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Hop latent viroid (HLVd)-caused pathogenesis: effects of HLVd infection on lupulin composition of meristem culture-derived *Humulus lupulus*

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Abstract

Season-dependent fluctuation of hop latent viroid in meristem tips enabled selection of viroid-free cultures from Osvald's clone 31, 72, 114, and cv. Premiant. These mericlones were used to evaluate effect of viroid infection on the composition of hop resins and essential oils in the first production year. Healthy plants were compared with naturally re-infected individuals under field conditions. On average, viroid infection decreased content of α-bitter acids by 40 %. The content of β-bitter acids, as well as the ratios of humulone/cohumulone and lupulone/colupulone was not influenced by viroid infection. The content of all monoterpenes was for 29, 37.4 and 41.6 % higher for myrcene, α-and β-pinene, respectively, in infected plants compared with the healthy controls. The contents of sesquiterpenes like β-caryophyllene, α-humulene, α-copaene, γ-muurolene, β-bisabolene, γ-cadinene, and δ-cadinene decreased by 13.7, 13, 14, 18.5, 29, 21.7 and 18.5 %, respectively, due to viroid infection. The possible influence of some oxidative-reduction processes activated by viroid-caused pathogenesis was assumed to be involved in the accumulation of terpenes alcohols like geraniol and methylgeranate, and in the reduction of the contents of the majority of ketones detected in the spectra of essential oils.

Additional key words: hop, *in vitro* mericlones, RT PCR diagnosis, viroid elimination.

Introduction

Hop latent viroid (HLVd) was characterised by Puchta *et al.* (1988) as world-wide distributed hop pathogen including the most countries in the Europe, Asia, Africa and America. It follows from our previous work that HLVd spreading and re-infection occurs in some materials with very high rate. For instance, the increase of HLVd incidence in Czech Osvald's clone 72 from zero to 65 % within three years was observed (Matoušek and Patzak 2000). In this respect two practical questions have to be solved. The first problem is dealing with the elimination of HLVd and the second question is dealing with the real assessment of "latency" or "pathogenicity" of HLVd infection in different hop cultivars and genotypes.

Despite the absence of characteristic morphological symptoms of infection, HLVd infection has been associated with changes in the composition of some secondary metabolites in lupulin glands (Barbara *et al.* 1990a,b, Adams *et al.* 1991, 1992) suggesting great practical significance of HLVd. Adams *et al.* (1991) reported that viroid infection significantly decreased α-bitter acid contents approximately by 11 %, increased α-bitter acid contents approximately by 8 % and myrcene approximately by 38 %. In addition, the cones of viroid infected

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hops were by 11 % smaller than those from viroid-free plants. Adams *et al.* (1992) reported a decrease in α-bitter acid contents ranging from 20 - 50 % varying with cultivar. These results suggest genotype-dependent effects of HLVd infection, however, neither HLVd structure responsible for this reaction has been identified, nor detailed analysis of biochemical response of the HLVd infected host plants has been performed. Such study appeared to be rather problematic, because it required a complete elimination of virus infections, which could interfere with viroid pathogenesis.

In our previous work, we partly characterised horizontal (Matoušek *et al.* 1994) and vertical (Matoušek *et al.* 1995) distribution of HLVd in Czech hop Osvald's clones 31 and 72. However, no information was available about the influence of HLVd infection on lupulin composition of these economically important clones. In this study we report isolation and selection of viroid-free materials from meristem cultures, which provided suitable genetically invariant materials for detailed study of the influence of viroid infection on chemical characteristics of lupulin.

