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Monitoring of Important Virus and Viroid Infections in German Hop (*Humulus lupulus* L.) Yards

Viroids and viruses can cause significant loss of yield and quality in hops. Several viruses are endemic in hop growing regions around the world but are tolerated because of high costs of replanting. A major problem is that viroids and viruses cannot be controlled by chemical plant protection measures. In addition, resistant hop cultivars are not available. But propagation of certified planting material, combined with a systematic replanting and monitoring program can impede the spread of viruses and viroids. Monitoring and close cooperation with plant health services are of utmost importance to detect and eradicate at an early stage first sources of infection with pathogens that have potentially catastrophic impacts on hop production. This is especially true with regard to *Hop stunt viroid* (HpSVd) and *Citrus viroid IV* (CVd IV), two dangerous viroids which must not be introduced in Germany's hop production. Therefore, monitoring was conducted from 2008 to 2013 comprising all German hop growing regions to detect possible primary sources of viroid infections. From 2011 onwards, in this monitoring tests for *Hop mosaic virus* (HpMV), *Apple mosaic virus* (ApMV), *Arabis mosaic virus* (ArMV), *Hop latent virus* (HpLV) and *American hop latent virus* (AHpLV) were included to elucidate the virus situation regarding economically important hop viruses. Hop leaves from breeding yards, field trials and cultivar collections from the Bavarian State Research Center (LfL), from commercial hop gardens and a propagation facility were tested by ELISA and RT-PCR. While viruses are widely distributed, HpSVd has not been detected in Germany's hop industry. In the present study, nine HpSVd findings from the hop germplasm collection in Huell in 2010 were the only ones in a total of 1,444 samples. This result suggests that eradication measures taken in 2010 were successful. Tests for CVd IV began in 2013 on a small scale and to date this viroid has not been detected in Germany. Monitoring will be continued in 2014 with the main focus put on HpSVd and CVd IV tests providing knowledge for effective risk management of these dangerous diseases.

Descriptors: monitoring, hops, virus, viroid, RT-PCR, DAS-ELISA

1 Introduction

Hop production is an economically important industry in Germany. With a total of 16,800 ha acreage in 2013 Germany is the country with the largest acreage of hops. Stable yield and excellent hop quality are crucial factors for growers and brewers alike. Plant health is a fundamental contributor to both yield and quality. Hop pathogens such as fungi and arthropod pests can be controlled by a combination of horticultural practice and appropriate agricultural chemicals. Additionally, breeding for resistance has produced tolerant and potentially resistant hop cultivars. Viruses and viroids with significant negative economic impacts on hop production or brewing quality require a different approach. Viruses and viroids cannot be targeted with agricultural chemicals, and are easily transmitted during routine hop cultivation measures, by root grafting or by aphids. Over time, latent infections become widely distributed within hop gardens and to other locations before visible disease symptoms occur under stress situations. Virus and viroids infections must be managed with a combination of monitoring, eradication and replanting with healthy (virus and viroid free) stock.

Hop stunt viroid infections in the USA were first reported in 2008 [1]. In response to this, the LfL started a monitoring programme for this pathogen with the objective of detection and elimination of any potential primary sources of HpSVd infections in hop gardens in Germany. This monitoring continued from 2009 till 2013 (and is ongoing). From 2011 onwards, economically important viruses were integrated in our survey. In 2013 first tests for the dangerous *Citrus viroid IV* (CVd IV) recently detected in Slovenia [2] were performed.

In this paper the monitoring programme and the results gained over the period 2008 to 2013 are presented. Based on the findings and results of our monitoring, advice can be provided to hop growers about how best to manage these diseases.

