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Virological assessment of stock planting material of apple and raspberry cultivars

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Abstract

Unlike fungal and bacterial diseases, viruses, and related organisms causing the infection are systemic in nature, and symptoms depend on the variety, type and concentration of the agent. Viral diseases cause significant economic damage in orchards and berry plantations, because there is no effective safety-net of viral diseases. Only healthy, virus and related organisms (phytoplasma, viroid, rickettsia) – free plants can produce high yields of quality fruit. Monitoring of apple and raspberry planting material for initial virology condition was made during 2004–2010 at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Plant Propagation Centre. Garden plants were tested according to the European Plant Protection Organization pathogen testing requirements, developed for *Malus* and *Rubus* plants. 22 apple varieties, 4 apple rootstocks and 21 raspberries varieties, 4 raspberry hybrids with high economic value, grown at Institute of Horticulture cultivar collection orchards, were tested using an enzyme-linked immunosorbent assay (DAS-ELISA) method. Economically important viruses of apple and raspberry were found in plants, grown in Institute of Horticulture's orchards. The prevalence of viruses was low: 1.7% of the analyzed apple trees and 3.2% of the analyzed raspberry bushes were infected. It was established that the apple cultivars 'Alva' and 'Auksis' were infected with *Apple chlorotic leaf spot trichovirus* (ACLSV) – 2.7% and 25% of the plants of the tested cultivars, respectively. *Arabis mosaic nepovirus* (ArMV) was detected in 18.2% to 20.0% of plants of the tested raspberry cultivars 'Raniaja sladkaja', 'Novokitajevskaja', 'Beglianka' and 'Zorinka'. *Tomato ringspot virus* (ToRSV) was detected in the cultivar 'Novostj Kuzmina' – 4.6% of plants of the tested cultivars were infected.

Fifteen apple cultivars are recommended as suitable for growing in Lithuanian climate conditions. Our aim was to certify these 15 (6 cultivars created in Lithuania and 9 introduced) cultivars. Apple cultivars were tested using three methods – DAS-ELISA, herbaceous and woody indicators. These plants were cultivated in the Institute of Horticulture's orchard, Plant Propagation Centre, during their annual monitoring.

Key words: apple, DAS-ELISA, herbaceous indicator, raspberry, virus, woody indicator.

Introduction

In Lithuania, the apple tree is one of the most common fruit trees, and the total apple tree orchard area in the country amounts to 3400 ha. Raspberries occupy 206 hectares of berry plantations in Lithuania. These orchard plants have been grown for decades; they are propagated exceptionally vegetatively; and due to intensive agrotechnology are often infected with fungal, bacterial or viral diseases. Virus-infected plants are more susceptible to fungal and bacterial diseases, and various environmental stresses (Zawadzka, 1989). Most of the orchard plant viruses belong to a group of latent viruses transmitted only with the planting material (Nemeth, 1986; Golis et al., 2000; Cieszlinska, Malinowski, 2002). There is no protection against viral diseases; therefore monitoring, isolation and propagation of healthy planting material play a very important part in viral disease prevention and control.

Sixteen virus or virus-like pathogens may be detected in apple and fifteen in raspberries, according to the European standards for plant protection. However, the international movement of plant propagation material enables the spread of non-indigenous vectors of viruses. Therefore, regular monitoring of stock planting material and orchards is required. Economically important viruses found in apple plants are *Apple chlorotic leaf spot trichovirus* (ACLSV), *Apple mosaic ilarvirus* (ApMV), *Apple stem grooving capillovirus* (ASGV), and *Apple stem pitting foveavirus* (ASPV). Fruit quality is decreased by other virus-like diseases, mostly occurring in fruit: rub-

bery wood, flat limb, rough skin, star crack. Raspberry viral disease often leads to clear signs on a raspberry plant or berries, and they are specific to the pathogen vectors. *Nepovirus* is the most widespread virus group in genus *Rubus* (vector is nematode) the main viruses are: *Raspberry ringspot virus* (RRV), *Strawberry latent ringspot virus* (SLRV), *Arabis mosaic virus* (ArMV), *Tomato ringspot virus* (ToRSV), *Tomato black ring virus* (TBRV).