Materials and methods

Plants: During 1995 to 1996 horizontal and vertical HLVd distributions were analysed in the Czech hop hybrid cultivar Premiant and Saaz semi-early redbine Osvald's clones 31 and 114 grown in maintenance breeding hop-garden in the Hop Research Institute, Žatec. Suitable donor plants were pre-selected for extraction of meristem tips. During 1996 to 1997 extracted meristem tips (about 0.5 mm) were transferred to *in vitro* culture according to procedure described by Svoboda (1991). Regenerated shoots were transferred to basal media without growth regulators and propagated for 4 weeks. Finally, one viroid-free mericlone of Osvald's clone 114, one of Osvald's clone 31, and one of cv. Premiant were selected. In addition to newly established mericlones, two viroid-free mericlones of Osvald's clone 72, which have been grown in *in vitro* conditions for 10 years were also used. Viroid-free meristem derived plants were multiplied *in vitro* to form genetically homogeneous groups and transferred to pots with peat, kept for one week under mineralcloth covers in the greenhouse. Rooted plants were transplanted to hop-garden in November 1997. Four re-infected and healthy plants of every meristem clone were analysed for their composition of hop resins and essential oils. Samples for quantification of HLVd infection were collected at the end of August 1998 and 1999, and stored frozen at -20 °C until used. Samples for chemical analyses were collected in August 1999 in the first production year and conventionally dried. Our analyses were in this study restricted to the first production year to guarantee the purity of the material, *i.e.* to avoid the problems with multiple pathogen and viroid re-infections.

Viroid and virus analyses: For viroid analysis we selected sensitive method of RNA-RNA dot blot hybridisation using ^{32}P [UTP]-labelled HLVd riboprobe (Matoušek *et al.* 1995). Viroid content was quantified by means of *STORM PhosphorImager* device and *ImageQuant* software (*Molecular Dynamics*, Sunnyvale, USA). If not otherwise stated, hop cones were used for evaluation of HLVd infection of plants under the field conditions and whole plants were used for evaluation of meristem *in vitro* cultures. Reverse transcription-polymerase chain reaction (RT PCR) diagnosis of viroid-free meristem *in vitro* cultures was performed by *Titan One Tube RT PCR* system (*Roche Molecular Biochemicals*, Manheim, Germany) as described Matoušek and Patzak (2000). RT PCR products were transferred onto charge modified nylon membrane (*Sigma-Aldrich*, Saint Louis, USA) by alkaline Southern blotting and analysed by hybridisation. Two viroid-free mericlones of Osvald's clone 72 and one of Galena isolated during our previous work were used as negative controls.

All plants were also tested by enzyme-linked immunosorbent assay (Clark and Adams 1977) for virus infections with alfalfa mosaic virus, apple chlorotic leafspot virus, apple mosaic virus, arabis mosaic virus, cherry leafroll virus, cucumber mosaic virus, hop latent virus, hop mosaic virus, petunia asteroid mosaic virus, prunus necrotic ringspot virus, strawberry latent ringspot virus and tabacco necrosis virus. It is important to note, that all plants selected for chemical analyses were virus-free throughout all experiments and the influence of other pathogens was eliminated by standard treatment with pesticides. In addition, hop mericlones were grown in the same field locality to eliminate environmental factors.

Quantity and quality of chemical compounds of hop cones: Lupulin compounds were analyzed in cones collected as mixed samples from either healthy or infected plants. Hop resins composition was determined by high pressure liquid chromatography (HPLC) according to *EBC 7.7* procedure (1997). Ten grammes (approx. 110 cones) of dry material was used for analysis. Method is based on extraction of hop resins in two-phase system diethylether-methanol-hydrochloric acid. α- and β-bitter acids were analysed directly from the ether phase on HPLC column *Nucleosil RP* C_{18} (*Machererey Nagel*, Düren, Germany, 5 mm, 250 × 4.6 mm). The mobile phase consists of the mixture containing methanol-water-phosphoric acid (85 %) 850:190:5 v/v, flow 0.8 cm^3 min^{-1}. Analyses were performed on liquid chromatograph

Shimadzu LC-10A (Tokyo, Japan) with diode array detector at the wavelength of 314 nm. Quantification was performed using external standard *ICE 2*.

Hop essential oils were isolated from hops by steam distillation method. One hundred grammes (approx. 1 100 dry hop cones) of ground hops and 2 dm^3 of water were inserted in 4 dm^3 round bottom flask. Mixture was boiled under reflux for 90 min. Hop oil, separated in collector, was analysed by temperature programmed (60 - 250 °C) gas chromatography on capillary column *DB 5* (30 m × 0.25 mm × 0.25 mm). Analyses were performed on gas chromatography-mass spectrophotometry system *Varian 3400 + Finnigan ITD 800* mass detector (Walnut Creek, USA). Individual components were identified with the help of analytical standards of pure substances and using mass spectra libraries. Contents of various compounds in healthy and infected cones were compared using the *t*-test.

