Relative concentration of *Apple mosaic virus* coat protein in different parts of apple tree

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Abstract

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The optimal conditions for detecting *Apple mosaic virus* were assessed by determining relative concentrations of viral coat protein in different tissues (leaves, flower petals, dormant buds, and phloem) in five selected symptomless *Apple mosaic virus*-infected apple trees of two cultivars at different terms during the vegetation period. Relative concentrations of *Apple mosaic virus* were calculated as the reciprocal value of the *Apple mosaic virus* coat protein titer determined by ELISA. The highest relative virus concentration and therefore the highest reliability of virus detection was obtained with young leaves in April before flowering. The reliability of the detection was proved by repeating tests of 80 apple trees of four cultivars in the two subsequent years. The presence of *Apple mosaic virus* was tested in young apple leaves before flowering taken from 472 apple trees from selected orchards of the Czech Republic. The association of the outcome with climate is also discussed.

Keywords: *Apple mosaic virus* (ApMV); ELISA; detection; virus titer; concentration; apple tree; leaf; flower; dormant bud; phloem; distribution; climate; orchard

Apple mosaic virus (ApMV), a species of the genus *Ilarvirus*, family *Bromoviridae* (Roossinck et al. 2005), is found worldwide. The virus is significant economically and is easily transmitted by infected propagating material (Mink 1992), with infection resulting in reduced production and tree decline (Desvignes 1999). Excluding the virus from apple trees in nurseries is necessary for the "Certification schemes of virus-free or virus-tested fruit trees and rootstocks", according to the recommendation of the European and Mediterranean Plant Protection Organization (EPPO 1991). Infection with ApMV is primarily symptom-free in apple trees, however some apple cultivars can show irregular white or yellow spots on leaves. The EPPO diagnostic procedure is based on using woody indicator plants, which takes a minimum of two years. ApMV can also be detected using molecular methods (Hassan et al. 2006; Lenz et al. 2008) and ELISA (Torrance, Dolby 1984; Turk 1996; Matic et al. 2008), which can serve as a quick screening approach. The virus concentration in an infected apple tree varies through the year similarly to other apple tree viruses such as *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem grooving virus* (ASGV), and is higher in the first half of the year (Fuchs 1982; Matic et al. 2008). Fuchs and Gruntzig (1994) reported that ApMV is only partially systemically distributed in woody hosts. Torrance and Dolby (1984) discovered that absorbance values using ELISA ApMV detection are greater for young leaves than for mature ones. Virus detection by ELISA is reliable when the absorbance

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value of positive samples is several times higher than that of negative samples. The value of absorbance depends on the concentration of the virus in a sample, the concentration of antibodies in a serum used, and the incubation time of ELISA microtiter plate filled with the substrate solution.

The objective of the current work was to quantify the ApMV relative concentration in apple tissue and then to determine the apple tissue with the highest viral relative concentration and the optimal time for the detection of ApMV by ELISA. The results of presented work were also used to find out the distribution of ApMV in selected apple orchards.

MATERIALS AND METHODS

Plant material

Five symptomless ApMV-infected apple trees of cvs. Kidd's Orange and Starkrimson, were selected in the orchard Ekofrukt near Slaný in the Czech Republic. Samples of various tissue (young leaves, flower petals, dormant buds, and phloem) were taken at different terms of the vegetation period. To prepare a tissue sample for testing, four single samples were taken from four different branches of individual apple tree and mixed in aliquots together as an average sample.

Eighty apple trees of eight cultivars (Idared, Kidd's Orange, Mc Intosh, Melrose, Spartan, Starkrimson, Stark Earliest, Vista Bella) were selected from two orchards (Horoměřice, Slaný) to confirm the results of the study on usefulness of various tissues for the detection of ApMV. Altogether 472 apple trees from different orchards both in Bohemia (Chelčice, Horoměřice, Loukovec, Mělník, Slaný) and Moravia (Brno – Starý Lískovec, Strachotín, Velké Bílovice, Velké Němčice) were selected for tests on the presence of ApMV. Samples of young leaves were collected in the years 2006–2008 in April regardless of the presence or absence of symptoms.

ELISA

Double-antibody sandwich ELISA (DAS-ELISA), described by Clark and Adams (1977), was used for the detection of ApMV in the mixed samples of apple tissue. From each mixed sample, 0.3 g of tissue was ground in 6 ml phosphate buffered saline pH 7.4 with the addition of 2% polyvinylpyrrolidone and 0.2% bovine albumin. Specific polyclonal antibodies at the same volume and dilution were used according to the manufacture's manual (Loewe Biochemica, Sauerlach, Germany) for each test. Positive and negative controls were included on each ELISA microtiter plate to improve the validity of the tests. Plates were incubated for one hour at 20°C after application of the substrate solution, and the absorbance value was read using the MR 5000 Dynatech reader at 405 nm. A reaction was considered positive when the absorbance value was at least five times higher than that for the healthy control; the absorbance value of the positive control (Loewe Biochemica) was above 1.6 and the absorbance value of the negative control (tissue taken from a healthy apple tree) was at most 0.01.

