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Morphological Diversity of *Phomopsis vaccinii* Isolates from Cranberry (*Vaccinium macrocarpon* Ait.) in Latvia

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Abstract. *Phomopsis vaccinii* cause a serious disease of blueberry, cranberry and other *Vaccinium* crops in the North America and Europe as well. Over 1000 species names are described by *Phomopsis* spp., but their biology and life style are mostly unknown. Identification of *P. vaccinii* by methods of classical phytopathology is difficult and complicate, because many species are morphologically similar to *P. vaccinii*, and *P. vaccinii* itself has diverse patterns of colony morphology. For this investigation *P. vaccinii* isolates were obtained from five cranberry plantations in different locations of Latvia (Babite, Alsunga, Rucava, Ape and Pargauja municipality) from berries affected by viscid rot at harvest and storage in 2010. Altogether 44 *P. vaccinii* isolates were cultivated on potato dextrose agar for description of colony morphology. In order to find some relationships between samples, the isolates were arranged in different groups on the basis of mycelium color and structure (zoning), reverse pigmentation, pycnidium formation time, size, location, number and size of conidia. During this study the colonies of *P. vaccinii* from cranberry in Latvia showed different morphological features in culture and no relationships between growing regions and groups of isolates were found. *P. vaccinii* is easy to confuse with other *Phomopsis* species if only classical phytopathological methods are used.
Key words: *Phomopsis vaccinii,* colony morphology, mycelium, pycnidia, conidia.

Introduction

The plantations of American cranberry in Latvia enlarge every year because of high production demand in the market of Latvia and Europe, but fruit rot reduces quality of berries in storage (Vilka and Bankina, 2013). Nine pathogens are detected from cranberry in Latvia. *Fusicoccum putrefaciens* and *Coleophoma empetri* are the most common in cranberry plantations and storage in Latvia (Vilka et al., 2009, Vilka and Bankina, 2013). One of the harmful pathogens in the future could be *Phomopsis vaccinii.* Although overall incidence of *P. vaccinii* is still low in Latvia, it is isolated from upright dieback, flowers and ovaries in cranberry plantations and mostly causes viscid rot in storage (Vilka et al., 2009).

Vaccinium macrocarpon (American cranberry) and *V. corymbosum* (highbush blueberry) were introduced from North America and now are widely commercially cultivated in Europe (Lombard et al., 2014). In Latvia, cranberry and blueberry growing has become more popular since 1990, and both are rated as some of the most succesful comercial fruit crops at the moment.

P. vaccinii cause a serious disease of blueberry, cranberry and other *Vaccinium* crops in the North America. *P. vaccinii* was considered to be of minor importance in the late 1940s (Bergman and Wilcox, 1936), but disease became serious in some areas of Wisconsin in 1966. From 1975 it was reported that pathogen could be epidemic in some southern regions and *P. vaccinii* was considered to be a serious pathogen under favorable conditions (Weingartner and Klos, 1975).

The genus *Vaccinium* contains approximately 450 species of woody, perennial shrubs belonging to the family *Ericaceae* (heath). Four *Vaccinium* spp. are regarded as economically important and indigenous to Europe, namely *V. myrtillus* (bilberry), *V. oxycoccus* (cranberry), *V. uliginosum* (bog bilberry) and *V. vitis-idaea* (lingonberry). Several *Diaporthe* and *Phomopsis* spp. have been reported from *Vaccinium* spp., including *P. vaccinii,* which is globally regarded as an important species of *Phomopsis/Diaporthe* (Lombard et al., 2014).

P. vaccinii is under a strict control in Europe as well as in Latvia and included into the A2 (2010 transferred from A1 (1995) list of Quarantine Pests of European and Mediterranean Plant Protection Organization (EPPO) and into the list of Quarantine Pests of Latvia. *P. vaccinii* is also listed in Annex II AI of Directive 2000/29/EC (European Food Safety Authority, 2014).

In Europe, *P. vaccinii* has been eradicated in Germany, Romania, the United Kingdom and the Netherlands (European Food Safety Authority, 2014, *Diaporthe vaccinii,* 2009), but in Lithuania it

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was isolated also from wild species (Kačergius and Jovaišienė, 2010).

