ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 24(5) 2025, 71–87
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2025.5525
RESEARCH PAPER
Received: 12.04.2025
Accepted: 14.09.2025
First published online: 23.10.2025
Issue published: 31.10.2025

BIOLOGY AND EPIDEMIOLOGY OF *Valdensinia heterodoxa* Peyronel IN POLAND

Elżbieta Paduch-Cichal[1], Wojciech Kukuła[1], Tadeusz Malewski[2], Anna Rucińska[3,4], Elżbieta Mielniczuk[5], Marcin Wit[1], Wojciech Wakuliński[1], Ewa Mirzwa-Mróz[1]✉

[1] Department of Plant Protection, Institute of Horticultural Sciences, Warsaw University of Life Sciences – SGGW, 159 Nowoursynowska, 02-776 Warsaw, Poland

[2] Department of Molecular and Biometric Techniques, Museum and Institute of Zoology, Polish Academy of Sciences, 64 Wilcza, 00-679 Warsaw, Poland

[3] Polish Academy of Sciences Botanical Garden-Center for Biological Diversity Conservation in Powsin, 2 Prawdziwka, 02-973 Warsaw, Poland

[4] Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, 05-870 Błonie, Poland

[5] University of Life Sciences in Lublin, Department of Plant Protection, 7 Leszczyńskiego, 20-069 Lublin, Poland

ABSTRACT

In Poland, the first description of the causal agent of the valdensia leaf blight fungus *Valdensinia heterodoxa* was on highbush blueberry in 2011. Our study identified this pathogen in highbush blueberry plantations and on bilberry plants to describe its biology, molecular characteristics, and disease epidemiology. The 115 fungus isolates yielded were divided into 2 groups based on the colony morphology differences on PDA (potato dextrose agar) medium. For the isolates from each group, morphology and growth dynamics were studied on PDA and WOA (weak oatmeal agar) with added highbush blueberry/bilberry/lily of the valley leaf decoction under different light and temperature conditions. The isolates' colony morphology differences were noted depending on the medium type. The best isolate growth was at 20 °C/19 °C-day, 12 °C-night, with a 12-hour photoperiod. Isolate identification using PCR showed nearly 100% nucleotide sequence similarity to reference isolates. On infected leaves, the release height of conidia was 18 cm. The presented results are novel in Poland and have a broader-than-domestic significance.

Keywords: *Vaccinium corymbosum* L., valdensia leaf blight, fungus morphology, mycelium growth rate, PCR detection and identification

INTRODUCTION

For many years, the highbush blueberry (*Vaccinium corymbosum* L.) was considered to be resistant to pathogen diseases. With the increasing number of plantations and the larger area of cultivation of this plant in Poland, the occurrence of new pathogens infecting the species has been reported more frequently. The results of research conducted by Paduch-Cichal et al. [2011] and Cieślińska [2020] revealed the presence of the following viruses in highbush blueberry plants: Blueberry shoestring virus (BSSV), Peach rosette mosaic virus (PRMV), Blueberry scorch virus (BlScV), Tobacco ringspot virus (TRSV), Blueberry

✉ ewa_mirzwa-mroz@sggw.edu.pl

red ringspot virus (BRRSV), and Blueberry mosaic associated virus (BlMaV). Various bacterial species from the genus *Pseudomonas* [Kałużna et al. 2013] and *Xanthomonas arboricola* [Kałużna and Pothier 2022] were detected in 2012–2013. Nevertheless, mycoses are the most numerous group of highbush blueberry diseases. The most frequent fungal pathogens of the plant include *Botrytis cinerea* Pers., which is a causal agent of gray mold, *Colletotrichum acutatum* J.H. Simmonds and *C. fioriniae* (Marcelino & Gouli) Pennycook, causing anthracnose in highbush blueberry, and *Godronia cassandrae* Peck., i.e. a cause of shoot blight in this plant species [Zalewska et al. 2007, Szmagara 2009, Meszka and Bielenin 2012, Mirzwa-Mróz et al. 2023].

In 2011, a new species of the fungus *Valdensinia heterodoxa* Peyronel, a causal agent of valdensia leaf blight, was detected in highbush blueberry cv. Bluecrop plants in one of the nurseries in Poland [Dzięcioł et al. 2014]. In the subsequent years, Kukuła et al. [2017] investigated the biology of this pathogen and carried out molecular characterization of 40 *V. heterodoxa* isolates originating from highbush blueberry shrubs growing in commercial plantations or nurseries (cv. Bluegold, cv. Bluecrop) in Mazowieckie Voivodeship and from bilberry plants growing in forests in Pomorskie and Lubelskie Voivodeships.

The available literature from Poland does not provide detailed information on the occurrence and harmful effects of valdensia leaf blight. In other European countries the main recommendation is prophylaxis based on maintenance work and fruit harvesting at a different time than at maximum plant wetting. In the case of the presence of the first symptoms of the disease in commercial cultivation, quick removal and burning infected plants are recommended [Annis and Yarborough 2009, Hildebrand et al. 2016].

The aim of the study was to assess the occurrence of *V. heterodoxa* in highbush blueberry plantations and bilberry plants growing in forests that were not adjacent to the highbush blueberry plantations. Additionally, the study was focused on examination of the dynamics of growth of *V. heterodoxa* isolates on various media, determination of the conditions for the production of conidial spores (conidia) by the fungus and the height at which conidia are released.

