Warsaw Agricultural University, Warsaw, Poland

DETECTION OF *APPLE CHLOROTIC LEAF SPOT VIRUS* (ACLSV) AND *APPLE STEM GROOVING VIRUS* (ASGV) IN DIFFERENT TISSUES OF 'MUTSU' APPLE CULTIVAR TREES BY ELISA

E. Paduch-Cichal and K. Tomala

Abstract

The detection of *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem grooving virus* (ASGV) by a modified-ELISA test was investigated. ACLSV was detected in breaking leaf buds, young leaves, flowers or phloem + periderm + cortex parenchyma samples. ASGV was found only in breaking leaf buds samples from Budziszynek. ACLSV and ASGV were detected in different parts of apple fruits.

Key words: ACLSV, ASGV, ELISA test, 'Mutsu' apple cultivar

Introduction

In 2003, a research was started to identify the possible virus or viruses causing 'Mutsu' apple cultivar tree disease. Two viruses, *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem grooving virus* (ASGV), were detected in tested apple trees by modified-ELISA technique described by Flegg and Clark (1979). These results were confirmed by immunosorbent electron microscopy (ISEM) + decoration technique (Paduch-Cichal et al. 2005).

The most common method to protect fruit trees against viruses is production of virus-free young trees that requires testing trees in the mother stock nurseries. The detection and identification of viruses is currently based on ELISA test (Flegg and Clark 1979, Fuchs 1982). ELISA is the most commonly used test in mass-testing of plant materials for the viruses (Spiegel et al. 1993). The samples for testing should be collected in April or May, when the virus concentration in trees is the highest (Fuchs 1982, Torrance and Dolby 1984, Kryczyński et al. 1995). However, little is known about whether the reliable results could be obtained with this de-

Phytopathol. Pol. 43: 53–59
© The Polish Phytopathological Society, Poznań 2007
ISSN 1230-0462

tection technique when different tissues are used and the tests are performed at various times of the year. Usually woody plants contain high amounts of virus inhibitors such as polyphenols and polysaccharides which interfere with the sensitivity of the virus detection (Mitra and Kootstra 1993). The amount of these components varies in particular tissues in different periods of the year (Fuchs 1982, Stewart and Nassuth 2000). Flegg and Clark (1979) worked out a modified-ELISA technique to check apple trees for ACLSV which was later successfully used by other researchers (Fuchs 1982, Cieślińska et al. 1994, Kryczyński et al. 1995). The highest ELISA readings were noted when leaf samples or flower petals were tested during spring (April, May). Polák and Zieglerova (2001) presented a detection of ASGV by a modified-ELISA technique in leaf dormant buds forced in the glasshouse from November to January, or leaves from April to May. Cieślińska et al. (1994) found the virus in pear fruit skin samples in August and September. James (1999), Kairby et al. (2001) and Kundu et al. (2003) detected ASGV in leaf or flower samples by a modified-ELISA and IC-RT-PCR techniques.

It happens sometimes that one has to test apple trees in other time than spring. Therefore it seems reasonable to check, which type of tissue and at what time may be used for the reliable virus detection. The aim of the present study was to check the possibility of ACLSV and ASGV detection in various plant tissues during growing season by ELISA test. The material from 183 'Mutsu' apple trees was tested. It was previously documented that these trees were infected by both or at least one of the two viruses (Paduch-Cichal et al. 2005).

Material and methods

Naturally infected 'Mutsu' apple trees from two orchards, Budziszynek near Chynów (orchard A) and Radom (orchard B) were tested. 96 trees grown in Budziszynek and 87 trees grown in Radom were selected for the test. It was found in the preliminary study that some of the trees were infected with ACLSV, ASGV or by both viruses together (Paduch-Cichal et al. 2005).

In 2004 plant material was collected from each tree separately. Eight breaking buds, eight young leaves, eight flowers were collected from different branches round the trees. The sample phloem + periderm + cortex parenchyma was a mixed sample of two or three different branches of each tested tree. The different types of tissue of apple trees were chosen according to the scheme:

Tissue type	Month
Breaking leaf buds	April
Young leaves	May
Flowers	May
Phloem + periderm + cortex parenchyma	November

In September 2004, eight apple fruit samples were collected from different 'Mutsu' trees infected with ACLSV or ASGV, grown in orchard A. Eight apples were selected for comparison from uninfected trees.

Three samples were taken from each apple fruit:

1 – fruit skin and fruit flesh of calyx depression,

2 – fruit skin and fruit flesh of the middle part of fruit,

3 – fruit skin and fruit flesh of stem cavity.

All plant tissues tested (breaking leaf buds, young leaves, flowers, phloem + periderm + cortex parenchyma, fruit tissues) were tested by ELISA immediately after collection.