The teleomorph stage of this fungus was described as *Diaporthe vaccinii* Shear, but in recent years researchers doubt its existence; therefore, they use more *Phomopsis* and sometimes *Diaporthe* like a synonym (Udayanga et al., 2011, Gomes et al., 2013, Udayanga *et al*., 2014a, 2014b).

In the recent years over 1000 species names are described in the genus *Phomopsis,* but their biology and life style are still unknown or not studied enough. The identification of *P. vaccinii* is difficult and complicated if only classical phytopathological methods are used, because many species have similar morphological characteristics, but *P. vaccinii* also shows diversity of colony morphology (Udayanga et al., 2011, Farr et.al., 2002).

The aim of the study was to compare *P. vacccinii* isolates from different cranberry locations in Latvia and to describe their morphology in culture.

Materials and Methods

The *P. vaccinii* isolates were obtained from five cranberry plantations in different locations of Latvia (Babite, Alsunga, Rucava, Ape and Pargauja municipality) from berries with typical symptoms of viscid rot at harvest and storage in 2010. The samples were taken from the cranberry cultivar 'Stevens'.

The number of isolates from plantations depended on incidence of viscid rot and differences of colony. Most samples were taken from Babite municipality (22 isolates; 50%), Rucava – 12 (27%), Alsunga – 7 (16%), Ape – 2 (5%) and Pargauja – 1 (2%).

For examination of colony morphology 44 isolates of *P. vaccinii* were cultivated on potato dextrose agar (PDA) in two replicates. Cultures were incubated at 23 °C in the dark for one month.

Growth rate of colonies was recorded after 4, 6, 7, 8 and 9 days; color and structure of surface mycelium; reverse pigmentation (color); zonate development of mycelia and pycnidia as described bellow (Ulloa and Hanlin, 2002).

During this study the isolates were arranged in different groups with similar descriptions to find some relationships between samples (Table 1).

Conidia from 26 isolates were described. The length and width of 100 conidia per each isolate were measured (μm).

In addition, the taxonomical identity of isolates from representative groups (n=15) was confirmed

Table 1

Morphological criteria used to group the isolates

Assessment timing	Morphological characteristics	Variations
After 4, 6 days and 1 month	Color of surface mycelium	grayish white (light) grayish brown (dark)
After 1 month	Reverse pigmentation	dark brown centre, at the edge grayish white creamy white dark gray dark brown
After 4, 6 days and 1 month	Structure of surface mycelia	zonate colony (concentric circles) non zonate colony
First time when observed	Appearance of pycnidia	in 7 days (after inoculation) in 8 days more than 8 days, about 1 month from inoculation
Once, after 1 month	Location of pycnidia	in the centre on circles scattered
Once, after 1 month	Number of pycnidia	0-10 11-20 >21
Once, after 1 month	Size of pycnidia	0-0.9 mm 1-3 mm

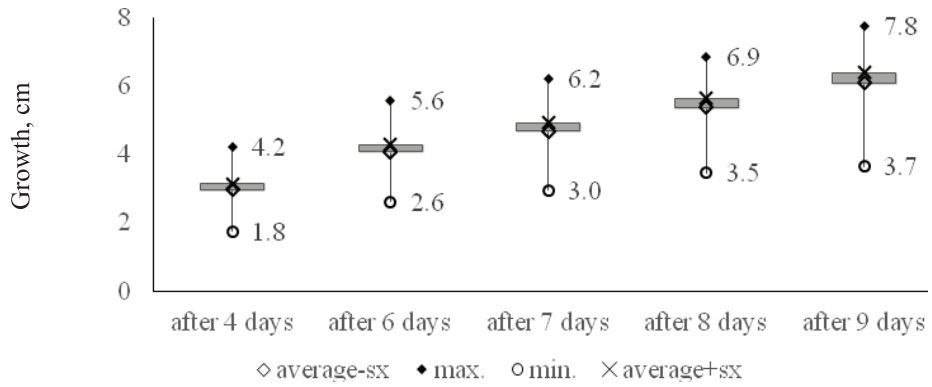

Figure 1. The dynamic of colonies growth (in diameter, cm) on PDA for different isolates of *P. vaccinii*.