1.1 Viruses affecting hops

Hops can be infected by a number of viruses [3, 4, 5, 6, 7] with the following recognized as being the most important (in alphabetical

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order): *American hop latent carlavirus* (AHpLV), *Apple mosaic ilarvirus* (ApMV-H = hop isolate; ApMV-I = intermediate isolate), *Arabis mosaic nepovirus* (ArMV-H = hop strain), *Hop mosaic carlavirus* (HpMV), *Hop latent carlavirus* (HpLV). No visual symptoms are known for any HpLV and AHpLV infected commercial hop varieties [4]. Virus infections associated with clear symptoms include for example 'nettlehead disease' caused by ArMV, vein clearing, mosaic and leaf deformation by HpMV as well as chlorotic and necrotic rings or arcs and oak-leaf line patterns on leaves caused by ApMV. These visual symptoms depend on the cultivar, the area of cultivation, on environmental and climatic conditions and may vary from year to year [3, 4, 5, 6]. Infections with ArMV, ApMV or HpMV can result in significant losses of yield and quality. Simultaneous infestation with different viruses is known to have synergistic detrimental effects. Viruses and viroids are spread by mechanical means within a hop garden. Additionally, carlaviruses are transmitted by aphids in a non-persistent manner and ArMV by nematodes [3]. Seed transmission is of little importance as hop is a clonal crop [7].

1.2 Viroids affecting hops

Viroids are small plant pathogenic particles known to infect a number of economically important crops with symptoms ranging from mild to severe. They consist of single-stranded RNA. They are propagated in host cells and are transmitted by mechanical means during harvest and cultivation operations. Symptom expression is due to interactions between viroid and host plant gene expression [8, 9].

To date, four viroids have been reported to infect hops: *Hop stunt viroid* [1, 3, 7, 10, 11], *Hop latent viroid* (HpLVd) [3, 6], *Apple fruit crinkle viroid* (AFCVd) [1, 6, 10] and *Citrus viroid IV* (CVd IV) [2]. AFCVd has only been found in Japanese hops grown in close proximity to apple cultivation. HpLVd is present in hop yards all over the world [3]. Negative effects of HpLVd on yield and quality have been reported for some hop cultivars [12], but due to its wide distribution eradication is unlikely.

HpSVd is considered as a serious problem in hops causing dramatic losses of yield and reductions in the levels of bittering substances [10, 11]. The same applies to CVd IV which seems to exceed HpSVd in its pathogenic power, particularly in the case of co-infections between the two viroids [2]. HpSVd is known to infect various crops (e.g. hop, cucumber, grapevine, citrus, plum, peach, apricot and almond; [10]). HpSVd from isolates from hop are most similar to isolates from grapevine and it is assumed that HpSVd in hops originated in Japanese grapevines [10]. In the 1940s HpSVd was observed in hop cultivars in Japan and Korea, but it was not until 1977 that HpSVd was identified as the causal agent of the hop stunt disease [10]. In the USA, HpSVd was detected in hop in 2004 [1]. Later, HpSVd infections of hop were also reported from China, in a cultivar imported from the USA [13]. In Europe HpSVd symptoms on hops have been observed in Slovenia since 2007. [14]

Yield losses attributable to HpSVd depend on weather conditions and cultivar. *Eastwell* [11] reported losses of 60 to 80 % in yield of alpha acid per acre in US cultivars. Typical symptoms for HpSVd infections are stunting, small cones, chlorosis and leaf curl. For HpSVd it is reported that latent infections may persist over three to five years before characteristic symptoms occur [11]. Thus, latently infected hops can be major sources of inoculum for infection. HpSVd is a real threat to all hop growing countries because:

i) hop may be propagated easily by root or softwood cuttings;
ii) there is an almost global exchange of hop germplasm; and
iii) the inevitability of mechanical inoculation within and between hop yards in the context of commercial production.

2 Material and Methods

2.1 Plant material monitored

Leaves of hop plants were taken from the breeding yards of the LfL, from plots of the EU PVR (Plant Variety Right) trial field and the international cultivar collection in Huell, from field trials in Straß, Tettnang, from one contract nursery of the Society of Hop Research (GfH), as well as from commercial hop gardens in the Hallertau, Tettnang and Elbe-Saale regions. Wild hops from the Huell wild hop collection and male hops were included. Approximately 250 samples were collected per year. Sampling was not random, but targeted by preferentially collecting material from plants showing suspicious symptoms such as chlorosis, reduced vigour and stunting. Commercial hop yards were sampled by collecting leaves from two plants, with one sample from each plant. In general, four to five young leaves were randomly collected from each individual plant. Leaf samples from the Huell breeding yards and the Huell plots were often composite samples of two plants from a particular genotype in a particular plot. Leaf material was kept at minus 80 °C until further testing.