Economic impact and economic value of viral diseases of virus-free and infected 'Shampion', 'Golden Delicious', 'Prima' and 'Florina' cultivars was investigated (Maxim et al., 2004; Kviklys, Stankienė, 2005; Cieszlinska, Rutkowski, 2008).

In recent years, new viruses, their strains, phytoplasmas, rickettsias and viroids have been discovered using sensitive methods (Malinowski et al., 1998). In this context, our Plant Propagation Centre required a systematic monitoring of orchard plants for pathogens. In order to certify apple and raspberry planting material in our collections, research on monitoring and virus elimination in economically important species and hybrids was carried out.

Materials and methods

The research was carried out in 2004–2010 in the cultivar collection orchard, greenhouse and laboratory of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. 22 cultivars of apple ('Alva', 'Shampion', 'Aldas', 'Delikates', 'Auksis', 'Štaris', 'Noris', 'Rudenis', 'Skaistis', 'Ligol', 'Feriside Red', 'Prima', 'Freedom', 'Antej', 'Florina', 'Rubin', 'Paulared', 'Vitos', 'Jonagold de Costa', 'Jonagold Red Prince', 'Elise', 'Early Queen') and 4 rootstocks (M26, MM106, B9, M111) were tested using DAS-ELISA for ACLSV, ApMV and ASGV detection. 21 cultivars ('Raniaja sladkaja', 'Sputnica', 'Novokitajevskaja', 'Beglianka', 'Zorinka', 'Glen Moy', 'Meeker', 'Siveli', 'Aborigen', 'Miraž', 'Otava', 'Solnyško', 'Novostj Kuzmina', 'Zviozdočka', 'Husar', 'Laška', 'Norna', 'Polesia', 'Polka', 'Pokusa', 'Babje leto') and 4 selection numbers (Ru-1, Ru-2, Ru-3, Ru-4) of raspberry were tested using DAS-ELISA for RRV, SLRV, ArMV, ToRSV, TBRV. 15 cultivars of apple ('Štaris', 'Freedom', 'Connel Red', 'Lodel', 'Vitos', 'Auksis', 'Aldas', 'Noris', 'Rudenis', 'Skaistis', 'Popierinis', 'Jonagold de Costa', 'Jonagold Red Prince', 'Early Queen', 'Elise') were tested using DAS-ELISA, mechanical inoculation to herbaceous indicators and graft inoculation to woody indicators with the purpose of certifying planting material and producing super elite stock planting material.

The samples of apple and raspberry cultivars were taken from collection orchards in May of 2004–2010. 100 mg of each sample leaf tissue was homogenized. Plant extracts were prepared in 0.05 M phosphate buffer (pH 7.4), with a 2% polyvinylpyrrolidone (PVP). The total sample was prepared by the primary analysis – tissue extracts of the same cultivar were mixed in equal parts. Upon detection of the pathogen, each sample of the same cultivar was tested separately. Mixed or individual samples were tested using DAS-ELISA for the detection of ACLSV, ApMV, ASGV, RRV, SLRV, ArMV, ToRSV and TBRV (Clark, Adams, 1977; Maat, De Bokx, 1978). The Swiss firm's "Bioreba" antibody kits were used for virus testing.

Herbaceous indicators (*Chenopodium quinoa* and *Cucumis sativus*) were used for testing of ApMV, ACLSV, ASGV and viruses of NEPO group in apple trees in a greenhouse (temperature range 18–25ºC). Leaf tissue extract of each apple cultivar was inoculated to five plants of each indicator. Plant extracts for inoculation were prepared: 50 g of leaf tissue was ground in 0.1 M sodium phosphate buffer (pH 7.5), containing 5% PVP and 0.12% sodium sulphite (Na_2SO_3). The development stage of the indicator plants was 4–6 fully expanded true leaves. Inoculated plants were grown for a minimum of four weeks. Leaves or buds of the herbaceous indicators were tested by DAS-ELISA.