A modified-ELISA procedure described by Flegg and Clark (1979) was used for the detection of ACLSV and ASGV. Commercial kits (Loewe Biochemica GmbH, Germany) were used throughout the experiment. Samples were prepared by grinding 0.25 g of plant material (breaking buds, young leaves, flowers, phloem + periderm + cortex parenchyma tissues and fruit skin, fruit flesh) with extracting buffer usually at 1/20 (w/v) dilution. The results were assessed by measuring absorbance at 405 nm. Negative and positive controls were always included. Readings for negative control never exceeded 0.100, whereas readings above 0.200 were regarded as positive.

Results

ACLSV was detected in all types of tissues although flowers were a better source of a virus than other tissues (Table 1). The virus was found in flowers from 63.9% tested trees. ACLSV detection was lower in the breaking leaf buds (35%), phloem + periderm + cortex parenchyma tissues (51.4%) and young leaves (54.1%). The number of trees, in which ACLSV was detected, was higher in orchard B than in orchard A. ASGV was undetectable by modified-ELISA test in young leaves, flowers and phloem + periderm + cortex parenchyma samples in all tested trees, except in breaking leaf bud samples from trees in orchard A. Altogether 40.62% of tested trees shown the presence of ASGV.

Table 1

Comparison of ACLSV and ASGV detection in different 'Mutsu' apple cultivar tissues by ELISA test (%)

Orchard	ACLSV				ASGV			
	1	2	3	4	1	2	3	4
Budziszynek	68.00	41.67	52.08	33.33	40.62	0	0	0
Radom	71.26	67.82	75.86	70.01	0	0	0	0
Average	34.97	54.10	63.90	51.37	21.30	0	0	0

1 – breaking leaf buds, 2 – young leaves, 3 – flowers, 4 – phloem + periderm + cortex parenchyma.

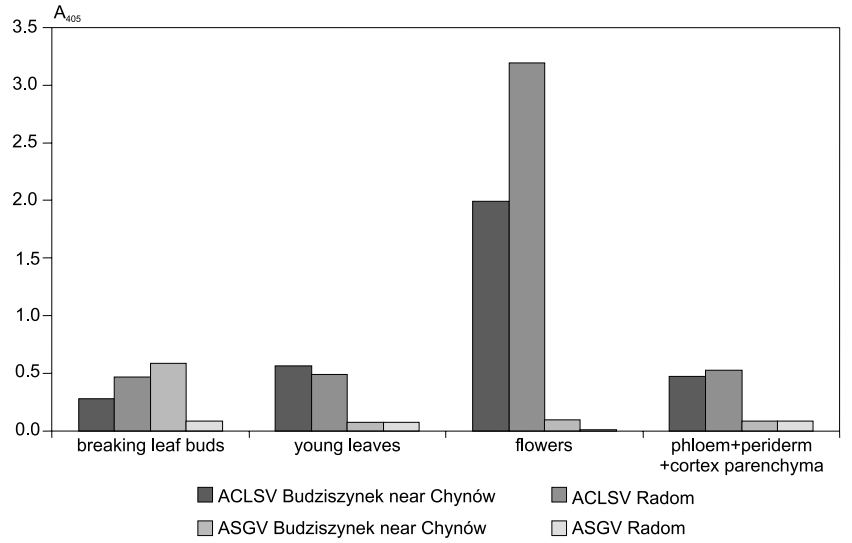

Fig. 1. ELISA absorbance values for ACLSV and ASGV detection in different tissues of 'Mutsu' trees

The highest absorbance readings were obtained in flower samples from trees infected with ACLSV from orchard B (A_{405} = 3.193) and orchard A (A_{405} = 1.996). There were no significant differences in readings for leaves, breaking leaf buds and phloem + periderm + cortex parenchyma samples collected from 'Mutsu' apple trees from orchard B. In orchard A a little lower A_{405} values were obtained for ACLSV in breaking leaf buds and phloem + periderm + cortex parenchyma samples than in leaf samples. ASGV was detected only in orchard A. The ELISA test reading for breaking leaf bud was 0.588 (Fig. 1).

No symptoms were observed in eight fruits tested, ACLSV and ASGV were detected in different parts of apple fruits (Table 2). ACLSV was found only in fruit skin and fruit flesh from the calyx depression and in the middle part of fruit. ASGV infection was detected in all parts of tested fruits, but skin and flesh of the stem cavity were a much better source of a virus than other areas.

