ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 10(4) 2011, 201-215*

DETECTION AND IDENTIFICATION OF VIRUSES OF HIGHBUSH BLUEBERRY AND CRANBERRY USING SEROLOGICAL ELISA TEST AND PCR TECHNIQUE

Elżbieta Paduch-Cichal[1], Elżbieta Kalinowska[1], Maria Chodorska[1], Kinga Sala-Rejczak[1], Barbara Nowak[2]

[1]Warsaw University of Life Sciences
[2]University of Agriculture in Kraków

Abstract. The problems in the cultivation of highbush blueberry and cranberry are diseases caused by infections factor, particularly by fungi and lately also by viruses. In the years 2008–2010 research concerning the detection and identification of viruses occurring on production plantations of the highbush blueberry located in the central and south-eastern Poland and the cranberry growing on the separate parts of the plantation in the central Poland using the serological ELISA test and PCR technique were performed. The results of the performed serological ELISA test showed the presence on the bushes of various cultivars of the *Blueberry shoestring virus* (BSSV) and *Peach rosette mosaic virus* (PRMV) (central and south-eastern Poland) and the *Blueberry scorch virus* (BlScV) and *Tobacco ringspot virus* (TRSV) (central Poland). During the observations carried out on the plantings of the highbush blueberry only the symptoms characteristic for the infection with the BlScV were noted (central Poland). This virus was also detected using DAS-ELISA test in the cranberry plants growing in the separate parts of plantations in the central region of Poland (Plantation A/W), which did not show any disease symptoms. In Europe it is the first report on the occurrence of BlScV in the cranberry bushes. What is more, it was established that the viruses can be detected in the leaves, the flowers and the phloem + periderm + cortex parenchyma samples in which the investigations could be performed in various months in the year. In the bushes of the blueberry of the Darrow and Herbert cultivars from Plantation A/W (central Poland) showing the symptoms in the form of red spots on the leaves or red spots and rings on the stems the presence of the *Blueberry red ringspot virus* (BRRSV) was confirmed using the PCR technique. In Poland it is the first report concerning the occurrence of the virus in the bushes of the highbush blueberry following those published in the Czech Republic and Slovakia.

Key words: *Viccinium*, *Ericaceae*, methods of virus detection

Corresponding author – Adres do korespondencji: Elżbieta Paduch-Cichal, Department of Plant Pathology, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland, phone: (+48) 22 59 320 39, e-mail: elzbieta_paduch_cichal@sggw.pl

INTRODUCTION

Highbush blueberry (*Vaccinium corymbosum*) belongs to the *Ericaceae* family of the *Vaccinium* genus. In Poland the area of plantations of the highbush blueberry systematically increases. In 2005 it amounted to 1.3 thousand and in 2009 it reached 2.4 thousand hectare. At the same time the level of fruit production increases by the year [acc. To the Main Statistical Office 2010]. The popularity of this plant is connected withits dietary and nutritional value of berries and their high antioxidant content. The reliability of yielding and the easiness of merchandising increase its attaction. The problems in the cultivation of highbush blackberry are diseases caused by infectious factors, particularly by fungi *Godronia casandrae*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and lately also by viruses. A few species of viruses were isolated from the bushes of blueberry: *Blueberry scorch virus* (BlScV), *Blueberry shock virus* (BlShV), *Blueberry shoestring virus* (BSSV), *Blueberry leaf mottle virus* (BLMoV), *Blueberry red ringspot virus* (BRRSV), *Tobacco ringspot virus* (TRSV), *Tomato ringspot virus* (TomRSV) and *Peach rosette mosaic virus* (PRMV). Some of those pathogens are polyphages, for which the highbush blueberry is one of the many host plants. They are: TRSV, TomRSV or PRMV. For BlShV, BSSV or BRRSV, that plant is the only so far recognized host [Caruso and Ramsdell 1995]. On the other hand BlScV also infects cranberry (*Vaccinium macrocarpon*), which is its symptomless host [Wegener et al. 2004], while BLMoV attacks *V. myrtillus* and *V. angustifolium* [Sandoval et al. 1995]. Due to the character of the diseases caused by viruses they comprise serious threat and causes great losses. Fruit yield from plants infected by the *Blueberry shoestring virus* decreased twice, four and even ten times, respectively in the first, second and third year after the infection [Bristow et al. 2000]. Similarly as other fruit trees and bushes, highbush blueberry is propagated vegetatively. Thus, if the lignificated seedling or herbaceous seedlings would originate from an infected plant, the material obtained from them will also be diseased. The first reports concerning the occurrence of viral diseases on the highbush blueberry plantations originated from the region of North America and Canada [Hutchinson and Varney 1954, Varney and Raniere 1960, Ramsdell and Stace-Smith 1979, Converse and Ramsdell 1982, Bristow and Martin 1987, Jaswal 1990, MacDonald et al. 1991]. In 2005, the BlScV was diagnosed in Italy [Ciuffo et al. 2005] while BRRSV in 2009 in Japan [Isogai et al. 2009], in 2010 in the Czech Republic [Přibylová 2010] and in Slovakia [Pleško et. al. 2010]. In Poland in the eighties of the 20^{th} century, first inspection of a few plantations of the highbush blueberry was performed by Prof. Selim Kryczyński [Lenartowicz 1978, Ejsmond 1980]. Due to the lack of proper diagnostic methods, it could not be confirmed whether the observed symptoms on the bushes were really the symptoms of viral diseases. In 2005, dr Barbara Nowak from the Department of Botany and Plant Physiology (Faculty of Horticulture, University of Agriculture in Krakow) while applying the serological ELISA test tested the blueberry bushes cultivated on plantations of private producers and those in the collection of the Faculty in Garlica Murowana for the presence of: BlMoV, BlShV, BlScV, BSSV, TRSV and PRMV. None of the viruses were found in the examined material. The results of the biotests done with the use of the indicator plants *Chenopodium amaranticolor* and *Ch. quinoa* were also not accepted as reliable [Nowak and

