# Occurrence of viruses on pepper plantations in the Czech Republic – Short communication

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**Abstract**

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A survey of viruses on capsicum plants in the Czech Republic was carried out in the years 2006–2010. Altogether, 375 leaf samples with symptoms suggesting viral infection were collected both from open fields and greenhouses. These samples were examined for the presence of *Alfalfa mosaic virus* (AMV), *Broad bean wilt virus*-1 (BBWV-1), *Cucumber mosaic virus* (CMV), *Pepper mild mottle virus* (PMMoV), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV) and *Tomato spotted wilt virus* (TSWV) by ELISA. Viruses detected in the samples were AMV, BBWV-1, CMV and PVY. The most prevalent were CMV and PVY which were present in 24 and 29% of tested samples, respectively. In some cases a complex infection of two viruses was detected. Gene sources of resistance against CMV and PVY are mentioned. The relation of virus occurrence on aphid incidence is discussed.

**Keywords**: alfamovirus; fabavirus; cucumovirus; potyvirus; *Myzus persicae*

Peppers (*Capsicum annuum*) are grown on nearly 300 ha in the Czech Republic with a yearly production of about 15,000 t (Smotlacha R. 2010, personal communication). Many diseases can decrease their yields and fruit quality. Among them, viral infections have a high importance, because they cannot be cured, therefore pepper disease management strategies are focused against viral vectors and on the use of resistant cultivars.

It was reported that *Alfalfa mosaic virus* (AMV; Chod et al. 1994) and *Tomato spotted wilt virus* (TSWV) were present on pepper plants. TSWV infected mainly vegetatively-propagated ornamental plants (Mertelik et al. 1996). In 2002, the $P_{1,2}$ pathotype of *Pepper mild mottle virus* (PMMoV; Svoboda et al. 2006) was found in the Czech Republic as well as in the nearby Hungary (Kalman et al. 2001).

Some pepper viruses occur throughout Europe. The most damaging are early infections. Avilla et al. (1997) informed that *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) drastically decreased fruit weight per plant, up to 70 and 80%, respectively, when they had been inoculated on the bell pepper cv. Yolo Wonder plants as early as one week after transplanting to the field. Marchoux et al. (2000) reported that five viruses were common on peppers in France: CMV, PMMoV, PVY, *Tobacco mosaic virus* (TMV) and TSWV. Besides two of them, CMV and PVY, also *Broad bean wilt virus*-1 (BBWV-1) is frequent in Sicily, Italy, which results in heavy yield losses (Davino et al. 1989). The highest loss of pepper production in Slovenia, nearly 100%, was caused also by BBWV-1 (Mehle et al. 2008). Additional viruses found on red pepper plants in Slovenia were CMV, PVY, AMV and

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TSWV (Vozelj et al. 2003) like in Serbia (Petrovic et al. 2010). *Tomato mosaic virus* (ToMV), CMV and PMMoV (Kostova et al. 2003) and TSWV (Neshev 2008) are the most widespread viruses on peppers in Bulgaria. Each year TMV, CMV, PVY and AMV infections had been reported in Hungary (Kiss et al. 2003), where TSWV also significantly reduced pepper yields (Jenser et al. 1996).

The goal of the presented work is to detect the most frequent viruses on capsicum plants in the Czech Republic, to which the resistant cultivars should be used.

## MATERIAL AND METHODS

**Plant material**. Pepper plants of various cultivars were inspected for the presence of symptoms suggesting virus infection both in fields and greenhouses. Samples were collected with a preference to symptomatic plants during the middle of the growing season, in July and August. Leaves showing yellow mosaic or pitting, discoloration, yellowing or stunting were collected from the pepper plants grown in the main production areas in Southern Moravia and marginal growing areas in Bohemia and Northern Moravia which were recommended for inspection by the State Phytosanitary Administration of the Czech Republic. Samples came from the districts of Benešov, Blansko, Břeclav, Česká Lípa, Hodonín, Jičín, Karviná, Litoměřice, Louny, Mělník, Olomouc, Praha-východ, Prostějov, Uherské Hradiště, Zlín and Znojmo.

**ELISA**. The double-antibody sandwich ELISA (DAS-ELISA), described by Clark and Adams (1977), was used for the examination of leaf samples. Samples for ELISA were prepared by grinding 0.2 g of leaf tissue in phosphate buffered saline, pH 7.4 with 2% polyvinylpyrrolidone and 0.2% of bovine albumin, in the ratio 1:20. Commercial AMV, BBWV-1, CMV, PMMoV, PVY, TMV and TSWV specific polyclonal antibodies were used according to the manufacture's manual (Loewe Biochemica, Sauerlach, Germany). Positive control (Loewe) and negative control (healthy pepper leaves) were included on each ELISA microtiter plate for validation of the test results. Plates were incubated for one hour at 20°C after pipetting the substrate solution, and the absorbance value was read at 405 nm using the MR 5000 Dynatech reader (Dynex Technologies GmbH, Denkendorf, Germany). As a substrate solution, 1 mg/ml 4-nitrophenyl phosphate Na-salt dissolved in a diethanolamine buffer, pH 9.8 with 0.01% magnesium chloride was used. A reaction was considered positive when the absorbance value was at least five times higher than that for the health control; the absorbance value of the positive control was above 1.6 and the absorbance value of the negative control was at most 0.02.