Four woody indicators: *Malus platycarpa*, 'Virginia Crab', 'Lord Lambourne' and 'Golden delicious' were grafted to select apple cultivars in accordance with Nemeth (1986) methodology. After five years, the indicators began to ripen. Viral damage on fruits was recorded every September.

Data was evaluated using a statistical program *ANOVA* (one-way analysis of variance) (Tarakanovas, Raudonius, 2003).

Results and discussion

The production of virus-free apple and raspberry propagation material included the monitoring and evaluation of the virological status of plants in the orchards of Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Analysis of 22 apple varieties and 4 rootstocks by DAS-ELISA showed that two cultivars 'Alva' and 'Auksis' were infected with the ACLSV – 2.7% and 25%, respectively (Table 1). 1.7% of the analyzed cultivars of apple trees were infected with pathogens. In comparison, 11.4% of ACLSV, 1.6% of ASGV and 4.5% of ASPV were found in Latvian apple orchards using DAS-ELISA (Pūpola et al., 2011). Monitoring of apple and pear collections in Czech Republic showed that 28 out of 29 tested cultivars were infected with viruses and the ACLSV was the most prevalent in apple trees (96.5%). In addition, monitoring in Czech Republic detected ASPV (89.7%) and ASGV (34.5%) (Hassan et al., 2008). In Turkey, 126 out of 174 tested samples of apple in orchards and commercial gardens were infected with at least one virus. A combination of four pathogens was found in 70.21% of the cultivars of collection orchards and 75% in cultivars of commercial orchards: ASPV + ACLSV (84.21%), ASPV + ASGV (36.8%), ACLSV + ASGV (26.32%) and ASPV + ApMV (5.2%), ASPV + ASGV + ACLSV (26.32%) (Caglayan et al., 2006). 36.8% of virus-infected plants were found in the apple collection in Romania (Popescu et al., 2004). The study of apple cultivars collection in Albania showed a very high level of virus contamination: ASPV (98.6%), ACLSV (97.8%), ASGV (91.4%) (Myrta et al., 2003).

Table 1. Spread of economically important apple viruses in the collection of apple cultivars and rootstocks Babtai, 2004–2010

Name of cultivar or rootstock	Analysed viruses ELISA (absorption at 405 nm wavelength)			Number of samples tested	Number of virus-free samples	Healthy plants %
	ACLSV	ApMV	ASGV			
'Alva'	+	–	–	74	72	97.3
'Shampion'	–	–	–	26	26	100
'Aldas'	–	–	–	14	14	100
'Delikates'	–	–	–	23	23	100
'Auksis'	+	–	–	20	15	75.0
'Štaris'	–	–	–	10	10	100
'Noris'	–	–	–	10	10	100
'Rudenis'	–	–	–	10	10	100
'Skaistis'	–	–	–	10	10	100
'Ligol'	–	–	–	10	10	100
'Feriside Red'	–	–	–	10	10	100
'Prima'	–	–	–	29	29	100
'Freedom'	–	–	–	16	16	100
'Antej'	–	–	–	19	19	100
'Florina'	–	–	–	8	8	100
'Rubin'	–	–	–	20	20	100
'Paulared'	–	–	–	10	10	100
'Vitos'	–	–	–	10	10	100
'Jonagold de Costa'	–	–	–	20	20	100
'Jonagold Red Prince'	–	–	–	11	11	100
'Elise'	–	–	–	12	12	100
'Early Queen'	–	–	–	10	10	100
M26	–	–	–	10	10	100
MM106	–	–	–	10	10	100
B9	–	–	–	10	10	100
M111	–	–	–	10	10	100
Total				422	415	98.3
$LSD_{0.05}$					1.63	

ACLSV – *Apple chlorotic leaf spot trichovirus*, ApMV – *Apple mosaic ilarvirus*, ASGV – *Apple stem grooving capillovirus*