Results

In order to assay effects of HLVd infection on the quality of lupulin, healthy and infected populations of hop were compared. Plants with low viroid content in hop cones, ranging from 0.03 to 0.3 ng g^{-1}(f.m.) were pre-selected at the end of the season for the next season, when meristems were isolated. Because vertical distribution of viroid changed during the season we collected meristem tips for *in vitro* culture at different times. Viroid-free mericlones were obtained at low frequency from meristem tips collected during May and the beginning of June from Osvald's hop (Table 1). Three viroid-free mericlones were also established from basal adventive (secondary) tip meristems of cultivar Premiant collected in the beginning of October (Table 1) *i.e.* after harvest. Viroid absence was verified by RT PCR combined with molecular hybridisation to confirm that newly established viroid-free mericlones did not contain any HLVd (Fig. 1). No viroid was detected in individual plants of these mericlone lines after two years of *in vitro* cultivation, suggesting that there is no prolong dormancy period of HLVd infection.

Table 1. Meristem cultures established and analysed for HLVd infection (Osvald's clone 31 and 114, cv. Premiant). [A] - the viroid negative mericlone was selected from meristem collected at the beginning of June, [B] - isolation only from lower adventive shoots, - not performed.

Period of isolation	Culture established / viroid-free mericlone		
	clone 31	clone 114	Premiant
April	48/0	104/0	-
May	18/1	117/3	-
June	97/0	117/1[A]	-
July	49/0	49/0	-
August	-	-	250/0[B]
September	-	-	-
October	-	-	72/3[B]

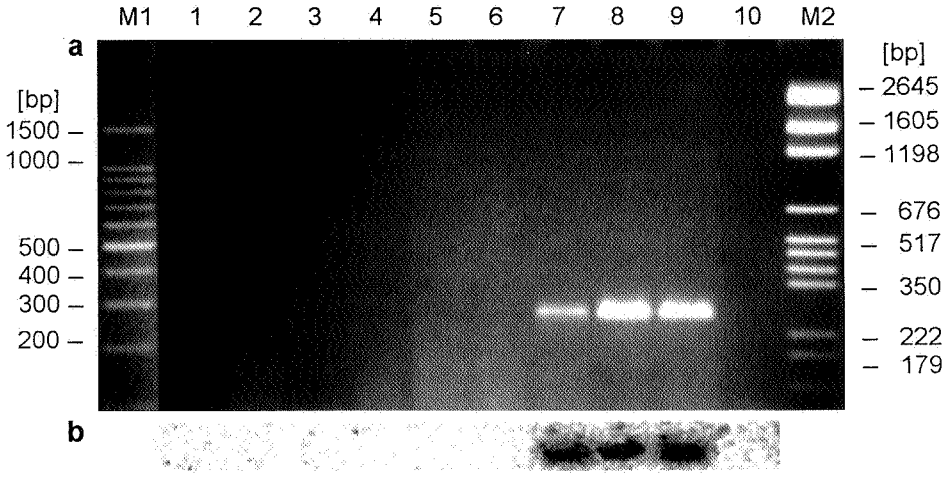

Fig. 1. An example of analysis of HLVd in hop mericlones by RT PCR and molecular hybridisation (*a*) RT PCR products in 2 % agarose gel stained with ethidium bromide (*b*) Southern blot analysis of the same products as in (*a*) hybridised to ^{32}P [UTP]-labelled HLVd probe. M1 - 100 pb ladder; M2 - pGEM molecular marker (*Promega*); *lane 1* - Osvald's clone 72 mericlone 1; *lane 2* - Osvald's clone 72 mericlone 2; *lane 3* - Osvald's clone 31; *lane 4* - Osvald's clone 114; *lane 5* - Premiant; *lane 6* - Galena; *lanes 7 - 9* - infected mericlones [0.05, 0.1 and 0.3 ng g^{-1}(f.m.)]; lane 10 - negative control (reaction without RNA) for analysis in agarose gel. For Southern blot analysis 0.01 cm^3 of samples were loaded except for positive samples (*lanes 7 - 9*), where only 0.001 cm^3 of reaction mixtures was loaded.