**Electron microscope**. Leaf samples with symptoms of viral infection were ground in a mortar with a 0.01M HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid) buffer, pH 8.2, in the ratio 1:2. The homogenate was filtered through a silon sieve and negatively stained by phosphotungstenic acid, pH 6.9, in the ratio 1:1. Then the mixture was used for the preparation of an electron microscope mount. Electron microscope grids were observed by means of the Philips 208S transmission microscope (Philips, Endhoven, The Netherland).

## RESULTS AND DISCUSSION

A survey of pepper plants for the presence of selected viruses in the Czech Republic was carried out in the last five years. Altogether, 375 leaf samples were examined by ELISA. Positive findings were observed in electron microscopy to confirm the findings in ELISA. Particles corresponding in size and shape to the detected viruses (Brunt et al. 2011) were observed. It was found that the most prevalent viruses were PVY and CMV followed by BBWV-1 and AMV with an average occurrence of 29, 24, 5 and 4%, respectively. Neither PMMoV, TMV nor TSWV were detected in the tested plants. Symptoms on samples which were negative in ELISA could have been caused by stress or by the presence of some other pathogen. In some samples a complex infection of CMV and PVY was detected. These two viruses were found both in Moravia and Bohemia whilst BBWV-1 and AMV were detected only in the main pepper growing area in Southern Moravia. CMV was the sole virus detected in the marginal growing districts of Blansko and Karviná (Table 1). The overall occurrence of viruses on capsicum plants during this survey is geographically demonstrated in Fig. 1.

The representative isolates of AMV, BBWV-1, CMV and PVY were deposited in the Virus Collection of the Crop Research Institute, Prague (Svoboda 2010).

All of the viruses detected are transmissible by aphids in a non-persistent manner (Brunt et al.

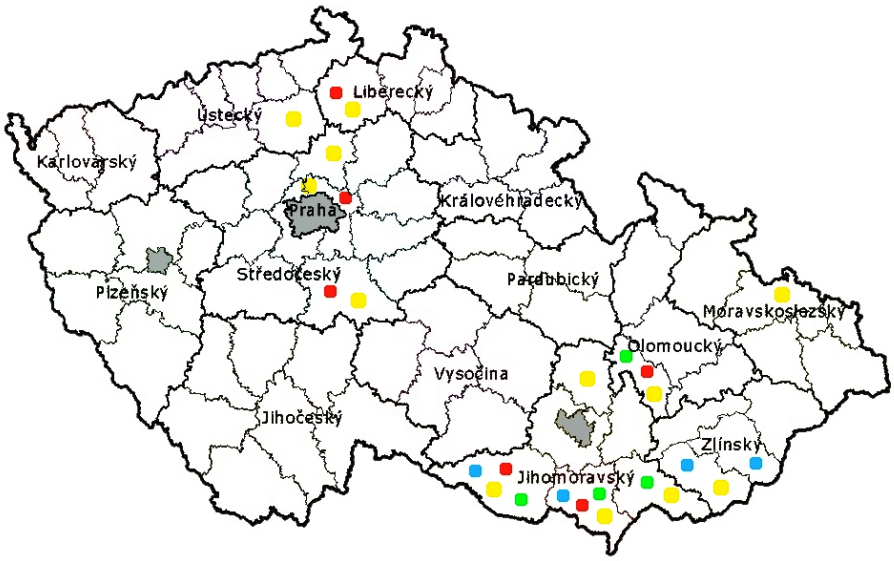


Fig. 1. Distribution of viruses on capsicum plants in the Czech Republic in 2006–2010
blue marks represent BBWV-1 findings; green marks represent AMV findings; red marks represent PVY findings; yellow marks represent CMV findings

