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# Influence of *Apple Mosaic Virus* on the Growth, Yield, and Qualities of Saaz Hop

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**Abstract:** The aim of this study is to elucidate the influence of *apple mosaic virus* (ApMV) infection on Saaz hop in the Saaz region of Czech Republic. The comparison between virus free and ApMV infection for the growth, yield and qualoties of Saaz hop was studied in one test garden in detail over two consecutive years (2010 and 2011). ApMV weakened vegetative growth and decreased hop cone yield. ApMV infection resulted in a decrease in the production of humulone (alpha acid) and essential oil. The composition of terpene compounds, which contribute to the hoppy aroma of beer, were also affected. ApMV-infected hop cones had lower ratio of mono-terpenes, and higher ratio of sesqui-terpenes than those derived from uninfected plants. Beer brewed using ApMV-infected hops had a diminished fruity aroma compared to beer brewed using uninfected hops. From these results, protecting hop plants from ApMV infection is important for maintaining both quantity and quality of hop. In some commercial gardens, it was confirmed that hop plants have been maintained virus-free for over 20 years. This fact suggests that good field practices can protect hop plants from virus infections.

**Keywords:** Beer, *Apple Mosaic Virus*, *Humulus lupulus*, Spatial Distribution

## 1. Introduction

Hop (*Humulus lupulus* L.) is a perennial, dioecious, climbing plant. In plantations, hop sprouts from its root during spring and grows upward on wires by twining, eventually reaching a height of 6–8 m within 3 months. Hops develop cones after an early summer bloom, which are harvested after 1–2 months. Hops provide bitterness, body, and flavor to beer, which are derived from bitter acids such as humulone, polyphenols and terpenes [3]. Brewers produce their desired beer type and flavors by selecting an appropriate hop cultivar, the type of processed hop product (such as hop pellets or extracts) and by optimizing brewing procedures [6, 18]. However, both yield and quality of hops vary by climate [7], age [9], type of viral infections present [11], and cultivation practices, such as method of fertilization and agrochemical [5], time of pruning, and time of harvest [10, 17]. Therefore, it is also important for both hop growers and brewers to control cultivation conditions described above to achieve both high yield and quality.

It has been reported that viral and viroidal infections reduce the vegetative growth of hops [1, 2, 11, 16]. Pethybridge et al. [11] summarized the effects of virus and viroids on hops relative to quantity, quality and epidemiology. It has also been reported that the hop stunt viroid detrimentally affects hop growth and quality, and so this viroid has become a significant problem in hop-producing regions of the world [1, 8, 16]. The influences of the *hop latent virus* (HpLV), *hop mosaic virus* (HpMV), and *apple mosaic virus* (ApMV) on hop varieties (Nugget, Opal, Pride of Ringwood, and Victoria) have been studied in Australian hop producing areas. These viruses weaken hop growth and decrease the content of humulone, a

key component for beer bitterness [15]. The incidence and spatial distribution of viruses have been studied in hop-producing areas in both the United States and Australia relative to the mode of transmission (e.g., via insect vectors or through sap transferred by agricultural machinery) [12, 13, 14].

The Czech hop variety, Saaz, is known as a fine-aroma hop owing to its high qualities of bitterness and aroma. A comparison of the chemical profiles between virus-free and infected Saaz hop showed that infection may decrease the amounts of humulone and essential oils produced by the plant [4], but the specific effect of ApMV is not yet known. This virus is among the most consequential for reducing yield and quality for many varieties of industrial hops [11]. However, the influence of ApMV on plant growth and beer quality have not been investigated for Saaz hops in Europe. Therefore, in this study we investigated infections by ApMV in Saaz hop and the influence of ApMV on vegetative growth, yield, chemical profile, and beer aromatic qualities. It was also observed the spatial pattern of ApMV and HpMV occurrence in Saaz hop plants in 17 commercial gardens in the Saaz region of the Czech Republic.

