citrus* spp, grapevine, pomegranate, mulberry, fig, jujube, pear and cucumber; see section 1 for disease names (hosts are listed in EPPO PQR). Additionally, there has been a recent report of HSVd in cultivated and wild apple in Greece (Kaponi *et al* 2012). Biolistic technologies, where high pressure is used to fire nucleic acids into plant cells, have been used to identify new potential experimental hosts (Matousek *et al.*, 2007). This study found high concentrations of HSVd were maintained in *Veronica arvenis* (corn speedwell) and *Amaranthus retroflexus* (an invasive tumbleweed), which indicates the extremely wide potential host range of the viroid.

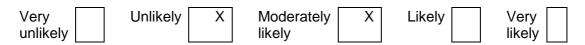
Hop, cucumber, *Prunus* spp. pear, apple and grapevine are the most economically important hosts to the UK, though no symptoms have been reported from grapevine or from the recent reports of HSVd infection in apple from Greece (Kaponi *et al* 2012) and pear from Tunisia (Hassen *et al.*, 2004). There are potential economic and environmental impacts due to the vulnerability of UK hedgerows (which comprise *P. spinosa* and hawthorn as major constituents) to HSVd as a consequence of their regular pruning. Wild hop also grows widely as a hedgerow plant in England (see Botanical Society of Britain and Ireland (BSBI) distribution map).

7. If the pest needs a vector, is it present in the UK?

HSVd, like other Pospiviroidae, are not considered to be vectored.

8. What are the pathways on which the pest is likely to move and how likely is the pest to enter the UK? (*By pathway*):

Plants for Planting

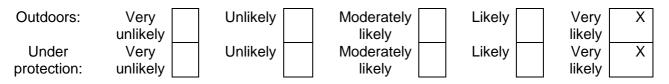


There is a very limited trade into the UK of hop varieties for commercial production from the USA. Plants imported from outside the EU have to be accompanied by a phytosanitary certificate declaring freedom from symptoms of the fungal diseases caused by *Verticillium albo-atrium* and *V. dahliae* (EC 2000) but there are no specific requirements for HSVd.

New potential breeding material and candidate nuclear stock imported from outside the UK is understood to be tested before use and infected material would not be used for further breeding or propagation (*P. Reed Pers. Comm.*). Potentially, there is a risk that individuals may illegally import hop plants from countries which have the disease (e.g. the US, Japan or China) in personal baggage.

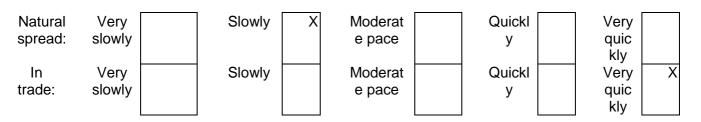
The most important known hosts which may potentially be infected and constitute an introduction pathway via plants for planting are hop, grapevine, cucumber and *Prunus spp.* Asymptomatic infection of grapevine has been reported widely from many countries including Japan, China, Germany, New Zealand, Australia, California (USA) Turkey, Iran, and Tunisia (Jiang *et al.*, 2012). Pale fruit disease of cucumber is not common in Europe. The first European outbreak of the viroid in hop has recently been reported from Slovenia where the outbreak is under eradication (Radišek *et al.*, 2012).

9. How likely is the pest to establish outdoors or under protection in the UK?



HSvd like other viroids and viruses has no free living stage and would be expected to be maintained in an infected host where ever the host can grow. However, more studies are required to establish if climatic conditions can influence *in planta* maintenance of HSVd carriage. A quantitative analysis of HSVd in apricot in Spain over the period of a year, found viroid levels fell after May to July but recovered by December (Amari *et al.*, 2001).

10. How quickly could the pest spread in the UK?



Opportunities for long distance natural spread are limited since there is no known vector for the HSVD. The viroid is transmitted by vegetative reproduction or mechanical means, e.g. pruning or other practices, which may lead to plant damage. Mechanical harvesting and other operations lead to spread along the rows in commercial hop plantations (Pethybridge *et al.*, 2008). The first disease report of HSVd in hop in Slovenia (EPPO Reporting Service 2012/055; Radišek et al., 2012) records spread of the disease as 10% per year.