2011). In practice it means that these viruses can be transmitted rapidly in several minutes without the need of any long-time acquisition and inoculation feeding. The highest incidence of viruses was observed in the year 2008 with the maximum for PVY and CMV at 70 and 38% in tested samples, respectively (Table 2). This seems to be closely related to the maximal presence of alate aphids caught by the State Phytosanitary Administration of the Czech Republic in 2008 (Kohler 2011), but it does not explain the observed high virus presence in the previous year 2007. Not every monitored aphid species feeds on the pepper plant or can transmit the found viruses. Among aphids, a very well known vector of all the found viruses is the green peach aphid, *Myzus persicae* (Matthews 1991). Development of its alate population during a certain year (Fig. 2) can better explain the observed viral maxims in both years. The increased *M. persicae* population observed by the State Phytosanitary Administration in the 21$^{st}$ week in both years represents the aphid migration from overwintering weed species to the grown pepper plants in the end of May, which poses potential for transmission of viruses from their reservoirs to cultivated plants. The second maximum amount of the *M. persicae* catches in about the 40$^{th}$ week (October) represents their migration back to weeds and does not influence the frequency of viruses on pepper plants. As *Myzus persicae* is the most efficient vector of the most frequently found PVY, other aphid species like *Acyrthosiphon pisum*, *Aphis fabae*, *Aphis nasturtii*, *Phorodon humuli* and *Rhopalosiphum padi* could also play an important role in the virus transmission (Verbeek et al. 2010). The ease of aphid transmission underlines the importance of protecting pepper crops against aphids after transplanting to the field in time according to the aphid population prediction made by the State Phytosanitary Administration of the Czech Republic. In the case of a non-persistant transmission, especially a protecting spray with a repellent effect like synthetic pyrethroids, may help the growers protect their plants from the virus infection.

Table 1. Incidence of viruses on capsicum plants in the Czech Republic in 2006–2010

| Virus | Quantity of positive samples* | Infection rate (%) | Origin of infected samples (shire) |
|---|---|---|---|
| AMV | 14 | 3.7 | Břeclav, Hodonín, Prostějov, Znojmo |
| BBWV-1 | 20 | 5.3 | Břeclav, Uherské Hradiště, Zlín, Znojmo |
| CMV | 91 | 24.3 | Benešov, Blansko, Břeclav, Česká Lípa, Hodonín, Karviná, Litoměřice, Mělník, Praha-východ, Prostějov, Uherské Hradiště, Znojmo |
| PVY | 110 | 29.3 | Benešov, Břeclav, Česká Lípa, Praha-východ, Prostějov, Znojmo |

*total number of tested samples was 375

Table 2. Comparison of the incidence of viruses on capsicum plants with the aphid presence in the Czech Republic in five subsequent years

| Year | 2006 | 2007 | 2008 | 2009 | 2010 |
|---|---|---|---|---|---|
| No. tested plants | 55 | 62 | 77 | 79 | 102 |
| AMV (%) | 1.8 | 4.8 | 2.6 | 10.1 | 0.0 |
| BBWV-1 (%) | 3.6 | 3.2 | 16.9 | 2.5 | 1.0 |
| CMV (%) | 25.5 | 29.0 | 37.7 | 7.6 | 23.5 |
| PVY (%) | 3.6 | 45.2 | 70.1 | 17.7 | 11.8 |
| Single infections | 19 | 47 | 77 | 28 | 29 |
| Mixed infections | 0 | 2 | 21 | 1 | 4 |
| No. aphids* | 128,271 | 95,513 | 142,821 | 104,792 | 89,854 |

*total number of alate aphids caught by the State Phytosanitary Administration of the Czech Republic

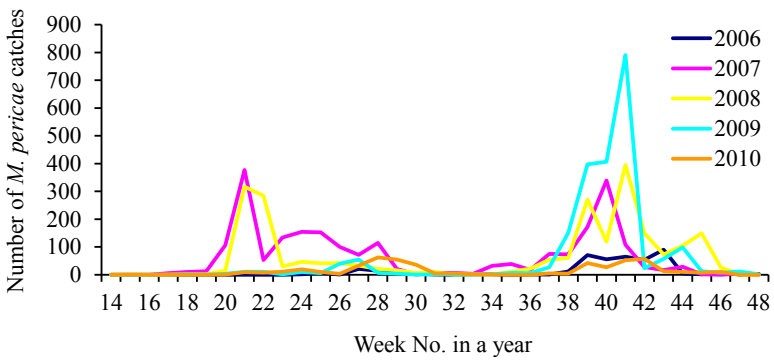


Fig. 2. Time behaviour of *Myzus persicae* catches in the Czech Republic in 2006 to 2010 [data taken from Kohler (2011)]

The most effective method of protection seems to be using resistant pepper cultivars. As CMV and PVY were the most frequently occurring viruses in our survey, cultivars with resistance genes to these viruses should be used. Mazourek et al. (2009) reported that they had developed a new tabasco pepper (*C. frutescens*) cv. Peacework with CMV resistance. Similarly Liang GengSheng et al. (2005) reported a new chilli pepper hybrid F1 cv. Tianjiao No. 4, highly resistant to CMV. Concerning PVY, efficient resistance to PVY (gene *Pvr4*) was identified in the wild hot pepper cv. Criollo de Morelos 334 (Janzac et al. 2009). Another new PVY resistant jalapeno pepper is cv. TAM Dulcito (Crosby et al. 2007). Pepper cultivars only rarely possess the complex resistance both to CMV and PVY, e.g. cv. Cecil F1 displays extreme resistance to both viruses (Horvath et al. 2000). The resistance genes are often connected with chilli peppers. Breeding sweet peppers with the combined resistance against CMV and PVY is the task for the future.

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