Selected samples of the four economically valuable rootstocks M26, MM106, B9, MM111 were virus-free according to DAS-ELISA data. ApMV, ACLSV and ASGV pathogens were not detected. Detection of virus or virus-like pathogens is a necessary step in creating a healthy planting material of apple rootstocks. Apple rootstock monitoring carried out in Romania showed, that 50% of the samples had viruses – ACLSV (37.2%), Spy epinasty and decline (25.5%), ASPV and ASGV (7.8% each) (Popescu et al., 2004). It can be concluded, that in warmer climate conditions, virus prevalence is much higher than at the Lithuanian Institute of Horticulture or Latvian apple orchards. Therefore, it is easier to get healthy planting material in our centre of virus-free planting material.

DAS-ELISA data of raspberry samples is presented in Table 2. ArMV was detected in these tested raspberry cultivars: 'Raniaja sladkaja', 'Novokitajevskaja', 'Beglianka' and 'Zorinka' (18.2%, 20.0%, 20.0% and 19.1% plants of the tested cultivars, respectively). ToRSV was detected in cultivar 'Novostj Kuzmina' – 4.6% of plants of tested cultivars. The tested viruses (RRV, SLRV, ArMV, ToRSV, TBRV) were not found in other raspberry cultivars and hybrids. Both detected viruses (ArMV and ToRSV) belong to the NEPO virus group which is widely spread in raspberry. This group of viruses was found in different raspberry varieties by Dale and Brown (1973) in West Midland region. ToRSV was studied in a red raspberry field in Washington State. Their data suggest that the rate of ToRSV spread in plants is limited when nematode-infested soil is not transported into the field (Pinkerton et al., 2008). High level of NEPO viruses in small fruit plantations was detected in Belarus. The virus infection level in cultivars varied from 0% to 52.6% in red raspberry plantation. Raspberry plants were mostly infected with the following viruses: 29.3% (of samples) with RRV, 21.9 % with SLRV, 16.7 % with ArMV. TBRV and ToRSV were absent in all tested raspberry plants (Valasevich, Kolbanova, 2009). The RRV was found in raspberry plants in East Scotland (Murant et al., 1968) and Belarus (Valasevich, Kolbanova, 2009); however we did not find this virus in raspberry collection at the Institute of Horticulture. Control measures of ArMV and ToRSV spread include the use of virus-free plant stock material, chemical control of vectors and planting resistant cultivars in raspberry plantations (Šutic et al., 1999). Even the cultivation of resistant varieties requires the monitoring of the virus because viruses mutate. In Britain, raspberry cultivars resistant to nepoviruses have been used successfully, although resistance-breaking strains of virus have been reported in a few fields (Harrison, Murant, 1996).

Table 2. Testing of virus diseases in the collection of raspberry varieties
Babtai, 2004–2010

Name of cultivar or hybrid	Analysed viruses ELISA (absorption at 405 nm wavelength)					Number of samples tested	Number of virus-free samples	Healthy plants %
	RRV	SLRV	ArMV	ToRSV	TBRV			
'Raniaja sladkaja'	–	–	+	–	–	22	18	81.8
'Sputnica'	–	–	–	–	–	23	23	100
'Novokitajevskaja'	–	–	+	–	–	25	20	80.0
'Beglianka'	–	–	+	–	–	20	16	80.0
'Zorinka'	–	–	+	–	–	21	17	80.9
'Glen Moy'	–	–	–	–	–	23	23	100
'Meeker'	–	–	–	–	–	24	24	100
'Siveli'	–	–	–	–	–	24	24	100
'Aborigen'	–	–	–	–	–	20	20	100
'Miraž'	–	–	–	–	–	25	25	100
'Otava'	–	–	–	–	–	21	21	100
'Solnyško'	–	–	–	–	–	21	21	100
'Novostj Kuzmina'	–	–	–	+	–	22	21	95.4
'Zviozdočka'	–	–	–	–	–	26	26	100
'Husar'	–	–	–	–	–	20	20	100
'Laška'	–	–	–	–	–	21	21	100
'Norna'	–	–	–	–	–	20	20	100
'Polesia'	–	–	–	–	–	24	24	100
'Polka'	–	–	–	–	–	25	25	100
'Pokusa'	–	–	–	–	–	25	25	100
'Babje leto'	–	–	–	–	–	21	21	100
Ru-1	–	–	–	–	–	22	22	100
Ru-2	–	–	–	–	–	24	24	100
Ru-3	–	–	–	–	–	23	23	100
Ru-4	–	–	–	–	–	23	23	100
Total						565	547	96.8
$LSD_{0.05}$							1.63	