## 2. Materials and Methods

### *2.1. Virus Test Garden*

Two block areas in a test garden in Deštnice village, Saaz, Czech Republic (managed by V. F. Humulus Ltd., Žatec, Czech Republic) were used for this study. Each block contained 40 Saaz hop plants of the Osvald's clone 72 variety planted in the autumn of 1999. Virus-free shoots produced by V. F. Humulus Ltd. in accordance with the standards of European and Mediterranean Plant Protection Organization (Certification Schemes, Pathogen Tested material hop, PM 4/16(1)), were planted in one experimental block. ApMV-infected shoots, derived from ApMV infection roots also produced by V. F. Humullus Ltd., were planted in the other experimental block. The two blocks were separated by six meters to prevent the infected plants from infecting the virus-free plants.

All hop plants were subjected to virus and viroid tests in spring 2000, described later in the paper. *Apple Mosaic Virus* (ApMV), *Hop Mosaic virus* (HpMV), *Arabis mosaic virus* (ArMV), *cherry leafroll virus* (CLRV), *hop latent virus* (HLV), *cucumber mosaic virus* (CMV), *petunia asteroid mosaic virus* (PAMV), *tobacco necrosis virus* (TNV), and *hop latent viroid* (HLVd) were not detected in the virus-free plants. ApMV was maintained in all the infected plants. However, the plants were not infected with other any viruses and viroid. The plants in both blocks were prevented from becoming infected by additional viruses by applying mechanical procedures, such as pruning and harvesting. After the year 2000, 10 randomly-sampled plants in each block were subjected to the virus test to confirm that no additional infections had occurred in the blocks. Random sampling, and virus and viroid infection checks described above were undertaken every year to ensure that the initial experimental conditions were preserved throughout the study.

In addition, 17 other hop production gardens in the Saaz region were examined to characterize the spatial distribution patterns of ApMV and HpMV. Young shoots were collected in 2010 from the 10 plants (Figure 1) from each production garden for virus assay.

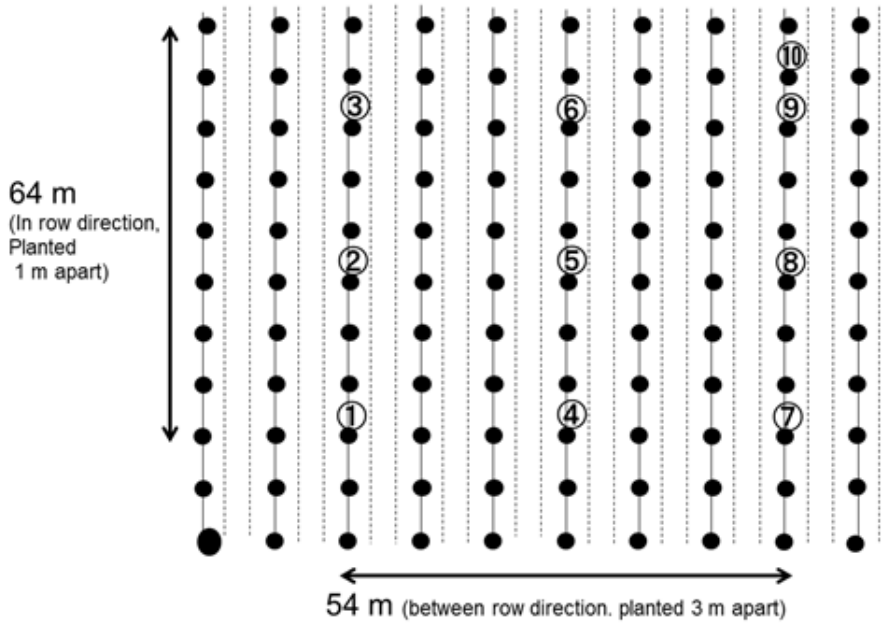


***Figure 1.*** *Spatial layout of hop gardens. ApMV and HpMV infection distributions were investigated at 17 commercial hop gardens. Numbers indicate the root position of sampled hop plants. Black dots show poles of hop trellises. Solid and dotted lines represent rows of hop plants.*

### *2.2. Commercial Gardens and Climatic Data*

15 commercial gardens planted with Saaz hop plants (Osvald's clone 72 variety) in the Saaz region were randomly selected to compare the general hop quality with virus infection. Yield data and samples for chemical analyses of humulone, essential oil, and terpene profiles were also collected. Climatic data in Saaz region were obtained from weather observation systems managed by the Hop Research Institute Co., Ltd., located 10 km south of the test garden, at Kneževes village.