To determine the concentration of the ApMV coat protein in apple tissues, a diluting series of ten steps (1:2, 1:4, 1:8, …, 1:1024) was prepared from each ground sample by dilution each step twice in phosphate buffered saline until the dilution of 1:1024 was reached. A sample from each dilution step was pipetted into two microwells of the ELISA microtiter plate. The titer of the virus in each assessed sample was evaluated as the highest dilution resulting in a positive reaction on ELISA. The concentration of the ApMV coat protein c is inversely related to the titer t, i.e. the less titer the higher concentration. Consequently it can be expressed as a specific constant k divided by the titer:

$$c = k/t$$

It is not necessary to know the exact value of the constant k for the comparison of concentrations of the same viral protein because it is identical for each sample using the same method and determining the same virus. Afterwards the compared concentrations are in the relation

$$c_1/c_2 = (1/t_1)/(1/t_2)$$

and the reciprocal value of the titer $1/t$ can be assumed for the relative concentration which does not have the exact value of the ApMV coat protein concentration in apple tissue but it is in the proper relation to the other relative concentrations. Consequently it can be calculated how many times one relative concentration is higher than the other.

First, this study concerns identification of the tissue with the highest viral concentration, and our second aim was to identify any association between

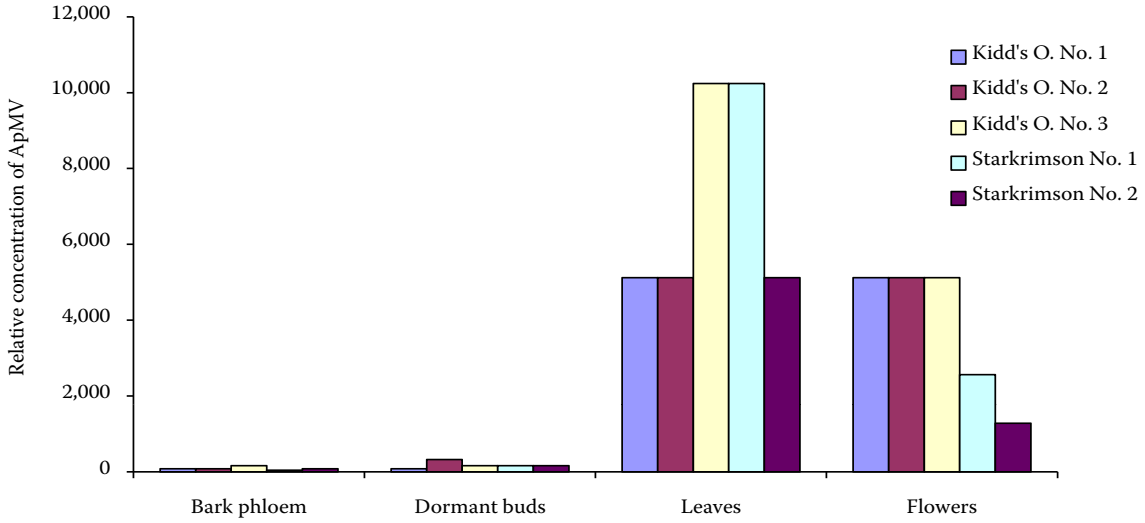

Fig. 1. Relative concentration of ApMV coat protein in different apple tree tissues

the highest virus concentrations and the term of samples collection.

RESULTS AND DISCUSSION

The presence of ApMV in three trees of cv. Kidd's Orange and two trees cv. Starkrimson was detected by ELISA and confirmed by RT-PCR (data not shown). Hassan et al. (2006) found that evaluated trees were simultaneously infected by one or more of three other viruses, ACLSV, ASGV, and *Apple stem pitting virus* (ASPV), none of which were expected to affect ELISA results in the current study.