Infection is likely to be restricted to the initial premises where infection occurred, except when infected propagating material is disseminated by trade or possibly through the use of shared machinery. A study

of the survival of HSvd in infected hop cones and leaves remaining in the field after the end of the season found that infectivity had been lost after 3 months (Yaguchi and Takahashi, 1984). This supports disease transmission mediated through vegetative reproduction or direct contact with infected material during hop management practices. Latent disease expression is conducive to spread as there may be a time lag before it is realised that infection is present, which allows the viroid to become established before management response measures can be implemented.

Whilst the epidemiology of HSVd infection is consistent with vegetative reproduction or mechanical transmission as the principal means of viroid spread, other potential transmission routes may occur as rare events.

Experimental transmission of HSVd by injection from grapevine to hop has been confirmed and grapevine has been described as a symptomless reservoir of HSVd infection (Matousek *et al.*, 2003, Kawaguchi-Ito *et al.*, 2009). The latter study also found HSVd mutation from a grapevine sequence variant to a hop sequence when grown in the hop host. Phylogenetic evidence also suggests that hop and *Prunus* strains of HSVd may be derived from grapevine strains (Sano *et al.*, 2001; Zhang *et al* 2012). Sequence analysis of HSVd from the recent Slovenian outbreak found four of five sequences determined were most similar to genotypes from cucumber pale fruit and *Citrus* spp.

Seed mediated HSVd transmission has been demonstrated in grapevine (Wan Chow, 1999), though it was not established if infection occurred through the ovule or pollen. In plum, HSVd seed transmission and infection of pollen has been demonstrated (Luigi *et al.*, 2010), though again, it was not clear if infection had occurred from the infected pollen. A small study has reported experimental transmission of cucumber pale fruit viroid via pollen to tomato (Kryczynski *et al.*, 1988). The potential for pollen mediated transmission of HSVd in hop was studied by Yaguchi and Takahasi (1984) who could not demonstrate viroid transmission either through the ovule or pollen. Moreover, the general experience of HSVd epidemiology in hop suggests that natural pollen transmission is not an efficient means of infection.

Although hop plants were grown close to infected grapevine in a study area in the Czech Republic there was no evidence of transmission and natural infection of the nearby hops (Matousek *et al.*, 2003). Analysis of 105 plant specimens representing 25 families that had been growing as weeds in a severely infected hop garden found no evidence of infection (Yaguchi and Takahhasi (1984). A survey of wild hop in southern Italy found no evidence of HSVd infection (Ragozzino *et al.*, 2008). The absence of HSVd in in hop in Europe (apart from the current outbreak) despite the presence of infected grapevine, indicates that transmission between these hosts in the environment does not occur at all or is very rare.

Together, these studies provide little evidence for the efficient natural spread of the viroid other than through vegetative propagation or mechanical transmission during cultivation. Spread of HSVd from imported *Citrus* or *Prunus* fruits to hop is unlikely because of the absence of an efficient means of viroid transmission. Consequently, spread of the viroid is likely to be restricted to the introduction of infected planting material.

11. What is the area endangered by the pest?

Hop production areas in England, (Herefordshire, Worcestershire, Shropshire, Kent, Sussex, Surrey, Hampshire, Oxfordshire and Yorkshire (British Hop Association information)). Cucumber production in glasshouses. *Prunus* trees are widely grown in the UK, are a major constituent of hedgerows and are commonly sold at nurseries.

12. What is the pest's economic, environmental or social impact within its existing distribution?

Very	Small	Medium	Large	Х	Very	7
small					large	

Significant economic losses to hop, Citrus, Prunus and cucumber production have been recorded.

Since HSVd symptom expression can vary significantly according to host species, host variety and viroid strain consequently, predicting the potential damage resulting from HSVd infection can be very difficult. The global occurrence of HSVd in grapevine has been reviewed by Jiang *et al.*, (2012) who identified reports from Japan, Germany, Australia, Turkey, Iran, New Zealand and China, additionally there is a report from the Czech Republic (Matousek *et al.*, 2003). However, minimal or no morphological symptoms have been reported in grapevine (Kawaguchi-Ito *et al.*, 2009; Wolpert *et al.*, 1996). Similarly, Jujube infections have not been associated with symptoms (Zhang *et al.*, 2009). HSVd infection of pear has been reported from Tunisia (Hassen *et al.*, 2004) and recently from wild and domestic apple from Greece (Kaponi *et al.*, 2012). Again it is not clear if HSVd infection produces symptoms in either fruit species.