### *2.3. Assays for Viruses*

Young shoots were collected in early April and homogenized in a 1.0-ml solution of 0.01 M phosphate-buffered saline (PBS) containing polyvinylpyrrolidone (20 g/L). The extracts (100 µl) were tested in duplicate by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), using polyclonal antisera to ApMV, HpMV, ArMV, CLRV, HLV, CMV, PAMV, TNV, and HLVd (Loewe Ltd., Sauerlch, Germany). Light absorbance at 405 nm was measured for each sample, and compared with the measurements for positive, negative, and buffer-only controls. The positive/negative threshold was set at twice the absorbance of the control wells. This threshold value was consistently higher than the value obtained using the average absorbance of control wells (plus three standard deviations) and so produced a more conservative estimate of virus incidence.

### *2.4. Measurement of Hop Growth and Yield in Test Garden*

Seven different hop plants were randomly selected in each test block, and vegetative growth characteristics were measured every 2 weeks from April to August. Measurements included hop height, bine (main stem of hop plant) diameter, and leaf length (at 1.5 m above ground). Transition from the vegetative to the reproductive phase at a height of 5.0 m from the ground was observed every week from the end of June to the middle of August. Raw hop cones from all plants were collected by a pilot-scale picking machine and their weights and moisture contents were determined in the end of August. Shortly after harvest, the picked hop cones were dried in a kiln at 55°C for approximately 8 h until their moisture content fell to 10% of wet weight. The weight of dried hop cones was measured to calculate an accurate yield. Dried hop cones were packed in shaded plastic bags with gaseous nitrogen and stored at -20°C in a dark place prior to being used in chemical analyses and brewing trials.

### *2.5. Chemical Analyses of Hop Samples*

Chemical analyses of hop samples were conducted at the Hop Research Institute Co., Ltd. (Žatec, Czech Republic). Hop humulone content was measured by the EBC 7.7 liquid chromatography (HPLC) method [10]. The essential oils of hops were extracted by steam distillation, and the composition of terpene content of the distilled oil was determined by gas chromatography (GC) using a Finnigan ITD 800 mass detector [10].

### *2.6. Brewing and Beer Sensory Evaluation*

Small-scale brewing mimicking commercial beer production was conducted in a 100-L pilot scale brewery (Suntory Beer Ltd., Tokyo, Japan) with a malt ratio of 100%. Hops were added twice: at the beginning (kettle hopping) and at the end (late hopping) of wort boiling. To compare differences in the characteristics of hop aromas, an identical amount of each hop test sample harvested in 2011 (milled hop) was added only at the late hopping stage. The amount of the hop extract typically used in the commercial brewing process was added at the time of kettle hopping to achieve an identical bitterness among beer samples. Worts were fermented at 10°C, using lager yeasts.

Seven well-trained panelists conducted sensory evaluations, based on hop aroma intensity, of the beers brewed using infected and uninfected hops [10]. An evaluation of each sample was carried out at least twice on different dates. The order in which beers were sampled was randomized for each panelist, and all evaluations were conducted with panelists blind to the status of the hops (infected vs. uninfected) they were testing.

### *2.7. Statistical Analysis*

Statistical tests were performed using the JMP 10.01 software (SAS, Cary, NC, United States). Statistical differences between means were determined by two-way ANOVAs (analysis of variance), by examining significances at $p < 0.05$, 0.01, and 0.001.

## 3. Result and Discussion

### *3.1. Influence of Apple Mosaic Virus on the Growth, Yield, and Qualities in Test Garden*

The presence of viruses and viroid were examined in the test garden in Deštnice. Ten hop plants were randomly selected from each test block. ApMV infection rates accounted for 0% and 100% in the uninfected and infected blocks, respectively. Other viruses and viroid were not detected both in uninfected and infected blocks.