Samples tested for respective viruses and viroids are described in table 1:

Table 1 Viruses and viroids tested in our monitoring programme

Virus/viroid	Acronym	Detection method
Hop stunt viroid	HpSVd	RT-PCR
*Citrus viroid IV**	CVd IV	RT-PCR
American hop latent carlavirus	AHpLV	RT-PCR
Apple mosaic ilarvirus	ApMV	DAS-ELISA
Arabis mosaic nepovirus	ArMV	DAS-ELISA
Hop latent carlavirus	HpLV	RT-PCR
Hop mosaic carlavirus	HpMV	DAS-ELISA

*Only twelve samples tested in 2013

Tests for HpSVd, ApMV, ArMV and HpMV were performed throughout the three years. Tests for ApHLV were conducted sporadically in 2011 and 2012. As results indicated a wider distribution of ApHLV than expected, all samples collected in 2013 were tested for ApHLV. Tests for HpLV were abandoned in 2013. The number of tests for CVd IV was very low as the detection method was only established after the first report [2] of its occurrence in Slovenia in 2012.

2.2 DAS-ELISA

500 mg of leaf material was homogenized in 4.5 ml of DAS-ELISA (double-antibody sandwich enzyme-linked immune

sorbent assay) homogenization buffer in homogenization bags using a homogenizer (Bioreba, hand model). One millilitre of the extract was further diluted with 1 ml homogenization buffer and centrifuged at 1000 g for 1 min. The resulting supernatant was used for DAS-ELISA. Each extract was tested twice. ApMV, HpMV and ArMV were detected using specific antisera from Loewe Biochemica, Sauerlach, Germany. Positive controls were obtained as freeze dried leaf material (Loewe Biochemica) and reconstituted according to manufacturer's instructions. As negative controls, leaves of healthy tobacco (*Nicotiana* sp.) plants grown under quarantine conditions were used and treated as per samples. DAS-ELISA was performed on Nunc immunoplates or strips following Loewe's advice with the following exceptions: volume of coating buffer and sample volume were 100 µl per well; volume of alkaline phosphatase antibody conjugate was decreased to 90 µl and volume of substrate solution (p-nitrophenyl phosphate 10 mg.ml^{-1} substrate buffer) was reduced to 80 µl in order to prevent non-specific reactions. Substrate incubation was 4 hours at 22 °C. Differentiation between positive and negative results was achieved according to the 'Bioreba data analysis' (http://www.bioreba.ch/files/Technical_Info/ELISA_Data_Analysis.pdf).

2.3 RT-PCR

For RNA extraction the QIAGEN RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) was utilised. 500 mg frozen plant material was ground in the homogenisation bag using a hand roller (Bioreba) with 4.5 ml RLC extraction buffer containing 10 µl ß-mercaptoethanol per ml. The RNA extraction procedure was continued as described for *Potato spindle tuber pospiviroid* [15] with one single modification: an additional washing step with 500 µl of RPE buffer was performed to remove possible RT-PCR (reverse transcriptase polymerase chain reaction) inhibitors in the extract. At the end, RNA was eluted from the spin columns using 50 µl of RNase-free water. In parallel to the samples, extraction controls were included in each extraction. As negative extraction control 500 mg of healthy leaves of tobacco grown under quarantine conditions were used and treated as per samples. 25–50 mg freeze dried leaf material infected with the respective virus or HpSVd provided by Dr. Ken Eastwell, Washington State University (WSU), USA, was used as positive extraction control after adding 4.5 ml RLC extraction buffer. RNA isolated from CVd IV-infected hop leaves provided by Dr. Sebastjan Radišek, Slovenian Institute of Hop Research and Brewing, Slovenia, was included as positive control in RT-PCR.