Witek 2006, Nowak 2009]. According to the information contained in the *Vaccinium* (Blueberry & Cranberry) Post – Entry Quarantine Testing Manual [2010], for detecting in the blueberry bushes the *Blueberry leaf mottle virus*, *Blueberry shoestring virus*, *Blueberry scorch virus*, *Blueberry shock virus*, *Tomato ringspot virus* and *Peach rosette mosaic virus*, the serological test ELISA should be applied. On the other hand, the *Blueberry red ringspot virus* can be only detected with the help of the PCR technique. So far, in Poland there are no legal regulations, which would define way of the assessment of the infection of the propagation material of the highbush blueberry which is in trade and there is no obligation of carry out such an assessment. However, due to the documented facts of the occurrence of viruses in the bushes of the highbush blueberry cultivated on plantations located in Europe, the UE markets may soon demand the preparation and performation of the defined procedures of the health assessment of the plant material. The aim of the present investigations was the detection and identification of viruses in the bushes of the highbush blueberry on plantations located in the central and south-eastern Poland and cranberry growing in the separate parts of plantations in the central region of part of the country using the serological ELISA test and the PCR technique.

MATERIAL AND METHODS

Detection and identification of viruses of the highbush blueberry and cranberry using the serological ELISA test

In the years 2008–2010 the bushes of highbush blueberry growing on plantations located in the region of central and south-eastern Poland were tested using the serological ELISA test for the presence of the following viruses: *Blueberry leaf mottle virus* (BLMoV), *Blueberry scorch virus* (BlScV), *Blueberry shock virus* (BlShV), *Blueberry shoestring virus* (BSSV), *Peach rosette mosaic virus* (PRMV) and *Tobacco ringspot virus* (TRSV).

Samples of plant material were collected individually from each plant of particular cultivar of the highbush blueberry: 8–10 leaves from randomly chosen shoots (leaf sample), 8–10 flower buds from randomly chosen shoots (flower sample) and samples of phloem + periderm + cortex parenchyma from 2–3 one year old and multi-annual shoots. Combined samples were prepared from the collected samples by putting together material from 5 bushes of a particular cultivar or material collected from a bush of a given cultivar treated as an individual sample. Testing the cranberry bushes, 10–20 leaves were collected from randomly chosen shoots from each plant of a particular cultivar (leaf sample). The presence of BlScV, BlShV, BSSV, TRSV and PRMV in the plant of highbush blueberry and BlScV in the cranberry plants was determined using the serological DAS-ELISA test. The presence of BLMoV in the bushes of the highbush blueberry was detected using the serological TAS–ELISA test. The diagnostic sets (Agdia Inc., USA) were used in accordance to the manufacture. The extract from the investigated plants was obtained by adding to 0.25–0.3 g of plant tissue the GEB buffer (sample buffer, dilution 1:10). The results of the DAS-ELISA test were read using the Labsystems Multiskan MS (Finlandia) and a filter of the wave length 405 nm for

BlScV, BLMoV, BlShV, PRMV and TRSV or the Dynatech Laboratories, MRX Microplate Reader (USA) with the filter for the wave length 650 nm for BSSV. As a treshold absorbance the value 0.2 was accepted. For the negative control samples and the buffer itself the absorbance value did not exceed the value 0.2. All absorbance values of above 0.2 confirmed the presence of the virus in the examined plant material.

Central Poland. In May 2008 collective samples from the bushes of the highbush blueberry growing on 7 production plantations located in the region of the central Poland were collected. In each plantation 50 bushes of a particular cultivar were randomly chosen. For each cultivar 10 collective samples were investigated (leaf sample). All together 2250 collective samples for 45 cultivars from 7 plantations were tested. The following cultivars were investigated in each plantation: Plantation 1/W: Bluecrop, Blueray, Darrow, Herbert, Jersey, Spartan; Plantation 2/W: Bluecrop, Brigitta Blue, Darrow, Duke, Earlyblue, Nelson, Patriot, Spartan; Plantation 3/W: Bluecrop, Chandler, Darrow, Duke, Nelson, Patriot; Plantation 4/W: Berkeley, Bluecrop, Blueray, Earlyblue, Nelson, Toro; Plantation 5W: Bluecrop, Bluegold, Darrow, Duke, Lateblue, Nelson, Toro; Plantation 6/W: Bluecrop, Blueray, Earlyblue, Lateblue, Nelson, Spartan, Sunrise; Plantation 7/W: Bluecrop, Brigitta Blue, Earlyblue, Nelson, Chandler.

During the May–June 2009 collective samples were collected from the bushes of the highbush blueberry growing on 4 production plantations in the region of the central Poland. On the A/W plantation from the randomly chosen bushes of three cultivars (Bluecrop cv. – 225 bushes, Darrow cv. – 75 bushes, Herbert cv. – 175 bushes) 95 collective samples were taken (leaf sample) and 70 collective samples (flower sample). On the B/W and C/W plantations 50 bushes of a particular cultivar were randomly chosen, and for each cultivar of the highbush blueberry 10 collective samples (leaf sample) and 10 collective samples (flower sample) were tested. There were 95 bushes on the D/W plantation out of which 19 collective samples (leaf sample) and 19 collective samples (flower sample) were taken. All together 603 collective samples including 334 leaf samples and 269 flower samples of 12 cultivars were tested. On each plantation the following cultivars were investigated: Plantation A/W: Bluecrop, Darrow, Herbert; Plantation B/W: Bluecrop, Bluejay, Lateblue, Meader, Sunrise; Plantation C/W: Bluecrop, Nelson, Spartan; Plantation D/W: Bluecrop.