The skin and flesh of the calyx depression and skin and flesh in the middle part of fruit were a good source of ASGV. The higher ELISA reading was reported in these samples for this virus than reading for ACLSV. High concentrations of

Table 2

Detection of ACLSV and ASGV in fruits of 'Mutsu' apple cultivar trees (number of fruits with virus detected/number of fruits tested)

Part of fruit	ACLSV		ASGV	
	skin	flesh	skin	flesh
Calyx depression	5/8	5/8	3/8	3/8
The middle part	5/8	5/8	3/8	3/8
Cavity area	0/8	0/8	5/8	3/8

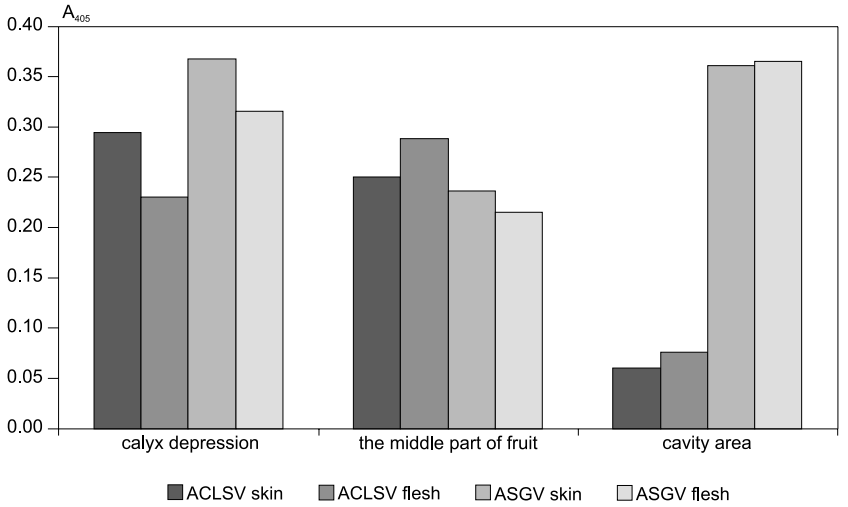

Fig. 2. ELISA absorbance values for ACLSV and ASGV detection in skin and flesh of 'Mutsu' apple

ASGV, measured by ELISA, were observed in skin (A_{405} = 0.361) and flesh (A_{405} = = 0.365) of cavity area. ACLSV was undetectable in these samples (Fig. 2).

Discussion

Our results concerning the detection of ACLSV and ASGV in 'Mutsu' apple trees agree with those obtained by other scientists. Flegg and Clark (1979), Cieślińska et al. (1994), and Kairby et al. (2001) reported that flower petals were the best source of ACLSV. In our experiment we obtained the highest value of absorbance while testing flowers. The virus was also detected in breaking leaf buds and phloem + periderm + cortex parenchyma samples taken from 'Mutsu' apple trees. These tissues showed lower ELISA readings. It agrees with the report by Cieślińska et al. (1994).

According to Kundu et al. (2003) the results obtained in leaves collected in April proved to be the most suitable for ASGV detection by the ELISA test as compared to bark, dormant buds and petals obtained by the modified-ELISA and IC-RT-PCR techniques. The virus was not detected in petals of cvs. 'Idared', 'Stark Earliest' although it was in 'Spartan' and 'Vista Bella'. ASGV was only found in 'Stark Earliest' bark. In the present experiments the virus was detected only in breaking buds (samples from Budziszynek orchard) but it should be kept in mind that our experiments were limited to 'Mutsu' cultivar only.

Flegg and Clark (1979) detected ACLSV in the calyx tissue of freshly picked apples of four dessert cultivars and two culinary cultivars, 'Bramley' and 'Lord Derby'. ACLSV was also detected in similar tissue from two months cold-stored 'Golden Delicious' apples. In experiment reported here, virus was detected in the calyx tissue but also in middle part of fruit. There were not differences in ELISA

test readings for fruits, breaking buds and phloem + periderm + cortex parenchyma samples (A_{405} > 0.200–0.400). Cieślińska et al. (1994) found high concentration of ACLSV measured by ELISA test in pear fruits tested in August and September (A_{405} > 0.300–0.500). No information can be found in literature concerning the detection of ASGV in apple fruits. The calyx and cavity tissues were used in our experiments for virus detecting with good results.

On the basis of the results presented in this paper we conclude that different apple tissues may be safely used for ACLSV and ASGV (only breaking leaf buds) detection by ELISA test. It could be helpful in establishing testing schedule for these viruses to screen candidate materials for virus-free apple trees in sanitary certification programs.

