The reliability of detection and the distribution of *Apple chlorotic leafspot virus* in pears in the Czech Republic

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ABSTRACT: The distribution of *Apple chlorotic leafspot virus* in pear cultivars in selected orchards of three different districts in the Czech Republic was evaluated by ELISA. The same procedure of DAS – ELISA as in apple trees, was used for the detection of ACLSV in pear trees. The detection of ACLSV in pear flower petals by ELISA was more sensitive than the ACLSV detection in leaves. ACLSV was detected in pear leaves in the reliable way only in June. The presence of ACLSV was not proved by ELISA in nine tested pear rootstocks. The different distribution of ACLSV was detected in pear cultivars Lucasova máslovka and Boscova lahvice grown in different orchards. Low occurrence of ACLSV was found in cultivars Konference, Pařížanka and Dicolor. ACLSV was not detected in cultivars Madame Verté and Bohemica.

Keywords: pear; orchards; *Apple chlorotic leafspot virus*; distribution; flower; cultivars and rootstocks; ELISA

Apple chlorotic leafspot virus (ACLSV) was found in apple trees in the USA (Mink, Shay 1959). ACLSV was characterized as a symptomless, mechanically transmitted virus of apple tree (Kirkpatrick, Linder 1964; Lister et al. 1964). ACLSV is distributed all over the world, transmitted mechanically and by grafting. It is the type of genus *Trichovirus* (Martelli et al. 1994). The ACLSV is present in infected trees irregularly in a low concentration and therefore the reliable detection is difficult. A suitable procedure of the detection of ACLSV by ELISA in apple trees was published by Flegg and Clark (1979) and Detienne et al. (1980). The virus was also detected in pears; e.g. Varveri and Bem (1995) found from 20% to 27% of pear trees infected with ACLSV in different orchards. Polák et al. (1997) studied the distribution of ACLSV in four commercial apple orchards and in one graft stool bed. The presence of ACLSV was proved in three orchards of 39 different apple cultivars. There were found trees of two apple cultivars completely free of ACLSV and three cultivars partly infected were found only in one orchard. ACLSV was detected only in several trees of six apple cultivars from forty-one tested in two graft stool beds lying out of recovered cultivars and rootstocks. The presence of ACLSV was not detected in 219 trees of 24 pear cultivars in graft stool bed. This propagation facility lies out of recovered cultivars and rootstocks. ACLSV was not also detected in individual pear trees in private gardens (Polák unpubl.). The aim of the presented work is on the one hand to improve the reliability of the ACLSV detection in pear trees by ELISA and on the other hand to prepare the procedure protocol of the ACLSV detection in pear trees that could be used in the certification system (testing of candidate, prebasic and basic plants). Another aim of this work is to contribute to the knowledge of the distribution of ACLSV in the Czech Republic.

MATERIAL AND METHODS

Plant material. Different cultivars of pear grown in commercial orchards were evaluated for the presence of ACLSV. The same procedure as for the detection of ACLSV in apple trees was used for the detection of virus in pear trees. Moreover the reliability of detection in leaves (April, May, June) and in flowers was compared. Leaves or flowers were sampled from four to ten different sites of a tree in the dependence on the size of each tree. Also nine

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Table 1. The results of ACLSV detection by ELISA (absorbance values) in leaves and flowers of pear cultivars

Orchard location, date of sampling	Cultivar row/tree number	Absorbance value A_{405}	
		leaves	flowers
Slaný, Ekofrukt, 22. 4. 2002	Lucasova máslovka 12/1	0.3	1.4
	Lucasova máslovka 12/10	0.0	0.5
	Boscova lahvice 6/1	0.0	1.2
	Boscova lahvice 6/2	0.3	0.8
	Boscova lahvice 6/3	0.0	0.0
	Boscova lahvice 6/4	0.0	1.0
	Boscova lahvice 6/5	0.0	0.5
	Boscova lahvice 6/6	0.0	1.1
	Boscova lahvice 6/7	0.9	0.9
	Boscova lahvice 6/8	0.6	0.4
	Boscova lahvice 6/9	0.0	0.6
	Boscova lahvice 6/10	0.0	1.2
Slaný, Station of RICP, 2. 5. 2002	Dicolor/1	0.1	0.3
	Dicolor/4	0.1	0.2
	US 625 – 63 – 4/1	0.0	0.8
	US 625 – 63 – 4/3	0.0	0.7
	Negative control *C. quinoa*	0.03	–
	Negative control – pear	0.01	–