by ITS1-5.8S-ITS2 region sequencing. Extraction of rDNA and PCR conditions performed as previously described (Farr et al., 2002, Kačergius and Jovaisiene, 2010). Purified PCR products were sequenced at Macrogen Europe (Amsterdam, Netherlands). Obtained sequences were compared to sequences of *P. vaccinii* and closely related taxa available in data base of NCBI GeneBank BLASTn. For more accurate identification additional phylogenetic test using sequences of CBS reference isolates were performed using Maximal parsimony method and bootstraping within the MEGA 5.1. software (Tamura *et al*., 2011). Preparing of samples for sequencing and data analysis was performed in the laboratory of Latvian Plant Protection Research centre. Sequences were deposited at NCBI GeneBank and are accessible by ID KP869876 – KP869890.

The results were statistically processed with Microsoft Excel for windows and the computer program SPSS 17.0 statistics package. The obtained data were analysed using Pearson correlation coefficient for every pair of traits in the SPSS 17.0 statistical package procedure ($p < 0.05$; $p<0.001$).

Results

Colony growth. After four days of cultivation different growth rate of *P. vaccinii* was observed. 16% of isolates, mainly from Rucava and Babite reached 1.8 - 2.5 cm in diameter. Slightly faster growth of colonies was observed in the main part of isolates (70%), the diameter reached 2.6 - 3.5 cm. Only for 14% of isolates the measured diameter was 3.7 - 4.4 cm.

Table 2

Characterizations of surface mycelium of *P. vaccinii* isolates from different cranberry growing regions

Region	**Mycelium color after one month**	Number of isolates	Percent	**Zonate (circle)**	Number of isolates	Percent
Rucava	grayish white	9	75	yes	9	75
	grayish brown	3	25	no	3	25
	Total	*12*	*100*	*Total*	*12*	*100*
Alsunga	grayish white	7	100	yes	7	100
Babite	grayish white	18	82	yes	21	95
	grayish brown	4	18	no	1	5
	Total	*22*	*100*	*Total*	*22*	*100*
Pargauja	grayish white	1	100	no	1	100
Ape	grayish white	2	100	yes	2	100

Table 3

Colony pigmentation of *P. vaccinii* in culture (reverse) divided in groups by geographical location

Growing region	Pigmentation, % of isolates				Number of analyzed isolates
	dark brown centre, grayish white periphery	creamy white	dark brown	dark grey	
Rucava	8.3	41.7	50	0	12
Alsunga	71.4	14.3	14.3	0	7
Babite	45.5	31.8	4.5	18.2	22
Pargauja	0	100	0	0	1
Ape	50	50	0	0	2
				Total	44

After six days the colony growth changed for some isolates and differences leveled off: 18% of isolates were slow growing (2.6 - 3.5 cm in diam.), 47% of isolates were medium in size (3.8 – 4.4 cm in diam.) and 34% of isolates showed faster growth (4.5 - 5.6 cm in diam.).

In the next days the colony diameter slightly increased, and at 8th day the growth of some isolates stopped at all. For 20% of isolates the colony diameter reached 3.5 - 4.5 cm; for another 20% – 4.9 - 5.5 cm and for 60% – 5.6 - 6.9 cm. In the last day the growth of most isolates stopped. In this study the growth of *P. vaccinii* significantly increased at first assessment, four days after inoculation (Figure 1). Although different growth of colonies was observed, no relationships between it and cranberry growing regions were found (r=0.183; p=0.233).

Mycelium color. At the first assessment (4 days after inoculation) all colonies produced white mycelia; it was not compact, flat, cottony and produced lobate and entire margin. After one month the surface mycelium of 84% of isolates became grayish white, only some isolates from Rucava and Babite produced grayish brown mycelia (Table 2), but no relevance between growing site and color of mycelia (r=-0.110; p=0.477).

One of the peculiarities of *P. vaccinii* is production of zonate colonies as a result of mycelium development in concentric circles. For some isolates first signs of thickened mycelia were observed at first assessment. The main part of isolates (89%) produced zonate colonies, including all isolates with light mycelia, except one from Pargauja (Table 2). The isolates with grayish white and grayish brown mycelium could also form zonate colonies, thereby no relevance was found between mycelium color and zoning (r=0.040; p=0.796).