Streszczenie

WYKRYWANIE WIRUSA CHLOROTYCZNEJ PLAMISTOŚCI LIŚCI JABŁONI (ACLSV) I WIRUSA JAMKOWATOŚCI PNIA JABŁONI (ASGV) W RÓŻNYCH ORGANACH DRZEW JABŁONI ODMIANY 'MUTSU' ZA POMOCĄ TESTU ELISA

Badano możliwość wykrywania wirusa chlorotycznej plamistości liści jabłoni (*Apple chlorotic leaf spot virus*, ACLSV) i wirusa jamkowatości pnia jabłoni (*Apple stem grooving virus*, ASGV) za pomocą testu serologicznego ELISA w różnych organach drzew jabłoni odmiany 'Mutsu'. Obecność ACLSV stwierdzano w pękających pąkach liściowych, w liściach, w kwiatach i w próbkach zawierających łyko + parenchymę + miękisz korowy. Obecność ASGV stwierdzono jedynie w próbach z pękających pąków liściowych zebranych z drzew z sadu w Budziszynku koło Chynowa. W sadzie w Radomiu ACLSV i ASGV wykryto tylko w trzech drzewach na 87 testowanych. Żaden z wirusów nie został tam wykryty w młodych liściach. ACLSV lub ASGV stwierdzano w różnych częściach owoców.

Literature

Cieślińska M., Malinowski T., Zawadzka B., 1994: Preliminary results of the studies on apple chlorotic leaf spot virus (ACLSV) isolates occurring in different fruit tree species. Phytopathol. Pol. 8, 20: 35–42.

Flegg C.L., Clark M.F., 1979: The detection of apple chlorotic leaf spot virus by a modified procedure of enzyme-linked immunosorbent assay (ELISA). Ann. Appl. Biol. 91: 61–65.

Fuchs E., 1982: Studies of the development of concentration of apple chlorotic leaf spot virus (CLSV) and apple stem grooving virus (SGV) in apple trees. Acta Phytopathol. Acad. Sci. Hung. 17: 23–27.

Grüntzig M., Fuchs E., 1987: Vergleich verschiedener Methoden zum Nachweis des Chlorotischen Blattfleckungvirus des Apfels (apple chlorotic leaf spot virus) in Pflaumenbäumen. Arch. Pflanzenschutz 23: 21–30.

James D., 1999: A simple and reliable protocol for the detection of apple stem grooving virus by RT-PCR and in a multiplex PCR assay. J. Gen. Virol. 83: 1–9.

Kairby M.J., Guise C.M., Adams A.N., 2001: Comparison of bioassays and laboratory assays for apple stem grooving virus. J. Gen. Virol. 93: 167–173.
Kryczyński S., Szyndel M.S., Paduch-Cichal E., Głowacki M., 1995: Virus-infection status of some apple propagative material plantations in Poland. Phytopathol. Pol. 10, 22: 75–84.
Kundu J.K., Svoboda J., Polák J., 2003: Detection of *Apple stem grooving virus* in different tissues of apple trees throughout the year. Plant Prot. Sci. 39: 93–96.
Mitra D., Kootstra A., 1993: Isolation of RNA from apple skin. Plant Mol. Biol. Rep. 11: 326-332.
Paduch-Cichal E., Szyndel M.S., Tomala K., 2005: Preliminary results of the study on viruses occurring in 'Mutsu' apple cultivar trees. Phytopathol. Pol. 37: 87–90.
Peňa-Iglesias A., Ayuso G.P., 1975: Preliminary identification of the viruses producing Spanish apricot pseudopox (virulea) and apricot mosaic diseases. Acta Hortic. 44: 255–262.
Polák J., Zieglerova J., 2001: Distribution of *Apple stem grooving virus* in apple trees in the Czech Republic. Plant Prot. Sci. 32: 1–4.
Spiegel S., Frison E.A., Converse R.H., 1993: Recent developments in therapy and virus-detection procedures for international movement of clonal germ plasm. Plant Dis. 77: 1176–1180.
Stewart S., Nassuth A., 2000: RT-PCR-based detection of *Rupestris stem pitting associated virus* within field-grown grapevines throughout the year. Plant Dis. 85: 617.
Torrance L., Dolby C.A., 1984: Sampling conditions for reliable routine detection by enzyme-linked immunosorbent assay of three ilarviruses in fruit trees. Ann. Appl. Biol. 104: 267–276.

Authors' addresses:
Dr. hab. Elżbieta Paduch-Cichal, Warsaw Agricultural University, Department of Plant Pathology, ul. Nowoursynowska 159, 02-787 Warsaw, Poland, e-mail: elzbieta_paduch_cichal@sggw.pl
Prof. Dr. hab. Kazimierz Tomala, Warsaw Agricultural University, Department of Pomology, ul. Nowoursynowska 159, 02-787 Warsaw, Poland

Accepted for publication: 27.03.2007