The field grown viroid-free mericlones of Osvald's clone 31 and 114, cv. Premiant and two control viroid-free mericlones of Osvald's clone 72 were used for evaluation of the influence of HLVd on the contents of hop resins and essential oils in the first production year. In the first year of hop growing in the field conditions, on average 0.4 ng (HLVd) g^{-1}(f.m.) was detected in approximately 25 % of naturally re-infected plants, which were then selected as positive controls. In the second year (*i.e.* in the first production year) the viroid content in these plants increased to an average of 1.8 ng(HLVd) g^{-1}(f.m.).

Viroid infection led to a significant reduction of the content of α-bitter acids, 40 % in the average (Fig. 2*A*). While the decrease in the content of α-bitter acids in infected plants was the major effect for the most of the mericlones, there was also some tendency observed in the increase of β-bitter acid contents (Table 2, Fig. 2*a*). The α/β ratio showed clear tendency to be lower in infected materials (Table 2, Fig. 2*A*). It is interesting to note that contents of cohumulone and colupulone were very stable and independent of viroid infection. The second important class of chemical compounds in hop cones are essential oils. Although their content is relatively small, there are around 200 individual components. The total content of essential oils was not significantly lower in cones of viroid-infected plants than in healthy plants (Fig. 2*a*), but the contents of some components were significantly different. For instance, the content of myrcene increased about 29 % in infected plants compared with healthy controls (Fig. 2*B*). Moreover the contents of α-pinene and β-pinene also increased and were, on average, 37.4 % and 41.6 % higher, respectively, in infected plants than in healthy controls. The contents of limonene and ocimene varied

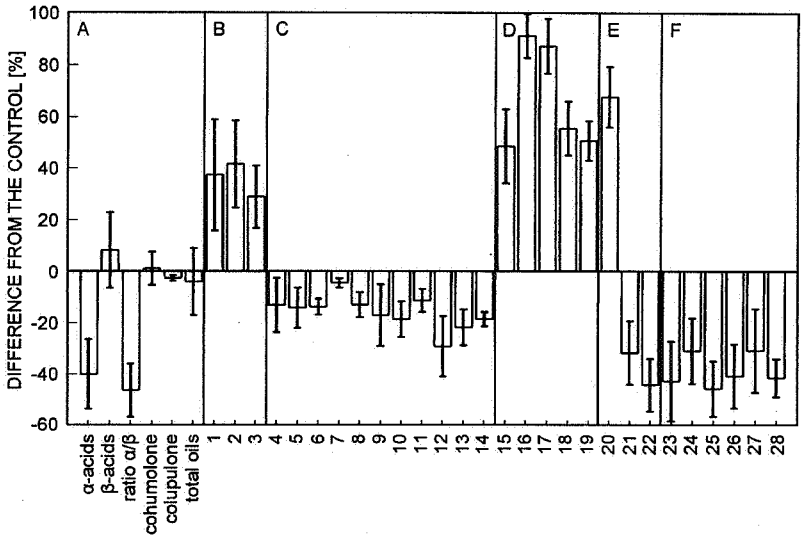

Fig. 2. Influence of HLVd infection on contents of hop resins and essential oils. The graphs show differences in the contents of individual compounds in hop cones of infected plants *versus* healthy controls. The differences are expressed in %: *A* - hop resins, *B* - monoterpenes - hydrocarbons, *C* - sesquiterpenes - hydrocarbons, *D* - terpenes - alcohols, *E* - esters, and F - ketones. Individual compounds of essential oils are designated by numbers as in Table 3. Confidence intervals are given at α = 0.05.

Table 2. Contents of bitter resins (α-acids = cohumulone + humulone; β-acids = colupulone + lupulone) in cones of healthy and HLVd-infected hop (* - significant differences at $P = 0.05$).

Cultivar/ clone	Mericlone	HLVd infection	α-acids [% (d.m.)]	β-acids [% (d.m.)]	Ratio α/β	Cohumulone [% (α-acids)]	Colupulone [% (β-acids)]
Osvald's clone 72	1	Negative	4.53	3.66	1.24	22.1	39.5
		Positive	3.15*	4.52*	0.70*	23.5	38.5
	2	Negative	4.95	3.77	1.31	21.3	39.0
		Positive	2.43*	3.78	0.64*	21.7	37.9
Osvald's clone 31		Negative	4.60	4.64	0.99	21.3	39.5
		Positive	1.69*	4.01	0.42*	24.2*	39.3
Osvald's clone 114		Negative	5.59	4.07	1.37	22.8	38.7
		Positive	4.38*	4.14	1.06	21.0	38.0
Premiant		Negative	9.00	3.28	2.74	17.9	38.7
		Positive	5.96*	4.99*	1.19*	16.9	36.8