After 9 days the colonies showed different pigmentation in reverse. The pigmentation varied by isolates. 41% of isolates from Rucava showed creamy white pigmentation. Mainly isolates with dark brown centre and grayish white periphery were observed from Alsunga (71.4%) and Babite (45.5%) (Table 3, Fig.3), but no relevance was found between growing site and reverse pigmentation (r=-0.192; p=0.211). In all inspected plantations, isolates with creamy white pigmentation were present. Positive correlation was detected between color of mycelia and pigmentation (r=0.719; p<0.001) - colonies with grayish white mycelia produced creamy white pigmentation.

The colonies produced slightly embedded, dark, spherical pycnidia, which started to form in 7 - 8 days (55% isolates) after plugs with mycelia were transferred on PDA. 45% of isolates showed pycnidia later than 8 days until the end of one month from inoculation (Figure 2). The correlation was weak negative between time and number of pycnidia (r=-0.308, p=0.042), so increasing days number of pycnidia will not increase (Table 3).

The number, size and placement of pycnidia on PDA were recorded after one month, and differences between isolates were found: 39% of isolates showed less than 10 pycnidia per plate, but the main part of isolates (62%) produced more than 10 pycnidia per plate. 42% of isolates from Rucava, 57% from Alsunga and 32% from Babite showed less pycnidia. A part (32%) of isolates formed more than 20 of pycnidia per plate; this ability was observed in 25% of isolates from Rucava, 14% from Alsunga and 41% from Babite, but no relevance was observed between

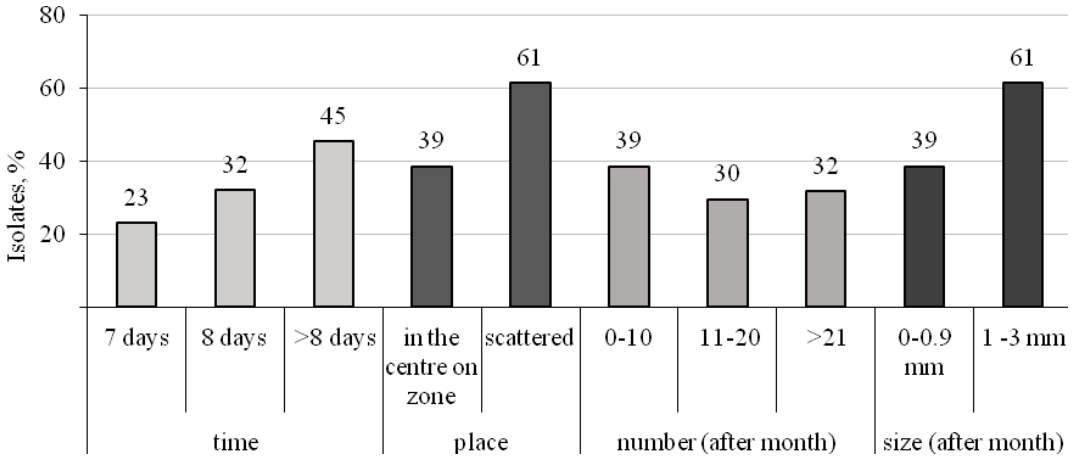

Figure 2. Characterization of pycnidial development of *P. vaccinii* isolates on PDA.

growing site and number of pycnidia (r=0.109; p=0.480).

In this study, 61% of isolates produced pycnidia not only arranged in circles, but sometimes also scattered (Figure 3; Table 4). The main part of isolates produced circles, but they did not always form pycnidia (r=0.137; p=0.375). 75% of isolates from Rucava and 68% form Babite, formed pycnidia scattered throughout the plate, but statistically no relevance was observed between growing site and place of formed pycnidia (r=0.131; p=0.396).

Size of produced pycnidia was mainly 1-3 mm (61%). The most isolates from Rucava (78%), Babite (57%) and Alsunga (71%) formed pycnidia with a size 1-3 mm arranged in circles (Table 4). 32% of all tested isolates formed pycnidia with a size less than 1 mm in circles, so no relevance was observed (r=-0.246; p=0.107).

Although statistically significant relationships between different morphological features of *P. vaccinii* isolates were not found (Table 4), some tendencies were observed (Table 5.). Colonies with creamy white pigmentation produced both types of circle (yes/no). Colonies, which produced circles, did not always form pycnidia and the number of pycnidia was not uniform at all plates.