RRV – *Raspberry ringspot virus*, SLRV – *Strawberry latent ringspot virus*, ArMV – *Arabis mosaic virus*, ToRSV – *Tomato ringspot virus*, TBRV – *Tomato black ring virus*

Table 3. Monitoring of apple cultivars, included into the list of promising plant varieties in Lithuania, using DAS-ELISA, herbaceous and woody indicators methods
Babtai, 2004–2010

Tested cultivars	Methods of virus detection	Tested viruses	Number of repetitions	Number of plants	Results
'Štaris'	ELISA (absorption at 405 nm wavelength)	*Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV)	2	3	no viruses found during testing
'Freedom' 'Connel Red' 'Lodel' 'Vitos' 'Auksis' 'Aldas'	herbaceous indicators (laboratory) (*Chenopodium quinoa, Cucumis sativus*)	*Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV) *Apple stem grooving virus* (ASGV), viruses of *Nepovirus* group	3	3	no viruses found during testing
'Noris' 'Rudenis' 'Skaistis' 'Popierinis' 'Jonagold de Costa' 'Jonagold Red Prince' 'Early Queen' 'Elise'	woody indicators (field) *Malus platycarpa,* 'Virginia Crab', 'Lord Lambourne', 'Golden delicious'	*Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), Scally bark, *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Apple mosaic virus* (ApMV), Rubberry wood, Flat limb, Chat fruit, Apple proliferation, Horseshoe wound, Leaf pucker, Star crack, Rough skin, Ringspot russet ring, Green crinkle, Dapple apple, Flat apple	3	3	no viruses found during testing

Vegetative propagation of infected planting material is considered the main transmission pathway of viruses (Pūpola et al., 2011). As no know natural vectors of economically important viruses of apple and no against for viral diseases, the production of virus-free reproductive and planting material and its use for establishment of new orchards are the main requirement for effective virus control (Paunovic, Jevremovic, 2004). 15 apple cultivars are included into the list of promising plant varieties in Lithuania. For the production of certified virus-free plant propagation material, these cultivars should be inspected for viruses and virus-like diseases in orchard. ACLSV, ApMV, ASGV pathogens were found in these cultivars using DAS-ELISA. In order to certify apple and raspberry planting material in our collections and to make them super elite plant propagation material, these varieties were additionally tested using the herbaceous and woody indicators methods. Data for these three testing methods are presented in Table 3.

Our research showed that economically important viruses of apple and raspberry were found in the Institute of Horticulture's orchard; however their prevalence is low compared to the pommes fruit orchards in warm climate countries. Certification system of planting material and a healthy material development and propagation of new cultivars is important for the development of healthy orchards.

Conclusions

1. Economically important apple and raspberry viruses were found in the orchards of Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. The prevalence of viruses was low: 1.7% of the analyzed cultivars of apple trees and 3.2% of the analyzed cultivars of raspberry bushes were infected.

2. Only one *Apple chlorotic leaf spot trichovirus* (ACLSV) was detected in the tested apple cultivars. Lithuanian cultivars 'Alva' and 'Auksis' were infected with the ACLSV – 2.7% and 25%, respectively. The cultivar 'Auksis' was the most sensitive to the ACLSV.