independently upon infection. Another group of compounds forming essential oils are sesquiterpenes. The content of most sesquiterpenes was significantly lower in infected than in healthy plants (Fig. 2*C*). For instance, the sesquiterpenes α-copaene, γ-muurolene, β-bisabolene, γ-cadinene and δ-cadinene were lower by 14, 18.5, 29, 21.7, and 18.5 %, respectively, in infected plants than healthy controls (Table 3, Fig. 2*C*). While contents of β-caryophyllene and α-humulene in the infected plants decreased, there was significant increase of their epoxides (Fig. 2*D*). Some terpenes alcohols, mainly geraniol and methylgeranate (Fig. 2*D*) which are often missing in healthy plants are present in infected materials. It is interesting to note that significant fraction of ketones is reduced due to infection (Fig. 2*F*). Some changes in composition of esters (Fig. 2*E*) were also observed. For instance, 1-octen-3-yl acetate and methyl dekanoate show clear tendency to decrease in infected mericlones, while the content of methyl non-6-enoate was enhanced in all infected materials. The contents of most esters identified show rather high variability independently on HLVd infection (Table 3). The contents of limonene, ocimene, α-bergamotene, β-selinene, isobutyl isobutyrate, 2-methylbutyl-isobutyrate, methyl heptanoate, methyl 6-methylheptanoate, methyl oktanoate, methyl nonanoate, methyl deca-4-enoate, methyl deca-4,8-dienoate did not significantly change.

Table 3. Contents of essential oils in hop cones of healthy and HLVd infected hops (* - significant differences at $P = 0.05$).

Compound [% (essential oils)]	No.	Osvald's clone72 1		2		Osvald's clone 31		Osvald's clone 114		Premiant	
		Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Monoterpenes - hydrocarbons											
α-pinene	1	0.04	0.06	0.04	0.07*	0.01	0.07*	0.05	0.05	0.06	0.08
β-pinene	2	0.41	0.54*	0.34	0.64*	0.13	0.59*	0.44	0.53	0.45	0.78*
myrcene	3	26.9	32.7*	28.1	37.0*	10.5	25.0*	29.9	35.1*	32.3	46.2*
Sesquiterpenes - hydrocarbons											
α-ylangene	4	0.07	0.07	0.08	0.07	0.09	0.09	0.09	0.07	0.10	0.07*
α-copaene	5	0.29	0.29	0.28	0.24	0.36	0.33	0.32	0.27*	0.38	0.26*
β-caryophyllene	6	8.05	7.07*	7.8	7.1	10.0	8.52*	8.81	7.73*	10.8	8.60*
β-cubenene	7	0.32	0.31	0.31	0.30	0.40	0.38	0.36	0.33	0.44	0.43
α-humulene	8	25.6	22.8*	24.4	21.9*	30.6	27.7*	24.7	22.5	33.2	24.9*
β-farnesene	9	17.2	17.7	17.2	13.0*	17.4	14.1*	16.1	15.0*	2.73	1.71*
γ-muurolene	10	0.73	0.66	0.79	0.60*	1.09	0.82*	0.79	0.71*	0.87	0.66*
α-selinene	11	0.44	0.44	0.45	0.38	0.65	0.56*	0.48	0.42	0.56	0.48*
β-bisabolene	12	0.65	0.54*	0.87	0.49*	0.91	0.51*	0.76	0.59*	0.59	0.48*
γ-cadinene	13	0.89	0.78*	0.92	0.71*	1.39	0.93*	0.95	0.82*	0.94	0.69*
δ-cadinene	14	1.62	1.31*	1.41	1.15*	1.92	1.61*	1.49	1.28	1.66	1.25*
Terpenes - alcohols											
linalool	15	0.27	0.43*	0.17	0.39*	0.11	0.46*	0.20	0.34	0.59	0.85*
geraniol	16	0.05	0.22*	0	0.25*	0.02	0.32*	0.01	0.06*	0	0.27*
methylgeranate	17	0.05	0.14	0	0.17*	0.03	0.19*	0.01	0.07*	0	0.42*
caryophyllene epoxide	18	0.12	0.31*	0.14	0.29	0.50	0.87*	0.12	0.22	0.02	0.08*
humulene epoxide-I	19	0.35	0.87*	0.35	0.88*	1.49	2.60*	0.37	0.61*	0.13	0.26*
Esters											
methyl non-6-enoate	20	0.06	0.11	0.02	0.08*	0.02	0.13*	0.02	0.07	0.02	0.05
1-octen-3-yl acetate	21	0.44	0.30*	0.50	0.44	0.52	0.33*	0.49	0.39	0.33	0.14*
methyl dekanoate	22	0.15	0.07*	0.23	0.15	0.23	0.09*	0.22	0.13*	0.16	0.11
Ketones											
2-nonanone	23	0.53	0.27*	0.76	0.56*	0.57	0.21*	0.69	0.55*	0.75	0.34*
2-decanone	24	0.38	0.27*	0.30	0.27	0.29	0.19*	0.41	0.32*	0.59	0.24*
2-undecanone	25	1.77	1.02*	2.55	1.75*	2.65	1.15*	2.46	1.57*	2.15	0.82*
6-undecanone	26	0.26	0.21	0.16	0.11	0.36	0.19*	0.25	0.14	0.24	0.09*
2-dekanone	27	0.22	0.17	0.19	0.16	0.23	0.15	0.22	0.19	0.28	0.09*
2-tridekanone	28	1.34	0.92*	1.89	1.26*	2.55	1.13*	1.89	1.05*	1.39	0.79*
Total oils [% (d.m.)]		0.68	0.68	0.61	0.49*	0.56	0.48*	0.73	0.64*	1.01	1.34*