Table 4

Relevance between morphological features of pycnidia produced in culture on PDA

		Time	Place	Number	Size
Time	Pearson Correlation	1	,068	**-,308***	,162
	Sig. (2-tailed)		,660	**,042**	,294
	N	44	44	44	44
Place	Pearson Correlation	,068	1	,270	-,246
	Sig. (2-tailed)	,660		,076	,107
	N	44	44	44	44
Number	Pearson Correlation	**-,308***	,270	1	-,065
	Sig. (2-tailed)	**,042**	,076		,677
	N	44	44	44	44
Size	Pearson Correlation	,162	-,246	-,065	1
	Sig. (2-tailed)	,294	,107	,677	
	N	44	44	44	44

* Pearson Correlation is significant at 0.05 confidence level

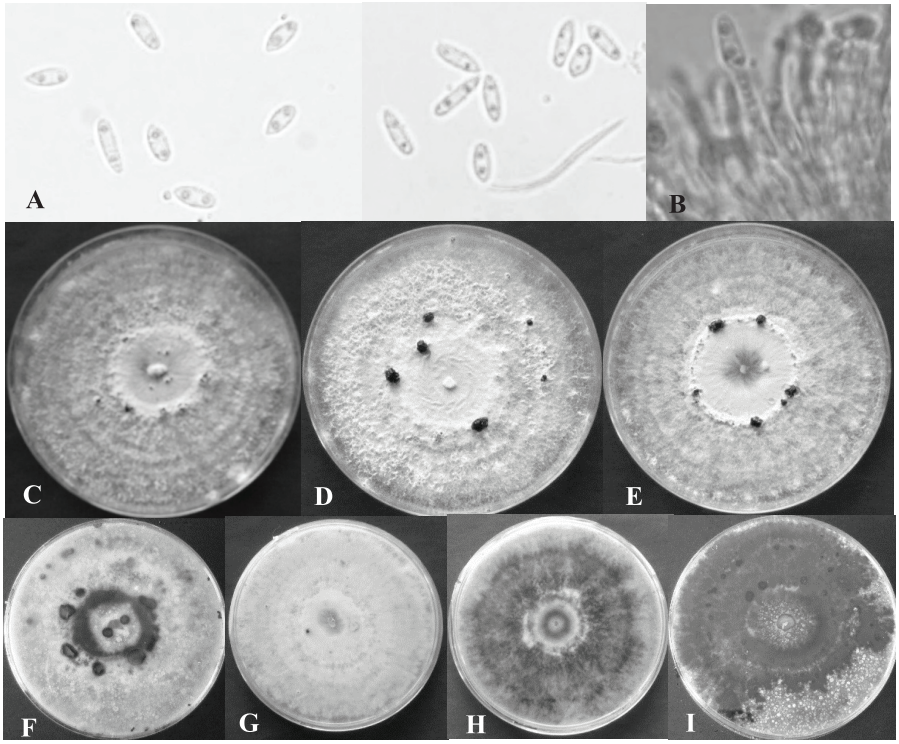

Figure 3. Morphological features of *P. vaccinii* isolates on PDA. A – α- and β-conidia in culture (400 x). B - conidiophores and α-conidia (400 x). C - grayish brown mycelia and pycnidia in circle. Grayish white mycelia, without zonate and scattered pycnidia (D) and grouped pycnidia in circle (E). Revers pigmentation: dark brown centre, at the edge grayish white (F), creamy white (G), dark brown (H), dark gray (I).

Table 5

Morphological characteristics of *P. vaccinii* isolates on PDA

Zonate	Colony pigmentation	Percent	Pycnidia	Percent	Number of pycnidia	Percent
Mycelium formed circles	dark centre and at the edge grayish white	43.6	in circles	41.0	0-10	38.5
	creamy white	30.8			11-20	28.2
	dark brown	15.4	scattered	59.0	>21	33.3
	dark grey	10.3				
	Total	*100.0*	*Total*	*100.0*	*Total*	*100.0*
		N=39		*N=39*		*N=39*
No circle	creamy white	60.0	scattered	100	*0-10*	*40.0*
	dark brown	40.0			11-20	40.0
					>21	20.0
	Total	*100.0*	*Total*	*100*	*Total*	*100.0*
		N=5		*N=5*		*N=5*