As shown in table 1, HpLV, AHpLV, HpSVd and CVd IV were tested by RT-PCR. Occasionally HpMV was tested by RT-PCR to verify doubtful ELISA results. For HpLV, AHpLV, HpSVd and HpMV a one-step RT-PCR protocol was applied using the Kit SuperScript® One-Step RT-PCR System with Platinum® Taq DNA Polymerase (LifeTechnologies, formerly Invitrogen, Germany), for CVd IV the SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase was used. Primers and RT-PCR protocols for HpLV, HpMV and AHpLV were developed by K. Eastwell, WSU, USA, and are confidential. Primers for the specific detection of HpSVd and CVd IV were according to [1] and [16], respectively. RT-PCR protocol for HpSVd published by Eastwell and Nelson [1] was adopted to the RT-PCR kit mentioned above: 55 °C for 30 min; 94 °C for 2 min followed by 40 cycles 94 °C for 15 sec, 59 °C for 30 sec, 72 °C for 60 sec and a final step of 72 °C for 10 min. RT-PCR protocol for CVd IV was as follows: 60 °C for 60 min, 94 °C for 2 min, followed by 40 cycles 94 °C/30 sec, 60 °C/30 sec, 72 °C/60 sec and at last a 5 min incubation at 72 °C. In each case RNA added to the RT-PCR reaction equals a volume of 1/25 of the total RT-PCR reaction volume. In each RT-PCR run besides the respective samples and extraction controls a sample containing water instead of sample RNA was included. RT-PCR products were analysed on 1.5 % agarose gels with ethidium bromide added to the gel solution [15].

In order to identify 'false negative' RT-PCR results due to failed RNA extraction or RT-PCR inhibitors, in addition to the virus/viroid specific primers each RNA extract was tested in parallel using specific primers to detect nad5 plant mRNA as an internal positive RT-PCR control (IPC) in a separate RT-PCR [17, 18]. Prior to 2013, in cases where the IPC failed and no viroid or virus was detected in the extracted RNA, the RNA was further diluted (1:10) and tested once again. In 2013, RNA of each sample was tested undiluted and in a 10^{-1} dilution. In cases where HpSVd could be detected in the undiluted and/or diluted RNA, the result was clear and declared as "positive". In cases where HpSVd was not detected while the IPC worked well with the undiluted and the diluted RNA, the result was 'HpSVd negative'. Results from all samples which tested HpSVd negative and gave positive IPC results only with the diluted RNA were unclear, as the pathogen RNA present might have escaped detection in the dilute RNA. The same approach of result interpretation was adopted with respect to the RT-PCR detection of the different viruses.

2.4 Virus and viroid identification

For verification and confirmation the RT-PCR products for AHpLV, HpLV and HpMV of selected virus samples were sequenced by Sequiserve, Vaterstetten, Germany. In the case of detection of viroids each amplicon was checked by sequencing to identify the viroid. Prior to sequencing, RT-PCR products were purified and concentrated using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The nucleotide order of the RT-PCR products were compared to reference sequences deposited in NCBI (National Center for Biotechnology Information) and homology confirmed the reliability and specificity of the RT-PCR results.

3 Results and discussion

3.1 Occurrence of HpSVd

Testing for infection began in 2008 using RT-PCR. Fifty-five samples were tested, with all samples producing negative results. Among these, 43 samples from US cultivars which were held under quarantine conditions in the greenhouse before being established in the EU PVR trial plots in Huell appeared to be HpSVd free. Additionally, eleven cultivar samples from the Huell breeding yard and one sample from a commercial hop farm in the Hallertau produced negative results for HpSVd.

In 2009, 224 hop samples were taken from breeding yards, the world hop collection and the PVR trial plots in Huell, a contract propagation facility of the Society of Hop Research and from commercial hop stands in the Hallertau, Tettnang and Elbe-Saale regions. There were no positive test results for HpSVd in 2009.