In the May/June 2010 material for the investigations originated from the A/W plantation. In 2009 the serological DAS-ELISA test of the collective samples gathered from the bushes of the Bluecrop, Darrow and Herbert cultivars grown in that plantings showed the presence of the BlScV, BSSV and TRSV in the biggest number of samples. The individual samples (leaf and flower) obtained from each bush of the above mentioned cultivars were tested. All together there were 465 individual samples including 155 leaf samples and 310 flower samples. In December 2010, material was received from an individual producer (E/W plantation, Mazovia province). There were 33 individual samples obtained from the following cultivars: Bluecrop (10 samples), Patriot (6 samples), Nelson (5 samples), Brigitta Blue (4 samples), Chandler (4 samples), Berkeley (2 samples), Duke (2 samples). The samples phloem + periderm + cortex parenchyma were also tested. In November 2010 and February 2011, leaf samples were collected from randomly chosen bushes of each of 8 cranberry cultivars grown in the marked of part of the A/W plantation: Ben Lear, Bergman, Early Richard, Howes,

McFarlin, Pilgrim, Stankiewicz, Stevens. All together 48 individual samples were tested on both dates.

South-eastern Poland. In May 2008 individual samples were collected from 28 bushes (28 leaf samples and 28 flower samples) from each of the cultivars growing on 3 production plantations from the region nar. Kraków, individual samples (leaf and flower samples) from each of 50 bushes from amateur plantings of unknown cultivars and from each of 4 cultivars from the experimental collection of the University of Agriculture in Kraków. In each plantation the following cultivars were tested: Plantation 1/K: Bluecrop, Darrow, Haidi, Patriot; Plantation 2/K: Bluecrop, Duke, Chandler, Patriot, Toro; Plantation 3/K: Bluecrop, Chandler, Patriot; Plantation 4/K: amateur plantings with the undefined cultivar status; University collection: Bluecrop, Croatan, Darrow, Herma.

In May 2010 the investigations included four new, so far not inspected plantations in the south-eastern Poland. In the first three plantations collective samples were gathered from 30 bushes of each investigated cultivar and in the fourth plantation – collective samples from 25, 15 or 5 bushes of the cultivar depending on their numer. All together the tests included 34 cultivars and 840 bushes from which 168 collective samples (leaf sample) and 168 collective samples (flower sample) were collected. In each plantation the following cultivars were tested: Plantation A/K: Darrow, Bluecrop, Blueray, Earliblue, Herbert, Jersey, Lateblue, Patriot; Plantation B/K: Bluecrop, Darrow, Haidi, Northblue,Northland, Patriot; Plantation C/K: Bluecrop, Duke, Elliot, Nelson, Reka; Plantation D/K: Bluecrop, Bluejay, Blueray, Bonifacy, Brigitta Blue, Chandler, Croatan, Darrow, Duke, Jersey, Lateblue, Northland, Puru, Sunrise, Toro.

Detection and identification of the Blueberry red ringspot virus of the highbush blueberry in central Poland using the PCR technique

In September 2010, symptoms showing the infection of plants by the *Blueberry red ringspot virus* were noted on the bushes of the Darrow and Herbert Plantation A/W (central Poland). They were red spots on the leaves and red spots and ringspots on the shoots. The extraction of the total DNA was performed from plant material comprising leaf samples as well as samples of phloem + periderm + cortex parenchyma obtained from 7 bushes of the Darrow cultivar and samples of phloem + periderm + cortex parenchyma originated from two bushes of the Herbert cultivar. Samples of plant material (0.1 g) were ground in the mortars. The extraction of the total DNA was carried out using the DNeasy® Plant Mini Kit (Qiagen Inc., USA) according to the producer instruction. In order to identify BRRSV the polymerase chain reaction was performed using the Taq PCR Core Kit (Qiagen Inc., USA). To detect BRRSV a pair of specific starters was used RRSV3/RRSV4 (Polashock et al. 2009) responsible for the amplification of a fragment of a transcriptional activator, TA gene, with the sequence: 5' – ATCAGTCCCAGAAGAAAAGAAGTA - 3'; 5' – TCCGAAAAATAGATAGTGTCAGC - 3'.

The matrix for reaction comprised ~20 ng of the extracted DNA. After the PCR reaction, 5 µl of the product was collected from each sample to which 1 µl of six fold concentrated buffer Loading Dye Solution (Fermentas, Lithuania) containing the dyes:

xylene cyanol FF and bromphenol blue was added. The electrophoretic separation of the PCR reaction products was performed in the buffer TBE in 1.2% agarose gel with the tension 50V for 35 minutes, using the apparatus for horizontal electrophoresis Easy Cast™ Horizontal System model B1A (Owl Separation Systems, USA). The results of electrophoretic separation were photographed on the UV transluminator using the system of documentation and image analysis UVI-KS400I (Syngen, Poland). The value of the obtained PCR products was established against the marker GeneRuler™ DNA Ladder (Fermentas, Lithuania).

RESULTS

Detection and identification of viruses of the highbush blueberry using the serological test ELISA

Central Poland. In the years 2008–2010, no *Blueberry leaf mottle virus* and *Blueberry shock virus* were detected on the tested bushes of highbush blueberry on any of seven plantations in central Poland. In May 2008, in the bushes of highbush blueberry growing on three out of seven inspected plantations viruses were detected only in one out of ten tested collective samples. BlScV was present in the bushes of the Darrow and Herbert cultivars which were additionally infected by PRMV (Plantation 1/W). The *Peach rosette mosaic virus* was also present in plants of the Bluecrop cultivar (Plantation 2/W). On the other hand, the *Blueberry shoestring virus* infected the bushes of the Spartan cultivar (Plantation 6/W) (tab. 1). In 2009, BlScV was detected in the bushes on all four investigated plantations. It was detected in the bushes of the following cultivars: Bluejay, Bluecrop, Herbert, Lateblue, Meader, Nelson, Spartan i Sunrise on the plantations A/W, B/W and C/W and in the bushes of Bluecrop cultivar on the plantation D/W.