Cucumber pale fruit disease (Sano *et al.*, 1981) has caused economic losses and in the 1970s it spread throughout cucumber production glasshouses in the Netherlands. However, the outbreak was associated with only a low incidence of symptomatic plants (Dorst *et al.*, 1974). A recent outbreak of the viroid in cucumber (from Finland) was effectively dealt with by removing infected plants (Lemmetty and Soukainen, 2011).

Symptoms of HSVd infection in hop have been reviewed by Pethybridge *et al.*, (2008) and include stunting, loss of vigour (leading to death of the vine) and reduction in flower cone metabolite quality (alpha acids), which leads to serious economic losses (Momma and Takahashi 1984). HSVd infection was detected in10 of 33 hop gardens in Washington State, a major hop growing region of the USA, where it has caused suppressed growth and canopy loss in the modern crop cultivars: 'Epinasty' and 'Glacier' (Eastwell and Nelson 2007). China is a major hop producer and HSVd infection was first reported in this host in 2007 when three of five cultivars were found to be infected in Xinjiang Province (Guo *et al.*, 2008). More recently, a survey of HSVd reported infection rate of 23.8 % among hop gardens sampled (Zhang et.al 2012). The first European report of HSVd in hop was recorded from Slovenia (EPPO Reporting Service 2012/055; Radišek el., 2012). Symptoms were found in hop gardens from 2007 involving three varieties- Celeia, Bobek and Aurora. Infection was confirmed by molecular testing from two hop gardens and eradication measures are being implemented. More information on the progression of the outbreak has been kindly supplied by Dr Sebastjan Radišek and this information is provided in Appendix 1.

The incidence and symptoms of HSVd infection in *Prunus* HSVd have been reviewed by Sano (2011). Although symptomless HSVd infections can occur in *Prunus* fruit discolorations known as peach and plum dapple diseases have been reported, that can seriously affect marketability. These diseases were first reported from Japan in the 1980s where it continues to be sporadic in plum growing areas of this country (Kusano and Shimomura (1997). Peach fruit with symptoms of 'dapple' disease are common in Chinese markets and HSVd infection has been confirmed from these fruit (Zhou *et al.*, 2006). HSVd infection has been confirmed in plum (*P. domestica*) with dapple fruit symptoms in an Italian orchard (Ragozzino *et al.*, 2002). In apricot HSVd infection can be asymptomatic, though a serious new disease associated with the viroid ('degeneracion') has been reported recently from Spain, which results in unmarketable fruits deficient in flavour characteristics (Amari *et al.*, 2007). The development of this new disease underlines the unpredictability of HSVd infection and the symptoms produced.

There are various diseases induced by HSVd infection in *Citrus* (including the significant disease cachexia) which are referenced in the disease names in section 1.

13. What is the pest's potential to cause economic, environmental or social impacts in the UK?



Introduction of HSVd would be expected to cause medium to large economic impacts, mainly through potential losses to hop and limited losses and disruption to cucumber production. In England hop growers supply over 1000 UK brewers and exports widely around the world in a trade estimated at £12 million at farm gate prices (British Hop Association data) and HSVd is considered to be an important threat to this industry. A recent confirmed report of cucumber pale fruit disease in Finland was effectively

controlled by removing symptomatic plants. Commercial hop growing is a 'large investment long-return' production system, and HSVd infection could be very damaging to this crop. The recent report of HSVd in hop in Slovenia found classic symptoms of the disease including stunting, leaf curl and small cone formation (Radišek *et al.*, 2012). The disease in the field progressed primarily along the hop rows where symptom incidence varied between 1 and 30%. There is considerable uncertainty as to the likely economic consequences of introduction of HSVd in other host species such as *Prunus*, where there is some risk to the ornamental tree trade, fruit production, and hedgerows.

Hedgerows may be vulnerable to HSVd infection because of their regular pruning. There have been no reports of HSVd infection in blackthorn or hawthorn though no large scale surveys have been reported and the seriousness of any diseases which may result from infection is difficult to evaluate. A small HSVd survey in Bosnia and Herzegovina did not detect the viroid from 11 *P. spinosa* trees tested (Matic *et al.*, 2005), similarly a Croatian survey which included *P. padus* and blackthorn found no infection (Skoric *et al.*, 2008).

14. What is the pest's potential as a vector of plant pathogens? None.

STAGE 3: PEST RISK MANAGEMENT

15. What are the risk management options for the UK? (Consider exclusion, eradication, containment, and non-statutory controls; under protection and/or outdoors).