In 2010, 377 samples were tested for HpSVd. Among these, HpSVd was detected in five hop plants of the cv. 'Horizon' (originating from the United States of America) in the Huell world hop collection. Additionally, four other hops (cv. Saphir, Glacier and two experimental lines) grown in close proximity to cv. Horizon, within the same row also tested positive for HpSVd. The other 368 samples investigated produced negative results for HpSVd. The specificity and reliability of the HpSVd findings were confirmed by sequence analysis of RT-PCR products. Amplicons obtained from 'Horizon' plants showed a length of 302 bp, the size of the four other RT-PCR products was 300 bp. In each case sequence was 98 % identical to a grape isolate of HpSVd (GenBank: HM357802.1).

No HpSVd infections were detected among the 788 hop plants sampled during the years 2011, 2012 and 2013. The nine hop plants from the hop yard in Huell which tested positive in 2010 were the only HpSVd detections in a total of more than 1,440 samples tested since 2008 in this study. There is strong evidence that the primary source of infection was a cultivar imported from the USA into the Huell germplasm collection in 2001, and it appears that the measures taken to eradicate this pathogen (elimination of the infected plants using Glyphosate) were successful.

Data presented here do not allow for a full estimation of the HpSVd incidence in German hop fields since the sampling strategy did not systematically cover all hop yards. Furthermore, the internal RT-PCR control failed in approximately 4 %, 9 % and 1 % of the samples, respectively, in 2011, 2012 and 2013 and therefore the true viroid status of these samples is not known. The failed amplification of the internal nad5 mRNA based positive RT-PCR control (IPC) may have resulted from endogenous RT-PCR inhibiting hop compounds that were not fully removed by the inclusion of an additional washing step during RNA extraction or additional purification (data not shown).

Nevertheless, taking all results together, the HpSVd data obtained in this study suggest that in Germany the HpSVd situation is different from that in countries such as Japan [10], China [13], USA [1] and Slovenia [2, 14] where HpSVd appears to be widespread in hop gardens causing significant economic losses. Moreover, it appears that HpSVd has not reached commercial hop production in Germany. To prevent the import of this viroid into the German hop production, continuous testing of introduced hop material is crucial, as HpSVd (as with CVd IV) is not listed as a quarantinable pathogen in the EU (Council directive 2000/29/EC). Further, testing for HpSVd is neither regulated nor obligatory. As discussed previously, testing for CVd IV was started quite late in 2013 after it was reported from Slovenia [2] that this viroid was detected in hop yards, and that the likely source was citrus material in compost. In Slovenia, CVd IV associated with HpSVd led to drastic decreases in yield and quality. The testing, in this study, of twelve samples from German hop yards where compost was used for years as fertilizer is not adequate to confirm the HpSVd and CVd IV free status of German hops with certainty, but testing will continue the coming years.

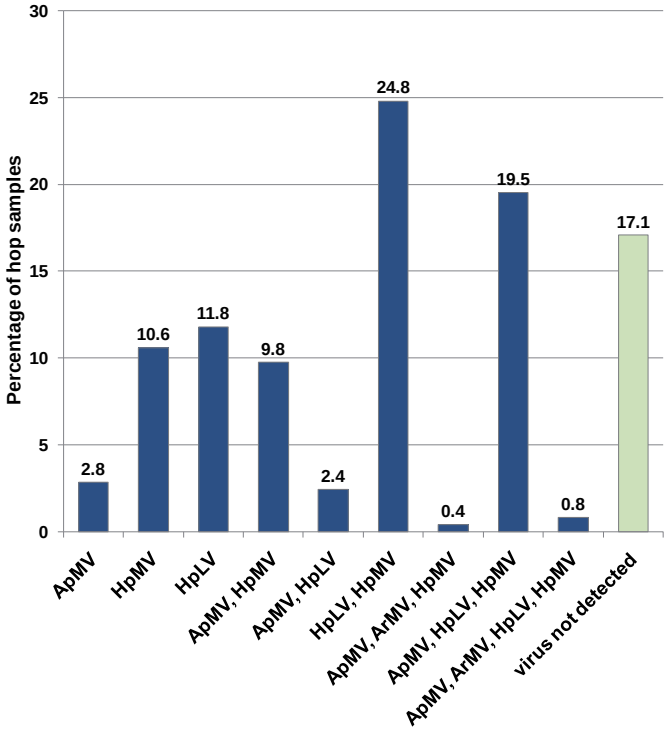

Fig. 1 **Percentages of hop samples infected with one or more viruses in 2011. A total of 246 samples was analysed for all viruses, except AHpLV which is not considered since only 10 samples were tested for AHpLV**