Heights of ApMV-infected plants were 10 to 15% shorter than those of uninfected plants (Figure 2a). Significant differences were first observed between the two groups by the middle of June in 2010 and by the end of May in 2011. Bine diameter at 1.5 m height of ApMV-infected plants was not significantly different in 2010 (Figure 2b1). However, in 2011, a significant reduction (20%) in infected plants was observed throughout the cultivation period (Figure 2b2). Length of leaves at 1.5 m height from the ground was 10% less in ApMV-infected plants than in uninfected plants in both 2010 and 2011 (Figure 2c), with significant differences appearing earlier in 2011 than 2010. Changes in leaf appearance, such as

chlorotic rings and necrotic spots, caused by ApMV were not observed in these tests (data not shown). The time between when plants sprouted in spring to the appearance of cones on 50% of plants did not differ between ApMV-infected and uninfected plants in 2010 (Figure 2d1), but was significantly longer in infected plants in 2011 (Figure 2d2).

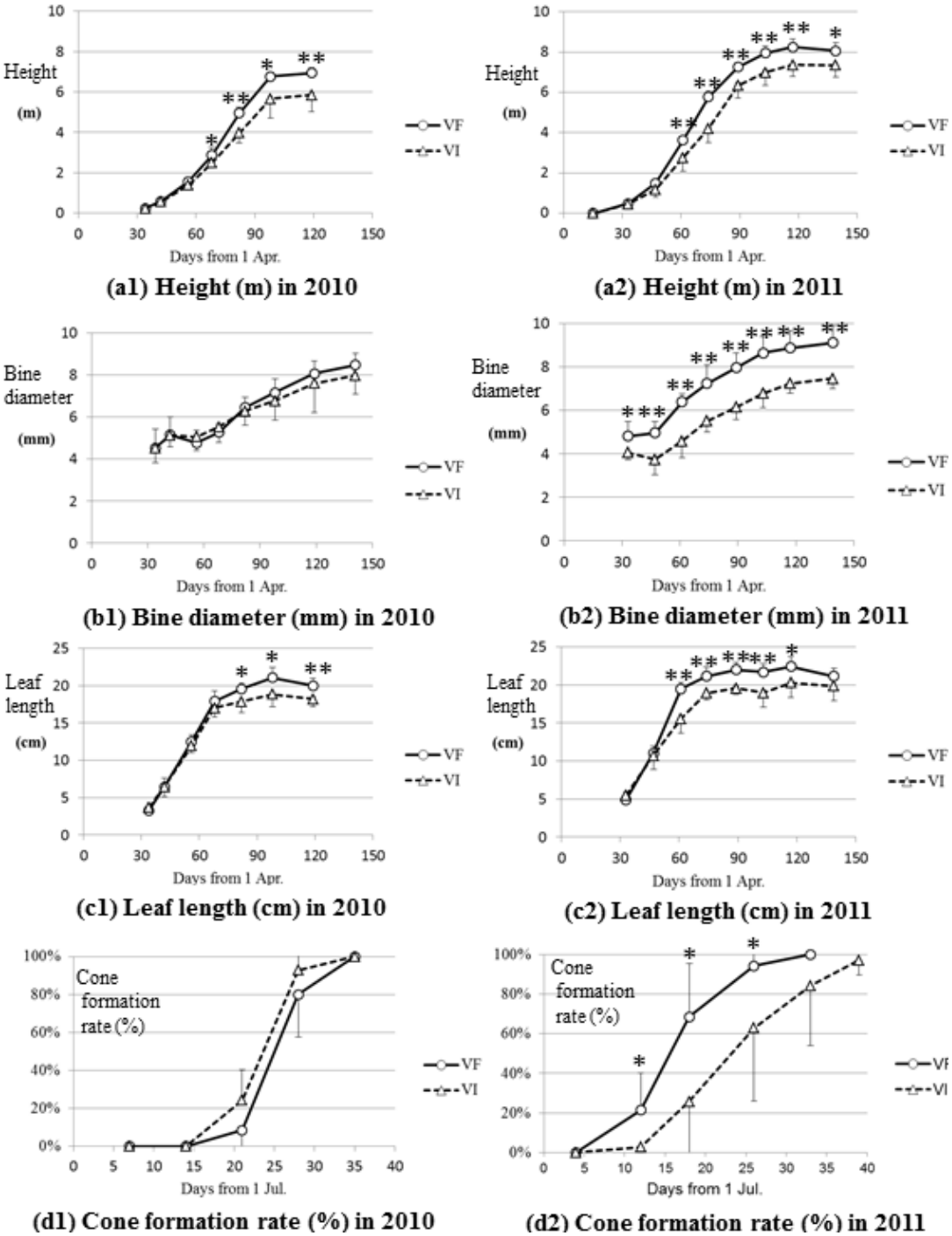


***Figure 2.*** *Behaviors of vegetative growth (height, bine diameter and leaf length) and cone formation during cultivation seasons in 2010 and 2011. VF and VI indicate virus-free (uninfected) and ApMV-infected hops, respectively. Each symbol represents data from 7 plants. Error bars show 1 standard deviation. Asterisk (*) indicates significant difference (* = $p < 0.05$; ** = $p < 0.01$).*

Yield of the ApMV-infected plants (0.6 ton/ha in 2010, 0.9 ton/ha in 2011) was lower than that of uninfected plants (1.9 ton/ha in 2010, 2.0 ton/ha in 2011). ApMV-infected yield was also below average in comparison to 15 commercial hop gardens in both 2010 and 2011 (Figure 3a). Humulone production in ApMV-infected plants (3.25% in 2010, 4.18% in 2011) was also lower than that in uninfected plants (4.95% in 2010, 4.99% in 2011), but was comparable to the average of 15 productive hop gardens (Figure 3b). The total amounts of essential oil produced tended to be roughly equal to the amounts of humulone (Figure 3c).