Exclusion

Management options for exclusion are limited to the prevention of introduction into the UK by infected planting material of hops, grapevine, *Prunus* and other major or minor hosts. This could be by the total prohibition of entry of these hosts into the UK and EU. However, the viroid has been known to occur in Europe on many hosts apart from hops for many years, during which time there has been no evidence of cross infection to hop despite the there being no restrictions on HSVd in these other hosts. This would make a prohibition seem unjustified.

The introduction of requirements specifying freedom from HSVd in EU legislation could be proposed by amending the Council Directive 2000/29/EC (Plant Health Directive) to include a listing for HSVd in either Annex IIAII or Annex IIB introducing protected zones. If listed in Annex IIAII, additional requirements could also be added to Annex IVAI and IVAII, for example that plants come from a pest-free area or a pest free place of production, or that plants are derived from material tested within a certain period.

Exclusion can also be enhanced by voluntary testing of new breeding or other material before use or dissemination within the UK and this is already carried out in breeding material. It is an assumption in the EPPO Certification scheme for hops that hop material imported to be used as nuclear stock will have been tested for pests not occurring in the EPPO region, such as HSVd (EPPO 2009).

Eradication

Eradication measures can be undertaken if any outbreaks are detected in the UK. If HSVd is introduced and detected before planting, material could be destroyed or returned to the supplier if of non-UK origin, and, if detected in the field, then eradication measures could be undertaken. This is the position at present, whereby under EU and UK legislation new unlisted pests that are "not normally present in Great Britain" can legally have statutory action taken to eradicate or contain them. Therefore any finding of HSVd in the UK in the glasshouse or field in hops (or other crops if deemed a risk to themselves or hops), could have eradication measures taken. This was done in the Slovenian outbreak in 2012 (Radišek el., 2012). The types of eradication measures that could be used in isolation or in combination would be destruction of infected plants and others considered 'at risk' of potential infection, disinfection of machinery and tracing of any associated stocks.

Containment

Containment could be considered as an alternative to large scale destruction of a hop garden if, for example, there was no risk of spread to neighbouring crops and hygiene and other measures such as prohibition of movement and propagation of plants was prohibited.

Non-statutory controls

HSVd could continue to be controlled as at present by voluntary industry actions such as testing of potential imported breeding material and inclusion in the voluntary PHPS certification scheme. This scheme presently covers nearly all the hop plants produced in the UK and requires visual freedom of all grades. However hop plants may occasionally be introduced from other parts of the EU with unknown testing requirements. Education of the main hop growers on the symptoms and risks from HSVd would be part of this voluntary industry practice and the sourcing of pathogen free material recommended. At the present time resistant hop varieties are not known.

16. Summary and conclusion of rapid assessment.

(Highlight key uncertainties and topics that will require particular emphasis in a detailed PRA) General / overall summary and conclusion and then specific text on each part of assessment...

This rapid assessment shows that:

HSvd has a large host range and can cause disease in taxonomically diverse hosts, though symptomless infections are common. In the UK, hop is vulnerable to economic losses from HSVd infection. There have been no reports of the viroid in the UK. In Europe the first outbreak of HSVd in hop has been reported recently from Slovenia, where the infection was associated with classic disease symptoms of stunting and foliar damage. Pale fruit disease of cucumber is now rare in Europe though the disease has been reported and eradicated recently in Finland (Lemmetty et al 2011). The absence of an efficient HSVd vector limits spread of the viroid away from the initial site of infection, except when infected material is passed on to other premises. Consequently, avoidance of introduction of HSVd infected material through use of certified plants largely provides an efficient means of disease control, though crop monitoring is also important to mitigate introductions which may arise as very rare events.

Risk of entry

There is a moderate risk of HSVd entry. Most commercial hop growers in England are aware of the risks of the viroid from imported plants and use certified plant material, which have complied with import restrictions. There may be some risk of illegal importation of hop plants for planting by individuals for domestic or small scale use. The viroid could also be imported from infected grapevine or *Prunus spp.*

Risk of establishment

There is a high risk of establishment from imported 'plants for planting' since infected plants would be expected to maintain the infection.

Economic impact

HSVd is evaluated to cause medium to large economic impacts mainly through potential losses to hop and cucumber production, though there is some uncertainty because of the unpredictability and insidious nature of the viroid.

Endangered area

Hop growing areas in England and glasshouse production growing cucumber. Premises involved with propagating *Prunus and* hedgerows.