Table 1. Virus detection in blueberry plantations (leaf samples – May 2008)
Tabela 1. Wykrywanie wirusów na plantacjach borówki wysokiej (próby liście – maj 2008)

Plantation Plantacja	Cultivar Odmiana	Virus detected/ A_{405}, A_{650} Wykryty wirus/ A_{405}, A_{650}	Number of collected samples with virus detected Liczba prób zbiorczych, w których wykryto wirus	Number of collected samples Liczba prób zbiorczych
Plantation 1/W Plantacja 1/W	Darrow	BlScV/ A_{405} = 0.340	1	10
	Herbert	BlScV/ A_{405} = 0.202 PRMV/ A_{405} = 0.291	1 1	10
Plantation 2/W Plantacja 2/W	Bluecrop	PRMV/ A_{405} = 0.331	1	10
Plantation 6/W Plantacja 6/W	Spartan	BSSV/ A_{650} = 0.310	1	10

Absorbance at A_{405} or A_{650} – Wartości absorbancji – A_{405} lub A_{650}

Only cultivar Darrow was free from BlScV (Plantation A/W). The virus was present in the leaf samples (37 samples) and flower samples (8 samples) (tab. 2). The values of absorbance pointing to the presence of the *Blueberry scorch virus* were within the limits A_{405} = 0.215–0.911 for leaf samples and A_{405} = 0.208–0.479 for flower samples. The *Blueberry shoestring virus* was present in 30 out of 105 tested collective samples collected from the bushes of the Darrow i Bluecrop cultivars (Plantation A/W) (tab. 2). The absorbance values confirming the presence of BSSV in the investigated plant material

Table 2. Virus detection in blueberry plantations (leaf samples – flowers May 2009)
Tabela 2. Wykrywanie wirusów na plantacjach borówki wysokiej (próby liście – kwiaty maj 2009)

Plantation Plantacja	Cultivar Odmiana	Plant material Materiał roślinny	Virus detected Wykryty wirus	Number of collected samples with virus Liczba prób zbiorczych, w których wykryto wirus	Number of collected samples Liczba prób zbiorczych
Plantation A/W Plantacja A/W	Bluecrop	leaves – liście	BlScV	2	45
		flowers – kwiaty		1	30
		leaves – liście	BSSV	13	45
		flowers – kwiaty		9	30
		leaves – liście	TRSV	1	45
		flowers – kwiaty		25	30
	Darrow	leaves – liście	BSSV	3	15
		flowers – kwiaty		5	15
		leaves – liście	TRSV	0	15
		flowers – kwiaty		10	15
	Herbert	leaves – liście	BlScV	13	35
		flowers – kwiaty		1	25
		leaves – liście	TRSV	1	35
		flowers – kwiaty		10	25
Plantation B/W Plantacja B/W	Bluecrop	leaves – liście	BlScV	2	10
		flowers – kwiaty	-	0	10
	Bluejay	leaves – liście	BlScV	5	10
		flowers – kwiaty	-	0	10
	Lateblue	leaves – liście	BlScV	3	10
		flowers – kwiaty	TRSV	1	10
	Meader	leaves – liście	BlScV	2	10
		flowers – kwiaty	BlScV	6	10
	Sunrise	leaves – liście	BlScV	2	10
		flowers – kwiaty	TRSV	1	10
Plantation C/W Plantacja C/W	Bluecrop	leaves – liście	BlScV	2	10
		flowers – kwiaty	-	0	10
	Nelson	leaves – liście	BlScV	1	10
		flowers – kwiaty	-	0	10
	Spartan	leaves – liście	BlScV	3	10
		flowers – kwiaty	-	0	10
Plantation D/W Plantacja D/W	Bluecrop	leaves – liście	BlScV	2	19
			TRSV	1	19
		flowers – kwiaty	TRSV	4	19

– No virus detected – Nie wykryto wirusa

were A_{650} = 0.202–3.105 for leaf samples and A_{650} = 0.209–1.648 for flower samples. *Tobacco ringspot virus* was noted in the bushes of the Bluecrop cultivar (Plantation D/W), Darrow, Herbert and Bluecrop (Plantation A/W) and Lateblue and Sunrise (Plantation B/W). TRSV was detected in 3 samples collected from the leaves and 51 samples collected from flowers. Out of all nine investigated cultivars, the biggest number of collective samples in which the virus was detected was noted in the Bluecrop cultivar (31 samples in which TRSV was detected out of 113 investigated samples) (tab. 2). The range of absorbance values confirming the presence of TRSV in the investigated plant material was A_{405} = 0.209–0.495 for leaf samples and A_{405} = 0.206–1.005 for flower samples. In the year 2010, the results of the DAS-ELISA test confirmed the presence of BlScV, BSSV and TRSV in the bushes of the highbush blueberry growing on the plantation A/W (tab. 3). Leaves of Bluecrop and Herbert cultivars were the best material for the detection of the *Blueberry scorch virus* and in the Darrow cultivar for the *Blueberry shoestring virus*. On the other hand, the higher number of plants infected with the *Tobacco ringspot virus* was noted when flowers were the testing material. The

Table 3. Virus detection in blueberry plantations (leaf samples – flowers May 2010)
Tabela 3. Wykrywanie wirusów na plantacjach borówki wysokiej (próby liście – kwiaty maj 2010)