different pear rootstock cultivars were tested for the presence of ACLSV in our work.

ELISA detection of ACLSV. The commercial ELISA kits of LOEWE Biochemica GmBH (Germany) were used for the detection of ACLSV in the pear trees. IgG and IgG conjugated by alkaline phosphatase, were diluted 1:200 in carbonate – bicarbonate buffer pH 9.6. ACLSV was detected by the direct double antibody sandwich ELISA (DAS – ELISA) described by Clark and Adams (1977). 0.2 g of flowers or leaves were homogenized in four milliliters of extraction buffer, pH 7.4 (phosphate buffered saline with 2% polyvinylpyrrolidone and 0.2% of egg albumin) in polyethylene bags. The hand homogenizer made by Bioreba Company was used for proper homogenization. Individual samples were filtered through cheesecloth and 0.2 ml of each was pipetted into a couple of ELISA microplate wells and incubated over night at 4°C. The immuno-enzymatic reaction was evaluated by reading the absorbance values at 405 nm in the MR 5000 photometer (Dynex Company) 30–45 minutes after adding the enzyme substrate.

RESULTS AND DISCUSSION

The presence of *Apple chlorotic leafspot virus* in different pear cultivars was detected in two orchards located near the town Slaný. For the detection of ACLSV in pear trees was used the same procedure as for the detection of this virus in apple trees (Polák et al. 1997). The incidence of ACLSV in pear trees

Table 2. The results of ACLSV detection by ELISA in leaves and flowers of pear cultivars sampled in different dates

Orchard location	Cultivar	Number of tested trees	Number of infected (ELISA positive) trees/date of sampling			
			leaves/22. 4.	flowers/22. 4.		leaves/5. 6.
Slaný, Ekofrut	Lucasova máslovka	10	1	2		2
	Konference	10	0	0		0
	Boscova lahvice	10	3	9		8
			leaves/9. 4.	leaves/2. 5.	flowers/2. 5.	leaves/5. 6.
Slaný, Station of RICP	Dicolor	10	1	1	2	2
	US 625 – 63 – 4	3	2	0	2	3
	Bohemica	3	0	0	0	0

Table 3. The results of ACLSV detection by ELISA in leaves and flowers of pear rootstocks sampled in different dates (Slaný, Station of RICP)

Rootstock	Number of tested rootstocks	Number of infected (ELISA positive) rootstocks/date of sampling			
		leaves/9. 4.	leaves/2. 5.	flowers/2. 5.	leaves/5. 6.
Quince S1	5	0	0	0	0
Seedling NSR – 94	6	0	0	0	0
Seedling TEH – 2	3	0	0	0	0
Quince Adamsova	5	0	0	0	0
Quince B – 29	5	0	0	0	0
Quince M – A	6	0	0	0	0
Seedling Farold – 69	3	0	0	0	0
Seedling Farold – 282	4	0	0	0	0
Seedling *Pyrus usuriensis*	6	0	0	0	0

was much lower than in apple trees. The sensitivity of ACLSV detection by ELISA in leaves and in flowers was compared (Table 1). Leaves and flower petals were sampled on the same day. The absorbance values of samples prepared from leaves were approximately three times lower than the absorbance values of samples prepared from flower petals. ACLSV was detected in greater number of flowers than of leaves. Sixteen trees of four pear cultivars were tested. ACLSV was detected in flowers of fifteen trees, but only in leaves of six trees. In the next trial leaves for the ACLSV detection were sampled from pear trees in a different vegetation period. ACLSV was proved in leaves of fifteen trees collected in June, but only in leaves of seven trees collected in April (Table 2). Very young pear leaves are not suitable for the detection of ACLSV by ELISA. The best samples for the detection of ACLSV in pear trees are the flower petals during the flowering time and the leaves in June. Problems with the ACLSV detection in pears are connected mainly with the period of sampling i.e. with the concentration of viral particles in the sample. ACLSV can be reliably proved by ELISA only in the leaves sampled during June.