Discussion

HLVd contents and distribution are known to change during the season and plant ontogenesis (Morton *et al.* 1993, Matoušek *et al.* 1995). It has been shown previously that the distribution correlates with the dsRNAse activity, which could influence viroid content (Matoušek *et al.* 1995). It is obvious that this natural fluctuation in viroid concentration in meristems is the major factor, which enables to select viroid-free mericlones. Our results supplement some data mentioned by Hataya *et al.* (1992) and Adams *et al.* (1996), who suggested the possibility to select HLVd-free materials from hop meristems, although such selection occurred with very low efficiency (Hataya *et al.* 1992, Adams *et al.* 1996). According to our results thermotherapy (Matoušek *et al.* 1995) and also cold therapy (unpublished) are by themselves insufficient methods to select viroid-free materials, although both treatments lead to significant reduction of viroid contents. Morton *et al.* (1993) and Adams *et al.* (1996) indicated some dormancy period characteristic for HLVd infection in cold-treated hop materials. It is important to note that these materials were not assayed in *in vitro* conditions. In our previous work (Matoušek *et al.* 1994) we compared field grown and *in vitro* grown plants of Osvald's clone 31 and found that viroid contents were much higher in *in vitro* mericlones than in the leaves of field grown plants. Thus, *in vitro* mericlones could serve as the early indicators of traces of HLVd infection. The fact that no viroid was detectable in selected hop mericlones clearly suggest a complete viroid eradication of from these materials, which were used as healthy controls. Approximately 25 % of mericlones became re-infected with HLVd after the first year in the field conditions and served as infected material for biochemical analyses in the second (production) year. Similar re-infection rate was observed earlier in our experiments in hop clones and hybrid materials (Matoušek and Patzak 2000).

Biochemical analyses revealed that viroid infection led to a significant reduction of the content of α-bitter acids, while contents of β-bitter acids slightly increased or remained unchanged in infected plants. Our finding agrees with the results of Adams *et al.* (1991, 1992). It is interesting to note, that contents of cohumulone and colupulone were very stable and independent of viroid infection. It is known that the ratio of the homologues of bitter acids is in general a relatively stable genetic trait (Peacock and McCarty 1992). The stability of the ratio of humulone/cohumulone and lupulone/colupulone could mean that viroid infection has no significant influence on expression and catalytic activity of phlorisovalerophenone synthase, which was recently identified by Paniego *et al.* (1999). This enzyme is involved in the synthesis of basic precursors for both analogues (Fig. 3). The humulone/lupulone and cohumulone/colupolone ratios could most probably be influenced *via* activity of some oxido-reductase, which could convert β- to α-bitter acids (Fig. 3) (Zuurbier *et al.* 1995). However, the putative enzyme has not been yet identified.