As reported by Torrance and Dolby (1984), ApMV can be detected in apple trees by ELISA from April to June. Samples of phloem, leaves, and flower petals were taken in the middle of April, and ELISA was carried out immediately following collection. Dormant bud samples were collected in November since Matic et al. (2008) recommend it for ApMV detection. The absorbance values obtained in these assays for different apple tissue showed high relative concentrations of ApMV in leaves and flower petals in contrast to phloem and dormant bud samples. The average value of the ApMV relative concentrations for leaves was 1.9 times higher than for flower petals, 41 times higher for leaves than for

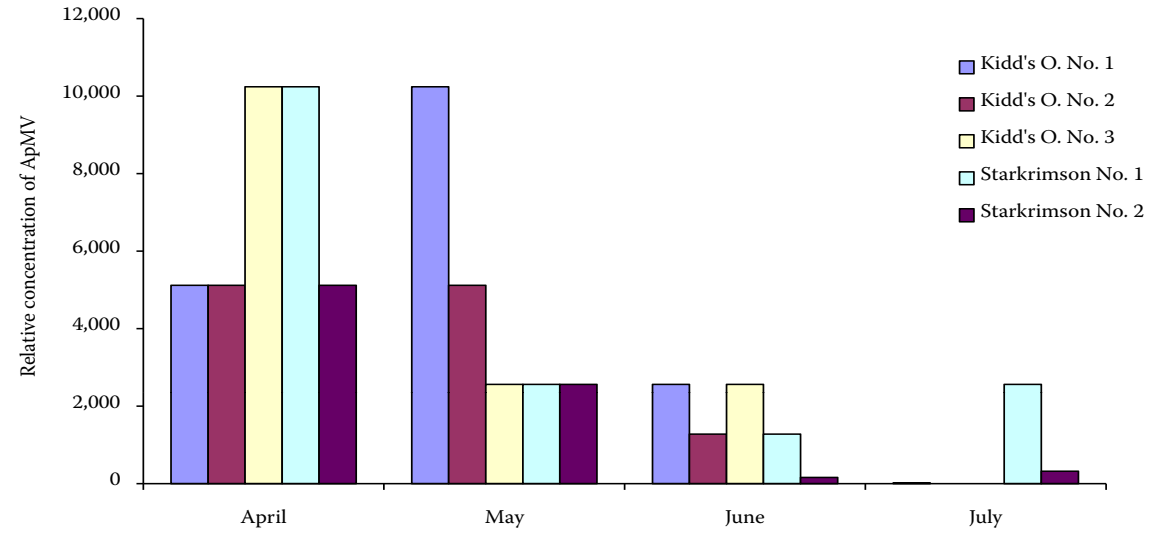

Fig. 2. Relative concentration of ApMV coat protein in apple tree leaves during the first half of the vegetation period

Table 1. Presence of ApMV in selected apple orchards in the Czech Republic during 2006–2008

Region	Location	Cultivar**	Infected/tested trees
Bohemia	Loukovec	Ang, Boh, Gold, Rub, Rbl, Top	19/60
	Mělník	GD, Nab, Rbl, Šam	7/32
	Chelčice	Id, Mu, Rub, Spa, Spe, Šam	5/90
	Horoměřice	Id, Spa, SE, VB	4/40
	Slaný	KO, Mel, MI, St	22/40
Moravia	St. Lískovec	GD, Glo, Id, JG, Spa, Šam	10/60
	Strachotín	GD, Id, Spa, St, Šam	13/50
	V. Bílovice*	El, GD, GS, Id, Jog	1/50
	V. Němčice*	Dia, GDS, Id, JG, Jog	0/50
Total			81/472

*New orchard; **Ang – Angold, Boh – Bohemia, Dia – Diadém, El – Elstar, GD – Golden Delicious, GDS – G. Delicious Smoothy, Glo – Gloster, Gold – Goldstar, GS – Granny Smith, Id – Idared, JG – James Grieve, Jog – Jonagold, KO – Kidd's Orange, Mel – Melrose, MI – Mac Intosh, Mu – Mutsu, Nab – Nabella, Rbl – Rubinola, Rub – Rubín, SE – Stark Earliest, Spa – Spartan, Spe – Spencer, St – Starkrimson, Šam – Šampion, Top – Topaz, VB – Vista Bella

dormant buds, and even 81 times higher for leaves than for phloem (Fig. 1).

Because leaf samples collected in the middle of April contained the highest ApMV concentrations and flowers were no longer available, the research continued using leaves for testing. Following samples were taken from the selected apple trees at the beginning of May, in the middle of June and at the end of July. The maximum ApMV relative concentration was obtained testing young leaves collected in the middle of April. Young leaves sampled later mostly showed a continuous decrease in relative concentrations of ApMV until a zero value in leaves collected in July. The average ApMV relative concentration for leaf samples was 1.6 times, 4.6 times, and 12.4 times higher in April than that in May, June, and July, respectively (Fig. 2).

These results determined further study focusing on the presence of ApMV in eighty selected apple trees of eight cultivars in the two subsequent years. The results showed that the same 26 trees were ApMV-positive in both years (data not shown). Both, positive and negative results were confirmed by RT-PCR (Hassan, unpublish data).