Table 6

The length and width of *P. vaccinii* α-conidia from different cranberry growing regions in Latvia (µm)

		Rucava	Babite	Alsunga	Ape	Pargauja
length	average	7.35	7.55	7.48	6.88	7.27
	min	5.16	5.19	5.72	5.02	5.14
	max	10.99	10.94	10.03	8.81	8.84
	standard deviation (SD)	0.86	0.89	0.85	0.67	0.69
	standard error (SX)	0.028	0.029	0.049	0.047	0.069
width	average	2.77	2.88	3.02	2.58	2.62
	min	1.49	1.75	1.70	1.89	1.79
	max	4.08	4.00	4,.40	3.55	3.43
	standard deviation (SD)	0.39	0.34	0.44	0.34	0.27
	standard error (SX)	0.013	0.011	0.025	0.034	0.019

Spore mass of conidia was creamy white, but the time when it appeared from pycnidia differed between isolates. *P. vaccinii* usually produced alpha (α) and beta (β) conidia, but in this study mainly alpha conidia were observed.

The length and width of conidia were measured only from 26 isolates, because some colonies produced pycnidia, but did not produce spore mass, and some isolates did not produce any pycnidia at all.

In all isolates *α*-conidia were hyaline, fusiform, ellipsoid, straight, aseptate, biguttulate. A few conidia of some isolates were relatively longer, with sharp apex, but most were with slightly rounded ends. The average size of α-conidia (n=2600) was recorded: 7.4 µm (5.0 - 10.9 µm) in length and 2.7 µm (1.4 - 4.4 µm) in width. The isolates from Ape produced significantly shorter (6.88 µm; SD=0.67; SX=0.047; $p<0.001$) *α*-conidia than conidia from other regions (Table 6), and the length was also significantly different ($p=0.001$).

Other typical type of conidia (β-conidia) was produced by only two isolates, one from Rucava and second from Ape (Figure 3, Table 7). The β-conidia were hyaline, filiform, straight or curved and aseptate. The length of β-conidia from Ape was significantly ($p<0.001$) higher 23.7 µm (±1.55 µm) than of those from Rucava 15.3 µm (±2.62 µm) and the width were significantly different ($p=0.043$) 1.6 µm (±0.19µm) and 1.5 µm (±0.11µm), respectively. Isolates were incubated at +21 °C in dark more than three months for conidia formed, but no more colonies produced β-conidia, probably the reason was air temperature, because optimal temperature for *P. vaccinii* is above 25 °C. Therefore, probably *P. vaccinii* cannot produce β-conidia in field conditions in Latvia. No information about relationships between α- and β-conidia of *P. vaccinii* on pathogenicity or aggressiveness was found in literature.

Table 7

Summary of morphological characteristics of *P. vaccinii* isolates in culture*

GenBank accession number	Isolate ID	Growing region in Latvia	Colony characteristics on PDA	Pycnidial formation in culture	Conidial size in culture (µm)	Mycelial growth (cm diam.)		
						after 4 days	after 7 days	after 9 days
KP869876	PV_2	Babite	Grayish brown mycelium; zonate; dark grey reverse	Appeared later more than 8 days, scattered, by number 0-10 with size 0-0.9 mm	not produced	3.0	4.2	4.8
KP869877	PV_11	Babite	Grayish white mycelium; non zonate; creamy white reverse	Appeared later more than 8 days, scattered, by number 11-20 with size 1-3 mm	α-conidia :7.59 (5.72 - 9.88) x 3.12 (2.21 - 4.00)	2.2	3.7	4.5
KP869878	PV_25	Babite	Grayish white mycelium; zonate; creamy white reverse	Appeared after 8 days, on zonate, by number >20 with size 0-0.9 mm	α-conidia: 7.71 (5.89-10.45) x 2.77 (2.13-3.43)	3.5	5.7	7.3
KP869879	PV_32	Ape	Grayish white mycelium; zonate; reverse: dark brown centre and grayish white periphery	Appeared after 7 days, scattered, by number 11-20 with size 0-0.9 mm	α-conidia: 7.06 (5.02-8.81) x 2.58 (1.79-3.34) β-conidia: 23.72 (21.54 – 26.03) x 1.63 (1.26 – 1.80)	2.9	5.4	7.1
KP869880	PV_36	Rucava	Grayish white mycelium; non zonate; dark brown reverse	Appeared after 8 days, scattered, by number 11-20 with size 0-0.9 mm	α-conidia: 7.23 (5.71-8.95) x 2.28 (1.49-2.83)	1.8	3.3	4.5
KP869881	PV_38	Babite	Grayish white mycelium; zonate; reverse: dark brown centre and grayish white periphery	Appeared after 8 days, on zonate, by number >20 with size 1-3 mm	α-conidia: 7.01 (5.19-9.19) x 2.51 (1.75-3.34)	3.8	5.7	7.3
KP869882	PV_17	Alsunga	Grayish white mycelium; zonate; reverse: dark brown centre and grayish white periphery	Appeared after 7 days, on zonate, by number >20 with size 0-0.9 mm	α-conidia: 7.17 (5.86-9.25) x 2.68 (1.70-3.66)	2.9	4.9	6.5