Risk management

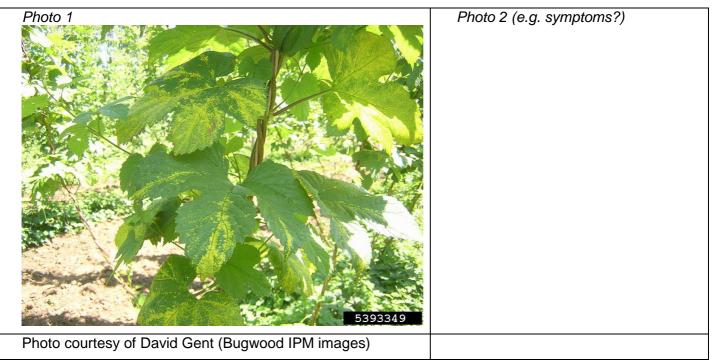
17. Is there a need for a detailed PRA? If yes, select the PRA area (UK or EU) and the PRA scheme (UK or EPPO) to be used. (for PH Risk Management Work stream to decide)

This Rapid Assessment covers the current known information on Hop Stunt Viroid. While there are uncertainties a more detailed PRA would not provide greater clarity at this time.

No	Х

Yes	PRA	PRA scheme:	
	area: UK	UK or EPPO	
	or EU		

18. IMAGES OF PEST



19. Given the information assembled within the time scale required, is statutory action considered appropriate / justified?

Due to the potential damage to hop production it is concluded that in the event of an outbreak or interception of HSVd in the UK that emergency statutory action is justified under UK legislation in order to eradicate or contain the disease. Current PHPS requirements appear to be effective in ensuring that UK produced hop propagating material and plants for growers are free of HSVd. While EU regulation could be considered in response to the risk that infected material could be imported from elsewhere within the EU, or from third countries, this would be technically challenging and costly. HSVd affects species other than hops and the possibility of asymptomatic infection means that any regulatory approach would need to involve a testing element, which would be very costly (to UK propagators and hop producers as well as those elsewhere) if it were to provide a satisfactory degree of assurance of pest freedom. While previous surveillance has not detected the presence of HSVd in UK hops, systematic surveillance of other hosts has not been undertaken. This would be needed for a UK protected zone, or to make pest fee area declarations, which would be relevant for exports as well as imports. Therefore, it is recommended that the current approach is retained, but supplemented by testing of imports by the PHSI, which will cover commercial imports as well as small quantities imported for amateur use. Awareness raising will also be initiated, targeted at both commercial and amateur growers, to highlight the risks of importing hops from areas where HSVd is present and highlighting the benefits of using PHPS certified material.

Yes Statutory action



No Statutory action



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Appendix 1.

Data on Slovenian HSVd outbreak supplied by Dr. Sebastjan Radišek*

Table 1: Hop stunt disease in Slovenia - Outbreaks, disease spreading and eradication in the period 2007-2012.

Year	Hop gardens with diseases plants in current year (ha)	Disease spreding to new hop gardens (ha)	Hop gardens with confirmed infection since firts disease discovery (ha)	Eradicated hop gardens (ha)
2007	6,55	/	6,55	*
2008	22,44	15,89	22,44	*
2009	29,84	7,4	29,84	*
2010	47,38	17,52	47,38	*
2011	50,1	11,15	58,51	10,80
2012	28,89	8,15	66,66	8,30

*Local eradication of affected plants

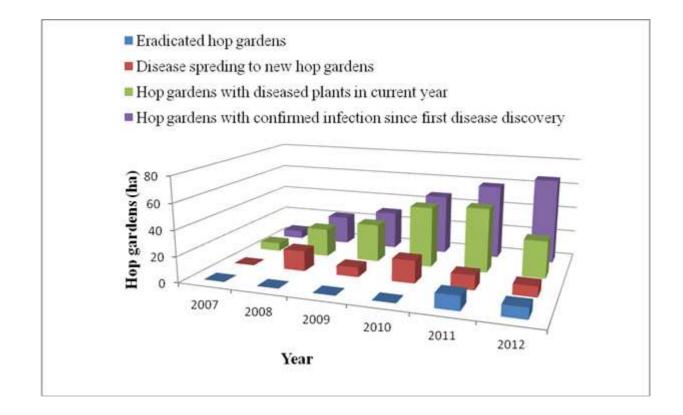


Figure 1: Hop stunt disease in Slovenia - Outbreaks, disease spreading and eradication in the period 2007-2012.

*Annual Report of systematic monitoring of hop stunt disease in Slovenia in year 2012.

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