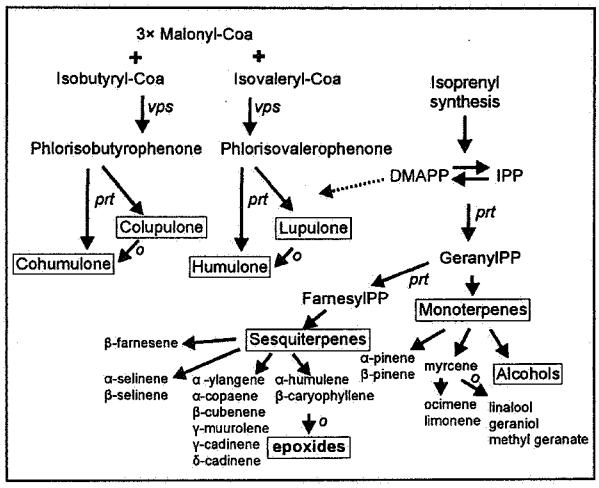

Fig. 3. Schematic drawing of biosynthetic pathways of hop resins and essential oils. The scheme was summarised according to Neve (1991), Zuurbier *et al.* (1995), Tressl *et al.* (1987) and Dieckmann and Palamand (1974). IPP - isopentenylpyrophosphate, DMAPP - dimethylallylpyrophosphate, PP - pyrophosphate, *vps* - phlorisovalerophenone synthase, *prt* - prenyl transferase, *o* - oxidation.

The second important class of chemical compounds in hop cones are essential oils. Every slight difference in essential oils can distinctly change aroma of hop and beer. The results of analysis of essential oils included 40 main components. The total content of essential oils was not significantly lower in cones of viroid infected plants than in healthy plants, but the contents of some components were significantly different. Adams *et al.* (1991) reported only about distinctive increase of myrcene. We found in addition that the contents of α-pinene, β-pinene also increased due to infection, while the contents of limonene and ocimene varied independently upon infection in spite of the fact that they are direct metabolites of myrcene (Neve 1991). According to published biochemical routes of terpene biosynthesis (Dieckmann and Palamand 1974) one can assume that myrcene was more rapidly converted in infected plants especially to linalool and geraniol than to limonene and ocimene (Fig. 3). Another group of compounds forming essential oils are sesquiterpenes. The content most sesquiterpenes, which are involved in the same biosynthetic pathway (Tressl *et al.* 1987) (Fig. 3), was obviously lower in infected than in healthy plants.

The decrease of content of sesquiterpenes could be correlated to the increasing content of monoterpenes, which are synthesised from the same precursor. The decrease of β-caryophyllene and α-humulene in the infected plants could be also correlated with the increase of their epoxides (Fig. 3) (Neve 1991). Very similar changes in the composition of essential oils, *i.e.* the increase of the content of myrcene and the general decrease of sesquiterpenes, was observed during hop maturation (Murphey and Probasco 1996, Menary and Doe 1983, Skinner *et al.* 1974). However, unlike to processes observed during hop maturation, viroid infection clearly caused the decrease of α-bitter acids. The possible influence of viroid infection on activation of some oxidative-reduction processes could lead to the increase of contents of terpenes alcohols mainly geraniol and methylgeranate, which are often missing in healthy plants. It is interesting to note that significant fraction of ketones is reduced due to infection. The conversion of ketones could be also caused by increasing activity of some oxidative pathway in viroid infected plants. The interpretation of changes in composition of esters is very problematic, because the information about their synthesis is still unknown. Some of them like 1-octen-3-yl acetate and methyl dekanoate show clear tendency to decrease in infected mericlones while the content of some like methyl non-6-enoate was enhanced in all infected materials.

Present observations suggest that HLVd pathogenesis probably promotes some disbalance in expression of host components involved in the synthesis of the secondary metabolites.

References

Adams, A.N., Barbara, D.J., Morton, A.: Effects of hop latent viroid on weight and quality of the cones of the hop cultivar Wye challenger. - Ann. appl. Biol. **118** (Suppl.): 126-127, 1991.

Adams, A.N., Barbara, D.J., Morton, A., Darby, P.: The experimental transmission of hop latent viroid and its elimination by low temperature treatment and meristem culture. - Ann. appl. Biol. **128**: 37-44, 1996.