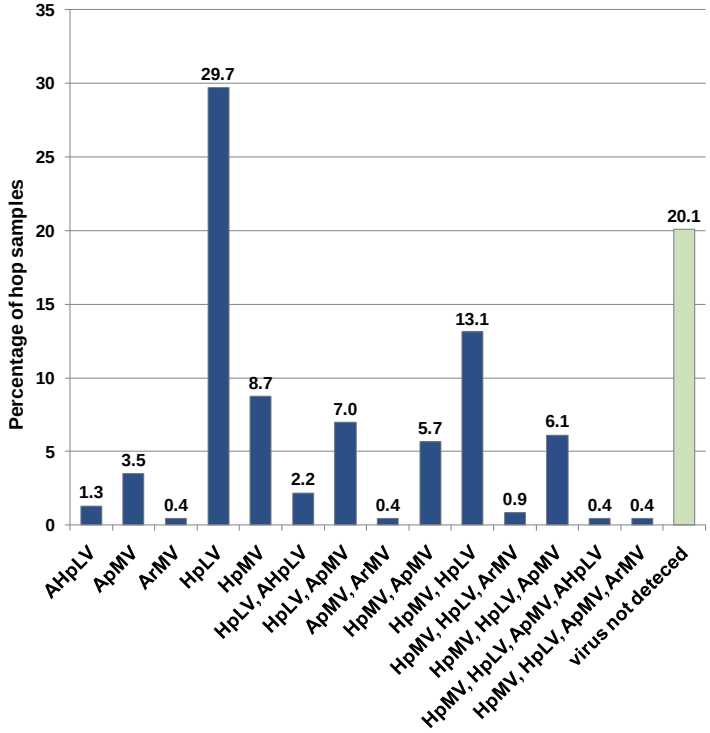

Fig. 2 **Percentages of hop samples infected with one or more viruses in 2012. 229 samples were tested, only the values for ApHLV infections are not fully comparable because only 53 samples were tested for this virus**

3.2 Occurrence of Hop Viruses

In 2011, a total of 246 hop samples was screened. Forty-two samples (17.1 %) produced negative results for all viruses. Using DAS-ELISA, HpMV and ApMV were found as single pathogens or in combination with other viruses in 65.9 % and 35.7 % of the samples, respectively (Fig. 1). Based on the RT-PCR detection method, HpLV was found in 59.3 % of all hop samples, often accompanied by other viruses (Fig. 1). AHpLV was not tested on a wide scale until after 2011 when reference material became available. Only ten US cultivars from our Huell hop collection were tested for this virus with six samples producing a positive result showing the characteristic 340 bp RT-PCR fragment. RT-PCR products of three samples were checked by sequence analysis and showed high homology (95–97 %) to the AHpLV GenBank isolates JQ245696.1 and JQ728538.1. The incidence of ArMV was negligible with 1.2 % of samples tested positive.

In the year 2012, 229 samples were examined for HpMV, ApMV and HpLV infections and 53 samples for AHpLV (Fig. 2). In 20.1 % of the samples no virus infections were discovered. The percentage of HpMV and ApMV infected samples was 35.3 % and 23.5 %, respectively, as single or mixed infections. The most predominant virus was HpLV with 59.8 % samples testing positive, occurring as single pathogen (29.7 %) or in mixed infections (Fig. 2). Thirteen hops at the Huell hop yard, most of them originating from the USA, were tested for AHpLV. In addition, 30 mother plants of the contract nursery originating from Huell and ten hop plants from commercial stands were screened for AHpLV. Four hops from the USA and one German hop at the Huell hop yard tested positive for AHpLV. In addition, four mother plants were AHpLV positive, while ten hop plants from commercial stands could be identified as negative for AHpLV. The low incidence of ArMV found in 2011 could be confirmed by detecting ArMV as single virus or in mixed infections in only 1.2 % of the samples (Fig. 2).