Plantation Plantacja	Cultivar Odmiana	Plant material Materiał roślinny	Virus detected Wykryty wirus	Number of individual samples with virus Liczba prób indywidualnych, w których wykryto	Number of individual samples Liczba prób indywidualnych
Plantation A/W Plantacja A/W	Bluecrop	leaves – liście	BlScV	8	10
		flowers – kwiaty		3	5
		leaves – liście	BSSV	20	65
		flowers – kwiaty		15	45
		leaves – liście	TRSV	4	10
		flowers – kwiaty		60	125
	Darrow	leaves – liście	BSSV	10	15
		flowers – kwiaty		5	25
		leaves – liście	TRSV	0	0
		flowers – kwiaty		5	50
	Herbert	leaves – liście	BlScV	30	45
		flowers – kwiaty		1	10
		leaves – liście	TRSV	1	10
		flowers – kwiaty		30	50
Plantation E/W Plantacja E/W	Berkeley	phloem+periderm+ cortex parenchyma łyko+miękisz korowy+peryderma	TRSV	2	2
	Bluecrop		BlScV	3	10
			TRSV	7	
	Brigitta Blue		TRSV	3	4
	Chandler		BlScV	1	3
	Duke		BlScV	1	2
			TRSV	2	
	Nelson		TRSV	5	5
	Patriot		BlScV	2	6
			TRSV	6	

mean values of absorbance pointing to the presence of the *Blueberry scorch virus* (Bluecrop and Darrow cultivar) was within the limits A_{405} = 0.305–1.011 (leaf samples) and A_{405} = 0.228–0.689 (flower samples), the *Blueberry shoestring virus* (Bluecrop and Darrow cultivar) amounted to from A_{650} = 0.321 to A_{650} = 2.457 (leaf samples) and A_{650} = 0.299 to A_{650} = 1.456 (flower samples) and the *Tobacco ringspot virus* (Bluecrop, Darrow and Herbert cultivars) was within the range A_{405} = 0.319–0.505 (leaf samples) and A_{405} = 0.226–1.125 (flower samples). In the samples phloem + periderm + cortex parenchyma collected from the bushes growing on the Plantation E/W the presence of the *Blueberry scorch virus* and *Tobacco ringspot virus* was demonstrated (tab. 3). TRSV was detected in the bushes of six cultivars (the mean absorbance value A_{405} = 0.509) with the exception of the cultivar Chandler. The *Blueberry scorch virus* was detected in eight bushes of the cultivars: Bluecrop, Chandler, Duke and Patriot (the mean absorbance value A_{405} = 0.368).

South eastern Poland. In the year 2008, none of the sought after viruses was detected in the examined bushes growing on four plantations and University collection with the help of the serological ELISA test. The investigations carried out in May 2010 on the successive four plantations also did not confirm the presence of any of the viruses with the exception of two collective samples obtained from the Darrow cultivar (Plantation A/K) and from the cultivar Croatan (Plantation D/K), in which the *Blueberry shoestring virus* (mean absorbance value A_{405} = 0.253) and *Peach rosette mosaic virus* (mean absorbance value A_{405} = 0.234) were detected, respectively. In the tests carried out in June 2010, the presence of BSSV and PRMV was confirmed in each plant of the investigated cultivars which comprise the collective sample.

Macroscopic observation of bushes of the highbush blueberry growing on plantations in the central and south-eastern Poland

In the years 2008–2010 (May–September) macroscopic observations of bushes of the highbush blueberry on plantations in the region of the central and south-eastern Poland were performed.

Central Poland. Symptoms were only observed on leaves of 9 out of 19 investigated cultivars in which a virus or viruses were detected (tab. 1, 2). They were red discolorations at the edges, chlorosis – in the cultivars Bluecrop, Bluejay, Darrow, Meader, Spartan, Sunrise, red discoloration of the leaf edges, necrotic spots, chlorosis – cultivars Nelson, Lateblue and mosaic, chlorosis and red discoloration of the leaf edges, red outlining of the veins in the shape similar to the oak leaves – Herbert cultivar.

South-eastern Poland. No macroscopic symptoms pointing to the BSSV or PRMV infection were noted on the bushes of the cultivars Darrow and Croatan (Plantation D/K, 2010). What is more there were no symptoms suggesting the presence of the virus in the bushes of the remaining cultivars tested in 2008 and 2010 for which the results of the ELISA test were negative.

Detection of the Blueberry scorch virus in the cranberry bushes in the central Poland using the serological ELISA test

In September 2010, *Blueberry scorch virus* was not detected only in the samples obtained from the cultivar Ben Lear and Stankiewicz. In February 2010 the results of the serological test DAS-ELISA were positive for all the investigated samples. Clearly higher absorbance values were obtained for the material tested in February. Mean absorbance values for positive samples obtained in November amounted to $A_{405} = 0.304$, and in February to $A_{405} = 0.803$ (tab. 4). On the tested cranberry plants there were no virus like symptoms confirming the presence of the virus.

Table 4. Detection of Blueberry scorch virus in cranberry plants
Tabela 4. Wykrywanie wirusa oparzeliny borówki wysokiej w roślinach żurawiny

Cultivar Odmiana	November 2010 Listopad 2010	February 2011 Luty 2011
Howes	1/3[1]/0.215/	3/3 /0.289/
Stankiewicz	-[2]	3/3 /0.559/
Ben Lear	-	1/3 /0.761/
Early Richard	1/3 /0.406/	3/3 /0.987/
Pilgrim	1/3 /0.206/	3/3 /0.366/
Stevens	2/3 /0.368/	1/3 /0.685/
Bergman	2/3 /0,290/	2/3 /1.073/
McFarlin	1/3 /0.294/	1/3 /0.418/

[1]Number of samples with virus detected – Liczba prób, w których wykryto wirusa; Number of samples – Liczba prób badanych; Mean absorbance at A_{405} – Średnie wartości absorbancji (A_{405}),
[2] No virus detected – Nie wykryto wirusa

Detection and identification of the Blueberry red ringspot virus in the central Poland using the PCR technique

As a result of the performed PCR reaction of the gene fragment coding the transcription activator using the starters described by Polashock et al. [2009] there were obtained specific products of the expected value for each isolate of the *Blueberry red ringspot virus*. The result of electrophoresis of the reaction products performed in 1.2% agarose gel was the product of about 450 pz value including a fragment of gene coding TA BRRSV.