The presence of ACLSV in nine different pear rootstocks was examined (Table 3). ACLSV was not proved by ELISA in any of them. The distribution of ACLSV in pear cultivars in selected orchards of three different districts in the Czech Republic was determined (Table 4). The district's distribution of ACLSV in the same cultivar grown in the different orchards was studied. Ten trees from twenty investigated of cv. Lucasova máslovka grown in orchard Horoměřice were infected by ACLSV. In orchard situated near Slaný only two trees from ten investigated of cv. Lucasova máslovka were infected by ACLSV and no ACLSV infection was detected in the orchard located in Dobré Pole. The high incidence of

Table 4. The results of ACLSV detection by ELISA in leaves of pear cultivars in some intensive pear tree orchards

Orchard location	Cultivar	Number of tested trees	Number of infected (ELISA positive) trees
Horoměřice, Prague-West district	Lucasova máslovka	20	10
	Konference	10	1
	Boscova lahvice	10	0
Dobré Pole, Kolín district	Lucasova máslovka	10	0
	Konference	10	0
	Madame Verté	10	0
	Paříženka	10	1
Slaný, Kladno district	Dicolor	10	2
	US 625 – 63 – 4	3	3
	Bohemica	3	0
	Lucasova máslovka	10	2
	Konference	10	0
	Boscova lahvice	10	8 (9 – flowers)

ACLSV was proved in pear cultivar Boscova lahvice (9 trees were infected from 10 trees tested), location Slaný. The presence of ACLSV was not proved in cv. Boscova lahvice, orchard Horoměřice. The high distribution of ACLSV was also proved in foreign pear cv. US 625 – 63 – 4, location Slaný. Two trees of cv. Dicolor were infected with ACLSV in Slaný, one tree of cv. Koference in location Horoměřice, and one tree of cv. Paříženka in location Dobré Pole. ACLSV was not detected in cultivars Boscova lahvice (Horoměřice), Lucasova máslovka, Konference, Madame Verté (Dobré Pole), Bohemica, and Konference (Slaný). ACLSV was detected total in 28 pear trees from 126 tested.

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Spolehlivost detekce a výskyt viru chlorotické skvrnitosti jabloně na hrušních v České republice

ABSTRAKT: Rozšíření viru chlorotické skvrnitosti jabloně v odrůdách hrušně ve vybraných sadech tří okresů České republiky bylo hodnoceno pomocí ELISA. Byl použit stejný postup DAS – ELISA jako pro stanovení ACLSV ve stromech jabloně a byla prokázána jeho vhodnost i pro stanovení ACLSV ve stromech hrušně. Stanovení ACLSV v okvětních lístcích hrušně pomocí ELISA bylo citlivější než stanovení v listech – stanovení ACLSV v listech hrušně bylo spolehlivé pouze v červnu. Přítomnost ACLSV nebyla prokázána v žádné z devíti testovaných podnoží hrušně. Rozdílné rozšíření ACLSV bylo prokázáno v odrůdách hrušně Lucasova máslovka a Boscova lahvice, pěstovaných v různých sadech. Nízký výskyt ACLSV byl zjištěn v odrůdách Konference, Paříženka a Dicolor. ACLSV nebyl nalezen v odrůdách Madame Verté a Bohemica.

Klíčová slova: hrušeň; sady; virus chlorotické skvrnitosti jabloně; rozšíření; květ; odrůdy a podnože; ELISA

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