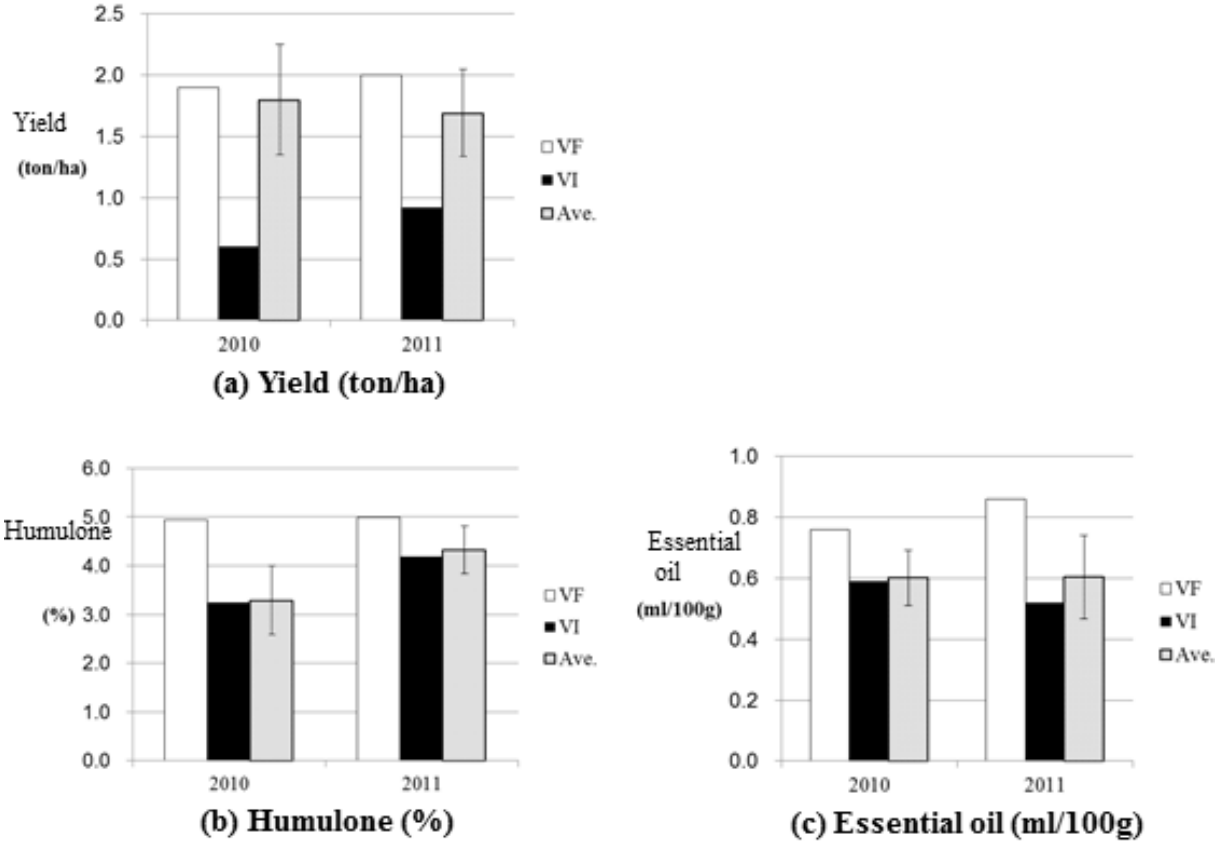


***Figure 3.*** *Yield, humulone, and essential oil levels in dried hop cones after harvest. VF and VI indicate virus-free (uninfected) and ApMV-infected hop, respectively. Ave. indicates the mean as obtained from 15 commercial hop gardens in each year. Error bars show 1 standard deviation.*

ApMV-infected hop cones had a higher proportion of sesqui-terpenes (farnesene, bergamotene, and caryophyllene) and a lower proportion of mono-terpenes (myrcene and β-pinene) than cones obtained from uninfected plants. This pattern was also true relative to average terpene composition in cones from 15 other productive gardens in the region (Table 1).

Beer brewed utilizing the hops derived from the ApMV-infected plants had a significantly weaker fruity aroma than beers brewed from the uninfected plants (Figure 4).

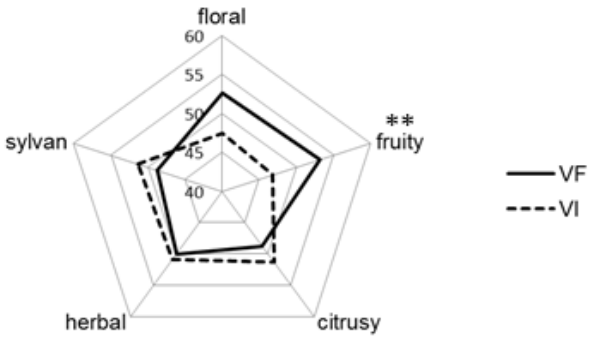


***Figure 4.*** *Scores for beer aroma. VF and VI shows virus free and ApMV infected hops, respectively. (** = $p < 0.01$).*

ApMV affected vegetative growth characteristics, such as height, bine diameter, leaf length, and the time of blooming (Figure 2). The difference in the timing of 50% cone formation between ApMV-infected hop plants and uninfected hop plants was within 10 days (Figure 2d) of one another. In previous studies, longer maturation periods resulted in an increase in essential oil and monoterpene contents in plants, but did not change the amount of sesquitepenes and humulone [10]. However, in this study, the amounts of humulone in virus-infected hops were less than those in virus-free hops for both years of study (2010 and 2011) (Figure 3). And ApMV-infected hop cones had a higher proportion of sesqui-terpenes and a lower proportion of mono-terpenes than cones obtained from uninfected plants (Table 1). We surmise that ApMV affects the biosynthetic pathway of secondary metabolites. This subsequently affects beer aroma quality (Figure 4), with a decrease in essential oil content and an alteration in the terpene profile (myrcene, β-pinene, farnesene, bergamotene, and caryophyllene). Generally, sesqui-terpenes (such as farnesene, bergamotene, and caryophyllene) confer a 'mild' hoppy aroma to beer, while mono-terpenes (such as linalool geraniol and myrcene) confer a 'floral' hoppy aroma [3]. The lower content of essential oils and the higher ratio of sesqui-terpenes of ApMV-infected hops resulted in weaker fruity aroma in beer. Therefore, ApMV infections are detrimental to the floral quality of the beer made from Saaz hop.