DISCCUSION

In Poland the investigations on viral diseases of the highbush blueberry inaugurated by prof. Kryczyński [Lenartowicz 1978, Ejsmond 1980], and resume by Nowak and Witek [2006] and Nowak [2009] ended in failure. The results presented in this paper point to the presence of viruses in the bushes of the highbush blueberry occurring in the

plantings located in the region of the central and south-eastern Poland and cranberry growing in the allocated part of plantation in the central region of the country.

In the central Poland the investigations included 12 production plantations out of which in eight viruses were detected using the serological test ELISA. Out of nineteen tested cultivars only in the bushes of the Bluecrop cultivar BlScV, BSSV, PRMV and TRSV were detected. In the remaining 18 cultivars the *Blueberry scorch virus* was detected in 11 cultivars, *Tobacco ringspot virus* in 8 cultivars and *Blueberry shoestring virus* in 3 cultivars. Out of 25 tested cultivars of the highbush blueberry growing on 8 plantations in the south-eastern region of the country only in one planting (Plantation D/K, 2010) BSSV was detected in the Darrow cultivar and PRMV in the Croatan cultivar. Clearly more intensive occurrence of viruses in the plants of the highbush blueberry in the central Poland may result from the fact that first plantations were established in the seventies of the 20th century just in that region and plant material was imported from the USA or Canada where the viral diseases had already been noted for a long time [Hutchinson and Varney 1954, Varney and Raniere 1960, Lockhart and Hall 1962, Ramsdell and Stace-Smith 1979]. The lack of health control of the imported plant material and the easiness of transmission during vegetative propagation resulted in the fact that the presence of viruses on the home plantations became a fact. On the other hand a bigger number of bushes infected by viruses in the central Poland may also result from different conditions of the development of the population of vectors, particularly aphids, which belong to the most important pests of the highbush blueberry. In the northern and central states of the USA and in Canada, the aphid most often occurring on the highbush blueberry is *Illinoia pepperi* (MacGillivray) which transmits BSSV [Garcia-Salazar 2002]. In the USA and Italy the effective vector of BlScV proved to be the species *Ericaphis scammelli* (Mason) [Brislow et al. 2000, Pansa and Tavella 2008]. It results from the information collected by Prodorutti et al. [2007] on the Canadian plantations of the highbush blueberry that apart from *E. scammelli* the possible virus vectors are such species as: *E. fimbriata* (Richards), *Aphis fabae* Scopoli, *Brachycaudus helichrysi* (Kaltenbach), *Hyalopterus pruni* (Geoffroy), *Hyperomyzus lactucae* (L.), *Myzus persicae* (Sulzer), *Rhopalosiphoninus staphyleae* (Koch) and *Rhopalosiphum padi* (L.). In Poland a few species of aphids are noted on the highbush blueberry. They are mainly: broad bean (*Aphis fabae* Scopoli), peach aphid (*Nectarosiphon persicae* Sulzer) and berry aphid (*Amphorophora borsalis*) [Krzewińska 2005, Łabanowska 2010]. Unfortunately, so far, there are no data concerning the relations between aphids and viruses of the highbush blueberry. The results of the investigations presented in this paper show that the good material for detecting the *Blueberry shoestring virus* were both leaves and flowers. In a bigger number of plants the *Blueberry scorch virus* was detected while testing leaves. In the case when the research material comprised flowers, the advantage was on the part of bushes infected with the *Tomato ringspot virus*. The samples phloem + periderm + cortex parenchyma can be also used for the detection of BlScV and TRSV. These observations are confirmed by the papers by Martin and Bristow [1988], Cavileer et al. [1994], Halpern and Hillman [1996], Martin [2001], Wegener et al. [2006] and Fuchs [2010]. During the inspection of plantations in both regions of the country no symptoms typical for BSSV, TRSV or PRMV were observed on any plants, despite the fact that the presence of those pathogens was confirmed by the serological

ELISA test. Only on plants in which BlScV was detected, symptoms similar to those already described in the USA and Canada [Martin and Brislow 1988, Caruso and Ramsdell 1995, Brislow et al. 2000] and in Italy [Ciuffo et al. 2005] were noted. The asymptomatic infection of various cultivars of the highbush blueberry by viruses were described in the works by Ramsdell [1983], Bristow and Martin [1999] and Wegener et al. [2006]. In 2003, the *Blueberry scorch virus* was detected by DAS-ELISA test on cranberry plantations in the Oregon and Washington state (USA) and in the region of British Columbia (Canada) [Wegener et al. 2004]. The plants were infected asymptomatically by BlScV. Out of bushes of eight tested cranberry cultivars growing in the separate part of the plantation A/W in the central Poland the presence of BlScV was detected. No symptoms of infection were observed on any of the tested bushes. It is the first report concerning the occurrence of the *Blueberry scorch virus* in the cranberry plants in Europe. Probably it was transferred from of the highbush blueberry plants growing in the same planting or it is possible that its primary source was cranberry. The explanation of that problem needs separate epidemiological investigations. Red-brown spots on the upper side of the older leaves, red spots or rings on the one year old or older shoots of the highbush blueberry infected with BRRSV were observed in the New Jersey state (USA) [Hutchinson and Varney 1954], Japan [Isogai et al. 2009], The Czech Republic [Přibylová 2010] and Slovakia [Pleško et. al. 2010]. Similar symptoms appeared on the bushes of the Darrow and Herbert cultivars originated from Plantation A/W (central Poland), in which BRRSV was detected using the PCR technique. It is the first in Poland and a successive in Europe report concerning the occurrence of the *Blueberry red ringspot virus*.