In 2013, 235 samples were analysed for ArMV, ApMV HpMV and AHpLV. Of these, 36.6 % of the samples showed no virus infection. In the previous years a considerable percentage (almost 60 %) of samples tested positive for HpLV, which was not tested in 2013, and therefore, the overall percentage of infected samples is likely to be higher. HpMV was detected in 45.1 % of the samples, ApMV in 28.5 % . Both viruses occurred in single or mixed infections (Fig. 3). AHpLV infections were checked on a large scale for the first time in 2013 (Table 2). AHpLV was detected in 28.5 % of the samples as single pathogen or in mixed infections with other viruses. No hop plant appeared to be ArMV positive.

In each of the three years mixed infections with two or more viruses were observed. As illustrated in figure 1, 2 and 3 in 2011 57.7 %, in 2012 36.2 % and in 2013 28.9 % of the samples were infected with two or more viruses. The lower percentage of mixed infections in 2013 may be due to the omitted tests for HpLV, as HpLV contributed to many of the identified mixed infections in previous years (see also Table 2). It is possible that the number of mixed infections is overestimated with respect to the carlaviruses (HpMV, HpLV and ApHLV) as cross reactions of the HpMV antiserum with the other carlaviruses may have occurred in DAS-ELISA. Figures 1 to 3 show a considerable number of samples tested positive for one of the three carlaviruses. This may be evidence that cross reaction due to non-specific antibodies is not the rule, and that different mixes of carlaviruses may be present in individual samples.

In all cases where AHpLV, ArMV, ApMV and HpMV were detected in hop samples from commercial yards (Table 2) eradication of the specific contaminated hop plants was recommended, especially when co-infection with different viruses was confirmed. If viroid infection had been found, the same advice would have been given for HpSVd or CVd IV infected hops. For all these viruses and viroids their deleterious impact is obvious and well documented [3, 4, 5, 6, 10, 11]. But for hops infected solely with AHpLV or HpLV the impact of virus infection on yield and quality is not clear. Eastwell and Druffel [21] reported a 10 % to 15 % decrease in yield and alpha acids contents for the cv. Chinook as a result of infection with AHpLV. However, data revealing the impact of infections with a single virus on growth, yield and quality of hops are limited [3]. At present, it appears that infection with HpLV should be tolerated in commercial hop gardens because the limited documented deleterious effects of this virus do not justify the cost of replacement of such plants.

Table 2 Number of hop samples tested from different origin in 2011–2013

	RT-PCR		DAS-ELISA		
Origin and nature of the sample material	**HpLV* positive**	**AHpLV** positive**	**HpMV positive**	**ApMV positive**	**ArMV positive**
LfL: Genetic resources and experim. lines	35 of 47 (74.5 %)	8 of 26 (30.8 %)	41 of 73 (56.2 %)	23 of 73 (31.5 %)	2 of 73 (2.7 %)
LfL: cultivar collections	152 of 248 (61.3 %)	53 of 105 (50.5 %)	198 of 353 (56.1 %)	119 of 353 (33.7 %)	0 of 353 (0 %)
GfH Hallertau propagation facility: mother plants	35 of 81 (43.2 %)	5 of 44 (11.4 %)	10 of 125 (8 %)	3 of 125 (2.4 %)	1 of 125 (0.8 %)
Commercial hop crops	58 of 99 (58.6 %)	1 of 60 (1.7 %)	101 of 159 (63.5 %)	63 of 159 (39.6 %)	5 of 159 (3.1 %)
Total	**280 of 475 (58.9 %)**	**67 of 235 (28.5 %)**	**350 of 720 (48.6 %)**	**208 of 720 (28.9 %)**	**8 of 720 (1.1 %)**

* testing of HpLV only in 2011 and 2012

**results only from 2013 when AHpLV was tested in full scale

4 Conclusion and outlook

In this study, HpSVd infections were not detected in German hop production and this study allowed elimination of HpSVd infected material from the world hop germplasm held in Huell. Breeding yards of the LfL and commercial hop fields showed high levels of virus infections with ApMV, HpMV and HpLV (Table 2) often with mixed infections which are known to be responsible for commercially relevant crop and quality losses. Although, it should be kept in mind that the occurrence of viruses might be overestimated in our monitoring due to the preferred testing of symptom showing hop plants. AHpLV was detected almost exclusively in Huell breeding yards and in mother

plants held at the contract nursery that were obtained from Huell (Table 2).