***Table 1.*** *Composition of aroma compounds in essential oil. Relative percentage was calculated by comparing peak area of each individual compound to the total peak area of all compounds. Mean and standard deviation were calculated using data collected from 15 commercial hop gardens.*

| | | | Mono-terpene | | | | | | Sesqui-terpene | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **linalool** | **geraniol** | **myrcene** | **ocimene** | **β-pinene** | **limonene** | **farnesene** | **bergamotene** | **humulene** | **caryophyllene** |
| 2010 | VF | (rel %) | 0.56 | 0.21 | 39.5 | 0.070 | 0.73 | 0.16 | 21.3 | 0.90 | 16.6 | 5.81 |
| | VI | (rel %) | 0.63 | 0.13 | 30.1 | 0.070 | 0.58 | 0.22 | 26.8 | 1.28 | 16.9 | 7.31 |
| | Mean | (rel %) | 0.62 | 0.28 | 34.3 | 0.099 | 0.57 | 0.15 | 23.9 | 0.89 | 16.0 | 6.63 |
| | STDEV | | 0.06 | 0.07 | 3.6 | 0.035 | 0.07 | 0.03 | 1.8 | 0.13 | 1.3 | 0.60 |
| 2011 | VF | (rel %) | 0.45 | 0.05 | 31.2 | 0.100 | 0.48 | 0.14 | 20.7 | 0.89 | 23.8 | 6.87 |
| | VI | (rel %) | 0.45 | 0.09 | 22.9 | 0.080 | 0.39 | 0.11 | 21.7 | 1.03 | 27.9 | 8.12 |
| | Mean | (rel %) | 0.53 | 0.14 | 34.7 | 0.106 | 0.55 | 0.15 | 18.4 | 0.85 | 23.4 | 6.49 |
| | STDEV | | 0.11 | 0.04 | 5.9 | 0.027 | 0.09 | 0.02 | 2.3 | 0.09 | 3.6 | 0.93 |

The difference in growth between ApMV-infected and uninfected plants in 2011 was larger than the growth difference in 2010, especially bine diameter and cone formation (Figure 2). It should be noted that differences in growth characters became evident earlier in 2011 than 2010 may have been due to differences in climatic conditions between years. Temperature in May 2011 was warmer than that in 2010 (Figure 5a). It may be that ApMV infection affects hop sensitivity to environmental conditions.

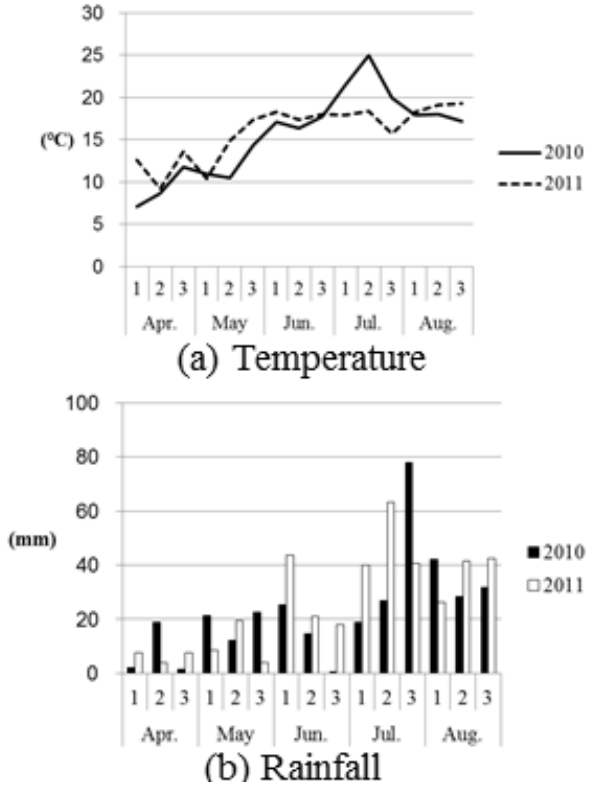


***Figure 5.*** *Weather condition in Kneževes village in 2010 and 2011. Kneževes village is located 10 km south from the test garden in Deštnice village.*

### *3.2. Spatial Pattern of ApMV and HpMV in Commercial Gardens*

Four adjacent plants were found to be infected by ApMV in one commercial hop garden (Table 2, garden M), but adjacent infections were not observed in other gardens. Average infection rates varied among hop gardens and growers (Table 2 and Figure 6).