CONCLUSIONS

1. In the bushes of various cultivars of the highbush blueberry growing on plantations located in the central and south-eastern Poland BSSV and PRMV were detected and identified using the DAS-ELISA test.

2. In various cultivars of the highbush blueberry growing on plantations located in the central Poland B1ScV and TRSV were detected and identified using the serological DAS-ELISA test.

3. It was established that leaves and flowers were the best samples for the detection of BSSV by DAS-ELISA.

4. Using the serological DAS-ELISA test it was demonstrated that in the bushes of the highbush blueberry the best material for the detection of BlScV are leaves and for TRSV – flowers. The detection of viruses was also possible in the phloem + periderm + cortex parenchyma samples.

5. It was also demonstrated that the alternative host for BlScV is cranberry. It is the first report of Blueberry scorch virus on cranberry in Europe. It is the first report of Blueberry red ringspot virus in cultivated blueberry in Poland.

6. It was established that the application of the PCR technique allows the detection and identification of BRRSV in Darrow and Herbert cultivars. In Poland it is the first

report on the occurrence of the virus in the bushes of the highbush blueberry following the reports published in the Czech Republic and Slovakia in Europe.

REFERENCES

Blueberry & Cranberry 2010. Post Entry Quarantine Testing Manual.

Bristow P.R., Martin R.R., 1987. A virus associated with a new blight disease of highbush blueberry. Phytopathology 77, 1721–1722 (Abstr.).

Bristow P.R., Martin R.R., 1999. Transmission and the role of honeybees in field spread of *Blueberry shock Ilarvirus*, a pollen-borne virus of highbush blueberry. Phytophatology 89, 123–130.

Bristow P.R., Martin R.R., Windom G.E., 2000. Transmission, field spread, cultivar response, and impact on yield in highbush blueberry infected with Blueberry scorch virus. Phytopathology 90, 474–479.

Caruso F.L., Ramsdell D.C., 1995. Compendium of blueberry and cranberry diseases. APS Press, St Paul, MN. 87 pp.

Cavileer T.D., Halpern B.T., Lawrence D.M., Podleckis E.V., Martin R.R., Hillman B.I., 1994. Nucleotide sequence of the carlavirus associated with blueberry scorch and similar diseases. J. Gen. Virol. 75, 711–720.

Ciuffo M., Pettiti D., Gallo S., Masegna V., Turina M., 2005. First report of Blueberry scorch virus in Europe. Plant Pathol. 54, 565.

Converse R.H., Ramsdell D.C., 1982. Occurrence of tomato ringspot viruses and dagger and other nematodes associated with cultivated highbush blueberries in Oregon. Plant Dis. 66, 710–712.

Ejsmond E., 1980. Stan zdrowotny plantacji borówki wysokiej w Polsce. Praca magisterska. Zakład Fitopatologii. Instytut Ochrony Roślin SGGW-AR, Warszawa, 54 pp.

Fuchs M., 2010. Association of *Tobacco ringspot virus* and *Xiphinema americanum* with decline of highbush blueberry in New York. Julius – Kühn – Archiv 427, 15–17.

Garcia-Salazar C., 2002. Crop timeline for blueberries in Michigan and Indiana. Prepared for the U.S. Environmental Protection Agency. 33 pp.

Halpern B.T., Hillman B.I., 1996. Detection of *Blueberry scorch virus* strain NJ2 by reverse transcriptase-polymerase chain reaction amplification. Plant Dis. 80, 219–222.

Hutchinson M.T., Varney E.H., 1954. Ringspot. A virus disease of cultivated blueberry. Plant Dis Rep. 38, 260–263.

Isogai M., Ishii K., Umemoto S., Watanabe M., Yoshikawa N., 2009. First report of blueberry red ringspot disease caused by *Blueberry red ringspot virus* in Japan. J. Gen. Plant Pathol. 75, 140–143.

Jaswal A.S., 1990. Occurrence of blueberry leaf mottle, blueberry shoestring, tomato ringspot and tobacco ringspot virus in eleven halfhigh blueberry clones grown in New Brunswick, Canada. Can. Plant Disease Survey 70, 113–117.

Krzewińska D., 2005. Metodyka Integrowanej Produkcji Borówki Wysokiej. Państwowa Inspekcja Ochrony Roślin i Nasiennictwa. 25 pp.

Łabanowska B., 2010. Szkodniki borówki wysokiej i możliwości ich zwalczania. Ogólnopolska Konferencja Nauka Praktyce: 'Intensyfikacja uprawy krzewów jagodowych przez wdrażanie najnowszych wyników badań' Uprawa Borówki Wysokiej, Skierniewice, ISiK, 21.04.2010, 59–63.

Lenartowicz A., 1978. Choroby borówki wysokiej (*Vaccinium australe* Small) wywoływane przez wirusy i organizmy mykoplazmatyczne. Praca magisterska. Zakład Fitopatologii. Instytut Ochrony Roślin SGGW-AR, Warszawa 133 pp.