This project widened the spectrum of viruses screened in Germany to obtain an estimation of the situation with respect to known suite of viruses that are economically important for hop production. The DAS-ELISA technique was confined to the detection of ApMV, ArMV and HpMV due to the availability of antisera. In this study, RT-PCR was used as an additional tool for the detection of HpLV and AHpLV, for the verification of doubtful ELISA results, and is the method of choice for viroid detection.

In general, in Huell breeding yards, in PVR trial fields and the world hop collection infection with HpLV, AHpLV, HpMV and ApMV is tolerated. Eradication of all affected plants would drastically reduce the genetic variability available to our hop breeding operation. Further, substitution of all such plants with meristem culture thermotherapy derived virus free plants would be unrealistic. High rates of infection for ApMV and HpMV in the world hop collection are also tolerated elsewhere as reported from the Hop Research Institute Žatec [22]. While tolerating virus infections in breeding yards, emphasis is put on the production of virus and viroid free mother plants for the contract nursery of the GfH by meristem culture and intensive virus/viroid testing. Moreover, since the maintenance of this virus/viroid free status in the nursery is crucial, eradication of any suspicious plants (with the exception of HpLV infected plants) in the nursery is obligatory. Virus and viroid free plant material is also mandatory for trial plots with advanced experimental lines in our breeding yard in Stadelhof and for on-farm trials.

The monitoring programme developed in this study will be continued on a broad scale for HpSVd and CVd IV, but significantly reduced for virus infections. HpSVd and CVd IV testing will be obligatory in Germany, for the introduction of hops from abroad, especially since these viroids are not listed as quarantine pathogens in the EU directive 2000/29/EG. Intensified cooperation with plant health services is necessary to mitigate the global biosecurity risk for hop production.

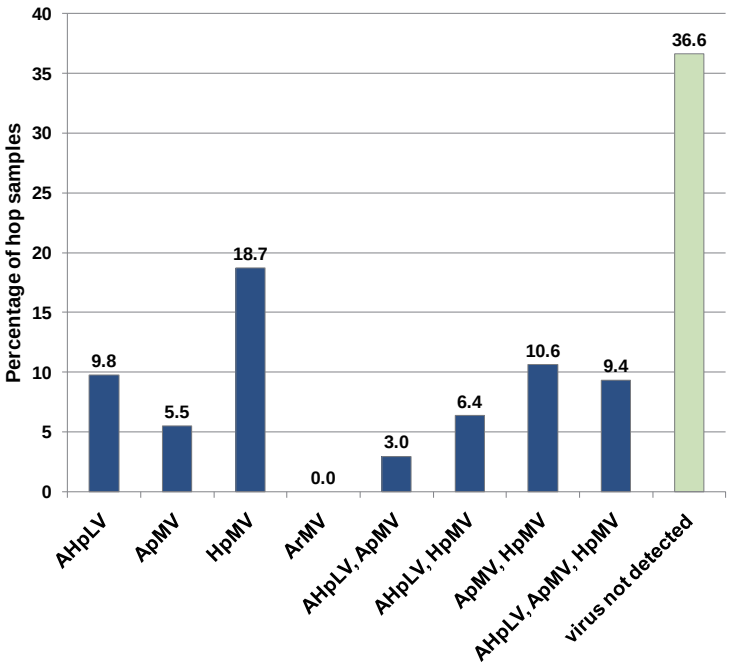

Fig. 3 Percentages of hop samples infected with one or more viruses in 2013. A total of 235 samples was analysed. As opposed to 2011 and 2012 samples were not tested for HpLV in 2013

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