GenBank accession number	Isolate ID	Growing region in Latvia	Colony characteristics on PDA	Pycnidial formation in culture	Conidial size in culture (µm)	Mycelial growth (cm diam.)		
						after 4 days	after 7 days	after 9 days
KP869883	PV_10	Rucava	Grayish white mycelium; non zonate; creamy white reverse	Appeared after 7 days, on zonate, by number 0-10 with size 1-3 mm	α-conidia: 6.94 (5.16-9.22) x 3.23 (2.69-4.06)	3.4	5.6	7.5
KP869884	PV_20	Rucava	Grayish brown mycelium; zonate; dark brown reverse	Appeared after 8 days, scattered, by number 0-10 with size 0-0.9 mm	not produced	3.0	4.6	5.5
KP869885	PV_13	Rucava	Grayish white mycelium; zonate; creamy white reverse	Appeared later more than 8 days, scattered, by number 0-10 with size 1-3 mm	α-conidia: 6.56 (5.41-8.16) x 3.02 (2.34 -4.08)	3.8	5.8	7.3
KP869886	PV_5	Babite	Grayish white mycelium; zonate; reverse: dark brown centre and grayish white periphery	Appeared later more than 8 days, scattered, by number >21 with size 0-0.9 mm	α-conidia: 7.3 (5.49 – 9.25) x 2.75 (1.94-3.55)	3.4	5.1	6.5
KP869887	PV_19	Rucava	Grayish white mycelium; zonate; creamy white reverse	Appeared after 8 days, scattered, by number 11-20 with size 1-3 mm	α-conidia: 6.68 (5.43-8.72) x 2.82 (2.11-3.93) β-conidia: 15.27 (12.25 – 19.73) x 1.47 (1.34 – 1.68)	2.3	3.9	5.1
KP869888	PV_37	Alsunga	Grayish white mycelium; zonate; reverse: dark brown centre and grayish white periphery	Appeared later more than 8 days, on zonate, by number 0-10 with size 1-3 mm	α-conidia: 7.09 (5.72-9.03) x 2.75 (2.21-3.39)	4.2	6.2	7.8
KP869889	PV_35a	Rucava	Grayish white mycelium; zonate; creamy white reverse	Appeared after 8 days, scattered, by number >20 with size 0-0.9 mm	α-conidia: 7.23 (6.04-9.79) x 2.91 (2.22-3.73)	3.0	4.7	6.4
KP869890	PV_ AL1	Ape	No data	No data	α-conidia: 6.70 (5.54-8.52) x 2.67 (1.98-3.43)	No data		

*isolates were incubated at 23 °C in the dark.

Discussion

P. vaccinii is associated with several diseases of plants from genus *Vaccinium*, but relationship between a host plant and pathogen can also be different like endophyte and nectrotroph life styles (Kačergius et al., 2004, Udayanga et al., 2011). Identification of *P. vaccinii* is often difficult and time-consuming using only classical disease identification methods, and with introducing molecular methods and data bases, progress in speed and accuracy of identification of *P. vaccinii* and other *Phomopsis* species has been achieved (Farr et al., 2002, Kačergius et al., 2004, Udayanga et al., 2011, Udayanga et al., 2014a, 2014b). Nowadays even conformation of taxonomical identity by molecular approach is needed. It is not always possible to analyze a large number of samples, and accurate selection of isolates by morphological features is still essential.