The results of this work were used to study the presence of ApMV in nine selected orchards using ELISA. This virus was detected on around 17% of apple trees out of 472 tested. The virus occurs more frequently in older orchards ranging from 5.6% in Chelčice to 55% in Slaný compared to 2% in the new orchards in Velké Bílovice and 0% in Velké Němčice (Table 1).

The fact that ApMV relative concentration in young leaves and flower petals reaches its highest level in spring in the Czech Republic suggests that the virus propagates better in colder weather. The high virus concentration was also found in flower petals, which supports this idea. Turk (1996), using ELISA to determine ApMV presence, reported even higher concentration of the virus in apple flower petals compared to young leaves. Mature leaves were not used for ApMV detection in the current study since Torrance and Dolby (1984) reported that their absorbance values obtained in ELISA for ApMV were low. As temperature rises from spring to summer, the relative concentrations of ApMV determined by ELISA decreased. The mean air temperatures in the years 2006–2008 ranged from 8.7°C to 12.2°C in April, from 14.2°C to 16.0°C in May, from 18.1°C to 19.3°C in June and from 19.1°C to 22.9°C in July (Climatic data, 2008). Matic et al. (2008) showed that in stone fruit trees in the Mediterranean climatic conditions ApMV was detected by ELISA in most samples of dormant buds collected in November, while only in 10% of these trees the virus was detected in leaf samples during a hot season. These results may indicate a possible thermolability of ApMV and correspond to the results obtained by Torrance and Dolby (1984) in England, who noticed the higher absorbance values in ELISA for samples taken from young apple leaves in May and June than for the samples tested in other months. According to our results, the virus relative concentration decreased in June and

various results obtained by Torrance and Dolby (1984) may be associated with colder climatic conditions in England. In conclusion, ELISA reliability appears to depend not only on the tissue used for the analysis and on the timing of the testing but also on the regional climatic conditions.

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References

Clark M.F., Adams A.N., 1977. Characteristic of the microplate method of enzyme-linked immunosorbent assay for the detection of plant virus. Journal of General Virology, *34*: 475–483.

Climatic data. Available at: http://www.vurv.cz/meteo/ (accessed November 25, 2008).

Desvignes J.C., 1999. Virus Diseases of Fruit Trees. Paris, Ctifl: 150–151.

European and Mediterranean Plant Protection Organization, 1991. Certification schemes Virus-free or virus-tested fruit trees and rootstocks. EPPO Bulletin, *2*: 267–277.

Fuchs E., 1982. Studies of the development of concentration of *Apple chlorotic leaf spot virus* (CLSV) and *Apple stem growing virus* (SGV) in apple trees. Acta Phytopathologica Academiae Scientiarum Hungaricae, *1–2*: 23–27.

Fuchs E., Gruntzig M., 1994. Investigations on the distribution of viruses in woody plant hosts. Kuhn Archiv, *1*: 40–53.

Hassan M., Myrta A., Polák J., 2006. Simultaneous detection and identification of four pome fruit viruses by one-tube pentaplex RT-PCR. Journal of Virological Methods, *133*: 124–129.

Lenz O., Petrzik K., Spak J., 2008. Investigating the sensitivity of a fluorescence-based microarray for the detection of fruit-tree viruses. Journal of Virological Methods, *1/2*: 96–105.

Matic S., Sanchez-Navarro J.A., Mandic B., Myrta A., Pallas V., 2008. Tracking three ilarviruses in stone fruit trees throughout the year by ELISA and tissue-printing hybridization. Journal of Plant Pathology, *1*: 137–141.

Mink G.I., 1992. Ilarvirus vectors. Advances in Disease Vector Research, *9*: 261–281.

Roossinck M.J., Bujarski J., Ding S.W., Hajimarad R., Hanada K., Scott S., Tousignant M., 2005. *Bromoviridae*. In: Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A. (eds.), Virus Taxonomy. Eight Report of the International Committee on Taxonomy of Viruses. Amsterdam, Elsevier/Academic Press: 1049–1058.

Torrance L., Dolby C.A., 1984. Sampling conditions for reliable routine detection by enzyme-linked immunosorbent assay of three ilarviruses in fruit trees. Annals of Applied Biology, *104*: 267–276.

Turk B.A., 1996. Detection of *Apple mosaic virus*, *Apple chlorotic leaf spot virus* and *Apple stem grooving virus* in apple trees by enzyme-linked immunosorbent assay (ELISA) using different plant tissues. Reports Biotechnical Faculty of the University of Ljubljana, *67*: 151–157.

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