Lockhart C.L., Hall I.V., 1962. Note on an indication of shoestring virus in the low-bush blueberry *Vaccinium angustifolium*. Can. J. Bot. 40, 1561–1562.
MacDonald S.G., Martin R.R., Bristow P.R., 1991. Characterization of an ilarvirus associated with a necrotic shock reaction in blueberry. Phytopathology 81, 210–214.
Martin R.R., 2001. Appendix I. Recommended procedures for detection of viruses of small fruit crops. Acta Hort. 551, 113–123.
Martin R.R., Bristow P.R., 1988. A carlavirus associated with blueberry scorch disease. Phytopathology 78, 1636–1640.
Nowak B., 2009. Badania nasadzeń borówki wysokiej w Polsce południowej pod względem porażenia przez wirusy. Zesz. Nauk. Inst. Sadown. Kwiac. 17, 17–23.
Nowak B., Witek A., 2006. Wstępne wyniki badań nad występowaniem wirusów na borówce w Polsce południowo-wschodniej. Mat. Międzynarod. Konf. 'Uprawa borówki i żurawiny'. Skierniewice 19–22 czerwca, 128–130.
Pansa M.G., Tavella L., 2008. Aphid population dynamics on highbush blueberry in relation to the spread of Blueberry scorch virus in Piedmont (NW Italy). Bull. Insect. 61 (1), 205–206.
Pleško M., Marn V., Koron P., 2010. Detection of *Blueberry red ringspot virus* in highbush blueberry cv. 'Coville' in Slovenia. Julius-Kühn-Archiv. 427, 2010, 204–205.
Polashock J.J., Ehlenfeldt M.K., Crouch J.A., 2009. Molecular detection and discrimination of *Blueberry red ringspot virus* strains causing disease in cultivated blueberry and cranberry. Plant Dis. 93,727–733.
Přibylová J., Špak J., Kubelková D., Petrzik K., 2010. First Report of *Blueberry red ringspot virus* in highbush blueberry in the Czech Republic. Plant Dis. 94, 1071.
Prodorutti D., Pertot I., Giongo L., Gessler C., 2007. Highbush Blueberry: cultivation, protection, breeding and biotechnology. Eur. J. Plant Sci. Biotech. 1(1), 44–56.
Ramsdell D.C., 1983. Blueberry diseases in Michigan. Extension Bulletin E-1731, 1–7.
Ramsdell D.C., Stace-Smith R., 1979. Blueberry leaf mottle, a new disease of highbush blueberry. Acta Hortic. 95, 37–47.
Sandoval C.R., Ramsdell D.C., Hancock J.F., 1995. Infection of wild and cultivated Vaccinium spp. with blueberry leaf mottle nepovirus. Ann. Appl. Biol. 126, 457–464.
Varney E.H., Raniere L.C., 1960. Necrotic ringspot. A New virus disease of cultivated blueberry. Phtytopathology 50, 241 (Abstr.).
Wegener L.A., Martin. R.R., Bernardy M.G., MacDonald. L., Punja. Z.K., 2006. Epidemiology and strain identification of *Blueberry scorch virus* on highbush blueberry in British Columbia. Can. J. Plant Pathol. 28, 250–262.
Wegener L.A., Punja Z.K., Martin P.R., 2004. First report of Blueberry scorch virus in Cranberry in Canada and in the United States. Plant Dis. 88, 427.

WYKRYWANIE I IDENTYFIKACJA WIRUSÓW BORÓWKI WYSOKIEJ I ŻURAWINY PRZY UŻYCIU TESTU SEROLOGICZNEGO ELISA ORAZ TECHNIK BIOLOGII MOLEKULARNEJ

Streszczenie. Jednym z istotnych problemów w uprawie borówki wysokiej i żurawiny są choroby infekcyjne powodowane przez grzyby i wirusy. W latach 2008–2010 prowadzono badania dotyczące wykrywania i identyfikacji wirusów występujących na plantacjach produkcyjnych borówki wysokiej zlokalizowanych w centralnej i południowo-wschodniej Polsce oraz żurawiny rosnącej na wydzielonej części plantacji w rejonie centralnym kraju

przy użyciu testu serologicznego ELISA oraz technik biologii molekularnej. Wyniki przeprowadzonych testów serologicznych ELISA wskazują na obecność w krzewach różnych odmian wirusa nitkowatości borówki wysokiej i wirusa mozaikowatej rozetowatości brzoskwini (centralna i południowo-wschodnia Polska) oraz wirusa oparzeliny borówki wysokiej i wirusa pierścieniowej plamistości tytoniu (centralna Polska). Podczas obserwacji prowadzonych w badanych nasadzeniach borówki wysokiej zanotowano jedynie objawy charakterystyczne dla infekcji przez wirus oparzeliny borówki wysokiej (centralna Polska). BlScV został także wykryty w niewykazujących jakichkolwiek objawów chorobowych roślinach żurawiny rosnących na wydzielonej części plantacji w centralnym rejonie kraju przy użyciu testu DAS-ELISA. W Europie jest to pierwsze doniesienie o występowaniu wirusa w krzewach żurawiny. Ponadto ustalono, że wirusy można wykrywać w liściach, kwiatostanach lub próbach łyko + miękisz korowy + peryderma i prowadzić w różnych miesiącach. W krzewach borówki wysokiej odmian Darrow i Herbert (Plantacja A/W, centralna Polska) wykazujących objawy w postaci czerwonych plam na liściach oraz czerwonych plam i pierścieni na pędach ustalono obecność wirusa czerwonej pierścieniowej plamistości borówki wysokiej (ang. *Blueberry red ringspot virus*, BRRSV) przy użyciu techniki PCR. W Polsce jest to pierwsze doniesienie o występowaniu wirusa w krzewach borówki wysokiej, a kolejne po Czechach i Słowacji w Europie.

Słowa kluczowe: *Viccinium*, *Ericaceae*, metody wykrywania wirusów

ACKNOWLEDGEMENTS

Research supported by grant NN 310 036038 from the Ministry of Science and Higher Education of Poland.

Accepted for print – Zaakceptowano do druku: 12.09.2011