Pethybridge et al. showed that the transmission of ApMV between plants in commercial gardens occurs predominantly by mechanical means (e.g., during the pruning process in spring [11]). This mode of transmission was observed in one garden (Table 2, garden M), but the hop plants in the majority of gardens remained virus-free over the course of this study, and one particular garden has seen no infection of ApMV or HpMV over its 20 years after hop planting (Table 2, garden Q). It is likely that Saaz hop plants of the Osvald's clone 72 variety have low susceptibility to ApMV and HpMV. Additionally, it was found that the infection rate varied among growers (Figure 6). It suggests that good field practices (GFP) such as the use of new blade for spring pruning, the protection against hop aphids and the sterilization of harrow and the equipment for harvest could be used to suppress the spread of ApMV and HpMV. A comparison of cultivation methods and field practices between gardens with high or low rates of infection may aid in evaluating the effects of such practices.

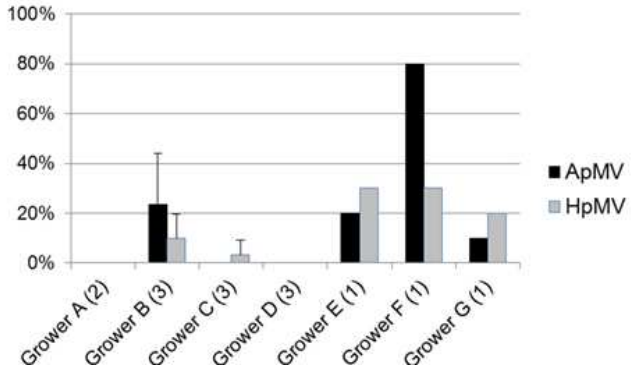


***Figure 6.*** *Infection rate of ApMV and HpMV at each grower in 2010. Number in brackets shows how many hop gardens were used to calculate infection rates. Error bars show 1 standard deviation.*

***Table 2.*** *Spatial distribution of ApMV and HpMV infection in 17 commercial hop gardens. Plant number corresponds to the number in Figure 1. Character 'P' indicates positive infection of ApMV or HpMV.*

**(a) ApMV**

| row | plant No | Hop garden (number indicates plant age) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q |
| | | 1 | 2 | 3 | 4 | 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 15 | 20 |
| row A | 1 | - | - | P | - | - | - | - | - | - | - | - | - | - | - | - | P | - |
| | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 3 | - | - | - | - | - | P | - | - | - | - | - | - | P | - | - | - | - |
| row B | 4 | - | - | - | - | - | P | P | - | - | - | - | - | - | - | - | - | - |
| | 5 | - | - | - | - | - | - | - | - | P | - | - | - | - | - | - | - | - |
| | 6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| row C | 7 | - | - | - | - | - | - | - | - | - | - | - | - | P | - | - | - | - |
| | 8 | - | - | - | - | - | - | - | - | - | - | - | - | P | - | - | - | - |
| | 9 | - | - | - | - | - | - | - | - | - | P | - | - | P | - | - | - | - |
| | 10 | - | - | - | - | - | - | - | - | - | - | - | - | P | - | - | - | - |

**(b) HpMV**

| row | plant No | Hop garden (number indicates plant age) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q |
| | | 1 | 2 | 3 | 4 | 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 15 | 20 |
| row A | 1 | - | - | - | - | - | P | - | - | - | - | - | - | - | - | - | P | - |
| | 2 | - | - | - | - | - | - | - | - | - | - | - | P | P | - | - | - | - |
| | 3 | - | - | P | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| row B | 4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 6 | - | - | - | - | - | - | P | - | - | - | - | - | - | - | - | - | - |
| row C | 7 | - | - | - | - | - | - | - | - | - | - | - | - | P | - | - | - | - |
| | 8 | - | - | - | - | - | - | - | - | - | - | - | - | P | - | - | - | - |
| | 9 | - | - | P | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

# 4. Conclusion

This study confirms the detrimental effects caused by ApMV infection on both hop production and quality for Saaz variety, which agrees with previous investigations of other hop varieties and production areas [13, 14]. The negative impacts of ApMV infection include decreased yield, humulone production, and essential oil content, and a change in aromatic compounds. And it is also confirmed that ApMV may negatively affects the hoppy aroma in beer.

ApMV transmission between plants in commercial hop gardens may occur by transferring viruses on field implements [11]. Future investigations into analysis of this method of transmission and other epidemiological considerations can contribute to GFP-based management of viral infections.

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