P. vaccinii colonies grew well on PDA, but previously reported growth rate differs between researchers: after 3 days they showed 3.5 cm (*Diaporthe vaccinii*, 2009), after 7 days colonies reached 6.2 cm (Kačergius et.al., 2004) on average, but after 8 days 2.7-4.8 mm in diameter, if grown at 25°C (Farr et.al., 2002), and these studies confirm data about the growth rate obtained in this study – colonies of *P. vaccinii* isolates could develop with different growth rate.

The main part of *P. vaccinii* isolates from this study in culture produced grayish white mycelia, like usually described in literature (Caruso and Ramsdel, 1995, Farr et.al.2002, Kačergius et al., 2004), but isolates of *P. vaccinii* with grayish (from cranberry) and yellow to brownish orange (from blueberry) mycelium were also reported (Farr et al., 2002). The other *Phomopsis* species can also produce similar colors and structures of mycelium (Gomes et al., 2013; Udayanga et al., 2014a, 2014b).

Although *P. vaccinii* has been described in literature as usually producing circle or zonate of mycelia and always forming pycnidia there (Caruso and Ramsdell, 1995, Kačergius et al, 2004), in this study the most part of *P. vaccinii* isolates formed pycnidia scattered, with a few pycnidia on circle. Some researchers (Farr et al., 2002) observed this feature, but others noticed that colonies on MEA usually produced scattered pycnidia (Kačergius et al., 2004). Three isolates from different geographic locations did not produce pycnidia (Farr et al., 2002).

P. vaccinii after 7-10 days formed 1-3 mm large pycnidia, partly embedded in substrate, maturing after 20-28 days (Caruso and Ramsdell, 1995; Kačergius et al., 2004, *Diaporthe vaccinii*, 2009).

Conidial size of *P. vaccinii* slightly differs as described by researchers in literature. Alpha conidia of *P. vaccinii* were 6-11 µm long and 2.5 µm wide as measured in 1931 (Shear et al., 1931) and also in 1995 (Caruso and Ramsdell, 1995) in the USA, but in recent years 5.9 – 11.3 µm long (SD=0.26, n=500) and 2.1-3.9 µm wide (SD=0.8) were recorded in the USA (Farr et al., 2002) and 7 – 8 x 2.5 µm in Lithuania (Kačergius et.al., 2004); hyaline, fusiform, straight, guttulate, aseptate, forming creamy white to yellowish spore mass (Farr et al., 2002, Kačergius et al., 2004). Beta conidia was recorded 12-18 x 0.75 µm (Caruso and Ramsdell, 1995) and 18-25 x 1.5 µm (Kačergius et al., 2004) hyaline, filiform, straight or curved, egguttulate, aseptate, but not always performed (Caruso and Ramsdell, 1995, Farr et al., 2002, Kačergius et al., 2004). Many of other *Phomopsis* species produce similar features of conidia like *P. vaccinii* (Gomes et al., 2013, Udayanga et al., 2004a, 2014b), it means characteristics of conidia do not permit precise identification of the species.

During this study many features of *P. vaccinii* isolates from viscid rot of cranberry in culture on PDA were described, but almost all isolates were slightly different (Table 7), and we were not able to provide one average uniform description, so this is the reason why it can be easily confused with other *Phomopsis* species if classical phytopathological methods only are used. 1000 species of *Phomopsis* have been described and many of them have morphological plasticy (Santos and Phillips, 2009, Udayanga et al., 2004a, 2014b).

The result of this study showed no geographic similarities between the isolates of *P. vaccinii,* as it was concluded by other authors, too (Farr et al., 2002).

Conclusions

Colonies of *P. vaccinii* from cranberry plantations in Latvia showed different morphological features in culture, which made the identification of *P. vaccinii* difficult and complicated.

P. vaccinii mostly produced grayish white mycelium with creamy white pigmentation in reverse and zonate in the centre, formed large size (1-3 mm) pycnidia after 7-8 days, but not always on zonate.

No relationship between *P. vaccinii* morphological features and cranberry growing regions was found.

Morphological diversity of *P. vaccinii* probably indicated to potential variability of pathogen.

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