Adams, A.N., Morton, A., Barbara, D.J., Ridout, M.S.: The distribution and spread of hop latent viroid within two commercial plantings of hop (*Humulus lupulus*). - Ann. appl. Biol. **121**: 585-592, 1992.

Barbara, D.J., Morton, A., Adams, A.N.: Assessment of UK hops for the occurrence of hop latent and hop stunt viroids. - Ann. appl. Biol. **116**: 265-272, 1990a.

Barbara, D.J., Morton, A., Adams, A.N., Green, C.P.: Some effects of hop latent viroid on two cultivars of hop (Humulus lupulus) in the UK. - Ann. appl. Biol. **117**: 359-366, 1990b.

Clark, M.F., Adams, A.N.: Characteristic of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. - J. gen. Virol. **34**: 475-483, 1977.

Dieckmann, R.H., Palamand, S.R.: Autooxidation of some constituents of hops. I. The monoterpene hydrocarbon, myrcene. - J. agr. Food Chem. **22**: 498-503, 1974.

Hataya, T., Hikage, K., Suda, N., Nagata, T., Li, S., Itoga, Y., Tanikoshi, T., Shikata, E.: Detection of hop latent viroid using reverse transcription and polymerase chain reaction (RT-PCR). - Ann. phytopathol. Soc. Jap. **58**: 677-684, 1992.

Matoušek, J., Patzak, J.: A low transmissibility of hop latent viroid through a generative phase of *Humulus lupulus* L. - Biol. Plant. **43**: 145-148, 2000.

Matoušek, J., Trněná, L., Svoboda, P., Růžková, P.: Analysis of hop latent viroid (HLVd) in commercial hop clones in Czech Republic. - Rost. Výroba (Praha) **40**: 973-983, 1994.

Matoušek, J., Trněná, L., Svoboda, P., Růžková, P., Lichtenstein, C.P.: The gradual reduction of viroid levels in hop mericlones following heat therapy: a possible role for a nuclease degrading dsRNA. - Biol. Chem. Hoppe-Seyler **376**: 715-721, 1995.

Menary, R.C., Doe, P.E.: Some morphological and chemical changes in hops during maturation. - J. Sci. Food Agr. **34**: 921-929, 1983.

Morton, A., Barbara, D.J., Adams, A.N.: The distribution of hop latent viroid within plants *Humulus lupulus* and attempts to obtain viroid-free plants. - Ann. appl. Biol. **123**: 47-53, 1993.

Murphey, J.M., Probasco, G.: The development of brewing quality characteristics in hops during maturation. - MBAA Tech. Quart. **33**: 149-159, 1996

Neve, R.A.: Hops. - Chapman and Hall, London 1991.

Paniego, N.B., Zuurbier, K.W.M., Fung, S.-Y., Heijden, R., Scheffer, J.J.C., Verpoorte, R.: Phlorisovalerophenone synthase, a novel polyketide synthase from hop (*Humulus lupulus* L.) cones. - Eur. J. Biochem. **262**: 612-616, 1999.

Peacock, V.E., McCarty, P.: Varietal identification of hops and hop pellets. - MBAA Tech. Quart. **29**: 81-85, 1992.

Puchta, H., Ramm, K., Sanger, H.L.: The molecular structure of hop latent viroid (HLVd), a new viroid occurring worldwide in hops. - Nucl. Acids Res. **16**: 4197-4216, 1988.

Skinner, R.N., Kavanagh T.E., Hildebrand, R.P., Clarke, B.J.: Hop maturation and resin formation: Field trials. - J. Sci. Food Agr. **25**: 1121 - 1133, 1974.

Svoboda, P.: [Clonal propagation of hop *in vitro*.] - Rost. Výroba (Praha) **37**: 705-708, 1991. [In Czech.]

Tressl, R., Kossa, M., Köppler, H.: Changes of aroma components during processing of hop. - In: EBC Monograph XIII - Symposium on Hops. Pp. 116-128. Elsevier Scientific Publishing Company, Amsterdam 1987.

Zuurbier, K.W.M., Fung, S.-Y., Scheffer, J.J.C., Verpoorte, R.: Formation of aromatic intermediates in the biosynthesis of bitter acids in *Humulus lupulus*. - Phytochemistry **38**: 77-82, 1995.