3. Raspberry cultivars 'Raniaja sladkaja', 'Novokitajevskaja', 'Beglianka' and 'Zorinka' were susceptible to the virus from NEPO group *Arabis mosaic nepovirus* (ArMV) (18.2%, 20.0%, 20.0% and 19.1% of the plants of tested cultivars, respectively) and cultivar 'Novostj Kuzmina' was susceptible to the *Tomato ringspot virus* (ToRSV) – 4.6% of the plants of the tested cultivars. These are high degrees of infection, given that the raspberries are quite short-lived garden plants, grown for up to 6–8 years.

4. For the purpose of certification and producing a super-elite planting material, 15 apple cultivars ('Auksis', 'Aldas',' Noris', 'Skaistis', 'Rudenis', 'Štaris', 'Popierinis', 'Freedom', 'Connel Red', 'Vitos', 'Lodel', 'Jonagold de Costa', 'Jonagold Red Prince', 'Early Queen', 'Elise') were monitored using DAS-ELISA, herbaceous and woody indicators methods. Virus-free trees of each cultivar were transferred to the elite mother plant nursery for further testing using molecular methods.

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Obelų ir aviečių veislių pradinės sodinamosios medžiagos virusologiniai tyrimai

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Santrauka

Kitaip nei grybinių bei bakterinių ligų, virusų ir jiems giminingų organizmų sukeliama infekcija yra sisteminio pobūdžio, o simptomai priklauso nuo augalo veislės, sukėlėjo tipo bei koncentracijos. Virusinės ligos daro didelę ekonominę žalą sodams ir uogynams, nes kol kas joms gydyti nėra veiksmingų priemonių. Tik sveiki, neužkrėsti virusais ir jiems giminingais organizmais (fitoplazmomis, viroidais, riketsijomis) augalai gali duoti gausų, geros kokybės derlių.

Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės instituto (LAMMC SDI) Sodo augalų dauginimo centre 2004–2010 m. atlikta obelų ir aviečių pradinės sodinamosios medžiagos virusologinės būklės stebėsena. Sodo augalai buvo testuoti remiantis Europos augalų apsaugos organizacijos parengtais testavimo dėl patogenų reikalavimais *Malus* ir *Rubus* šeimų augalams. Imunofermentinės analizės (DAS-ELISA) metodu testuotos SDI genetinių išteklių sode augančios ūkiniu ir ekonominiu atžvilgiu vertingos obelų 22 veislės bei 4 poskiepiai ir aviečių 21 veislė bei 4 selekciniai numeriai. SDI sode nustatyti ekonomiškai svarbūs virusai, aptinkami obelyse ir avietėse. Virusų paplitimo laipsnis buvo nedidelis – 1,7 % tirtų veislių obelyse ir 3,2 % tirtų veislių aviečių krūmuose. Nustatyta, kad veislių ‘Alva’ ir ‘Auksis’ obelys buvo užsikrėtusios obelų žiedinės chlorotinės dėmėtligės virusu (ACLSV) (2,7 ir 25 % tirtų augalų). Keturių veislių ‘Raniaja sladkaja’, ‘Novokitajevskaja’, ‘Beglianka’ ir ‘Zorinka’ avietėse buvo aptiktas vaistučio mozaikos virusas (ArMV) (18,2–20,0 % tirtų augalų), veislės ‘Novostj Kuzmina’ avietėse – pomidorų žiediškosios dėmėtligės virusas (ToRSV) (4,6 % tirtų augalų). Siekiant sertifikuoti 15 (6 sukurtų Lietuvoje ir 9 introdukuotų) obelų veislių, pripažintų perspektyviomis mūsų šalies klimato sąlygomis, jų testavimas atliktas trimis metodais – DAS-ELISA, žolinių ir sumedėjusių indikatorių. Šie augalai kultivuojami LAMMC SDI Sodo augalų dauginimo centre, atliekant jų kasmetinę stebėseną.

Reikšminiai žodžiai: avietė, DAS-ELISA, devirusavimas, obelis, sumedėjęs indikatorius, žolinis indikatorius, virusas.