MATERIALS AND METHODS

Inspection of plantations

The 5-year study was conducted in 18 commercial highbush blueberry plantations located in Mazowieckie (8 plantations), Łódzkie (5), Podlaskie (3), and Lubelskie (2) Voivodeships. Additionally, the other examined species included trees (*Acer macrophyllum* Pursh., *Betula pubescens* Ehrh., *Corylus avellana* L., *Fagus sylvatica* L., *Quercus robur* L., *Q. petraea* Liebl., *Sorbus aucuparia* L.), shrubs (wild raspberries, blackberries), and perennials (*Convallaria majalis* L., *Oxalis acetosella* L., *Polygonatum multiflorum* (L.) All.) growing in the vicinity or at a certain distance from some plantations. The study was also conducted in mixed forests located at some distance from the highbush blueberry plantations in Pomorskie (3 sites), Lubelskie (2) and Małopolskie (3) Voivodeships.

The plantations were inspected using the route method from June to the end of August. The research material consisting of leaves collected from different plant species with visible symptoms of *V. heterodoxa* infection was placed in a parchment envelope and stored at 4 °C for further analyses. Then, they were examined under a dissecting microscope (SZ11, Olympus, Tokyo, Japan) to find conidia of the pathogen. The conidia were viewed under a BX50 light microscope (Olympus, Tokyo, Japan) and photographed using a DP71 camera (Olympus, Tokyo, Japan).

In total, 100 conidia of *V. heterodoxa* collected from each plant species were measured. The total length (CL) and total width (HW) of each fully developed conidium were measured as in Zhao and Shamoun [2010]. The size of the spores was measured using the CellF program (Olympus, Tokyo, Japan), compatible with the camera and the microscope specified above. ANOVA analysis of variance was carried out, and homogeneous groups were determined with the Tukey test. The analysis was performed in Statistica 13.0.

The pathogen species was identified with the use of available mycological keys [Marcinkowska 2012, Farr and Rossman 2013] and based on the results of studies conducted by Peyronel [1923], Redhead and Perrin [1972a, 1972b], and Nekoduka et al. [2012].

Selected conidial structures were observed under a scanning electron microscope (FEI Quanta 200 ESEM

with an EDS EDAX analyzer). Sections with a size of approx. 3 × 3–4 mm were cut from conidia-bearing leaves and placed on a table covered with activated carbon tape. The preparation was then silver-coated in a vacuum sputter coater (JEOL JFC-1300).

Acquisition of *V. heterodoxa* isolates

Leaves with disease symptoms collected during the inspection were processed with two methods to obtain *V. heterodoxa* isolates.

Method I – sections (approx. 5 × 5 mm) were cut from the border between healthy and diseased tissue in the disease spots and disinfected in 96% ethyl alcohol and 10% sodium hypochlorite. Afterwards, they were transferred to Petri dishes (Ø 100 mm) with PDA medium (potato dextrose agar) [Gams et al. 1987], and incubated at 20 °C in daylight. Single-spore cultures were obtained (from the tips of single hyphae in the case of non-sporulating cultures) and transplanted into tubes onto PDA medium slants. The fungal collection was stored at 4 °C.

Method II – single-spore cultures were obtained from conidia that were actively released from the infected leaves [Redhead and Perrin 1972a, 1972b].

Testing Koch's postulates. Approximately 20 cm long leafy shoots were collected from healthy plants of highbush blueberry cv. Bluecrop and bilberry. Young leaves of these shoots (the second and third fully developed leaves; in total 6 leaves per isolate) were inoculated. Isolates differing in the colony morphology on the PDA medium slants were selected for the analyses. In the case of isolates with similar morphology, 10 isolates were selected randomly. After inoculation the plants were tightly closed in transparent polyethylene bags, and incubated at 20 °C. The pathogen was re-isolated from the forming spots, and the re-isolates were compared with the isolates used in the study.

Identification of the causal agent of valdensia leaf blight using the PCR technique

The material consisted of 14-day cultures of 115 isolates of the fungus *V. heterodoxa* and 2 reference isolates: HBI0401 from leaves of highbush blueberry cv. Jersey, Japan (MAFF No. 645023, NIAS – National Institute of Agrobiological Sciences, Genebank Japan) and DAOMC 186993 from leaves of *Gaultheria shallon* Pursh, Canada (Canadian Collection of Fungal Cultures, Biodiversity and Collections).

DNA isolation. Genomic DNA was isolated from 115 isolates obtained from the fungus *V. heterodoxa* parasitizing highbush blueberries and blackberries with the use of the C-tab procedure (cetyltrimethylammonium bromide) described by Doyle and Doyle [1987].

PCR technique. The pathogen species was identified with the use of the PCR technique. Amplification of non-coding rDNA fragments (ITS1 and ITS2) was carried out using primers ITS1F [Gardes and Bruns 1993] and ITS4A [Larena et al. 1999]. The reactions were carried out as described by Nekoduka et al. [2012] with some modifications. The primer annealing temperature was 59 °C instead of 57 °C, and 28 cycles were run instead of 30. The PCR reaction was carried out using a Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, USA).

Amplified fragments were separated electrophoretically in 1.2% agarose/TBE gels in the presence of ethidium bromide. The amplified fungal DNA fragments were sequenced in the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw. The sequences were analyzed and aligned to the sequences of this fungus available in GenBank using the BLASTn algorithm [https://blast.ncbi.nlm.nih.gov/Blast.cgi] and MEGA12 software.

Characterization of *V. heterodoxa* isolates with classical methods

Effect of temperature and light on the growth of selected *V. heterodoxa* colonies. Based on the morphology of the cultures grown on PDA medium, 115 fungal isolates were preliminarily divided into groups. Further analyses were performed using isolates VhVal3A, VhVal4, and VhBg/1z/16 from highbush blueberry and isolates VhDM1, VhKir1, VhK26/2a from bilberry, which differed in the morphology and origin (northern, central, and southern Poland). Reference isolates HBI0401 and DAOMC 18699 were also included in the study.

The inoculum consisted of 5 mm diameter disks of PDA medium overgrown by the mycelium of the analyzed isolates and cut out with a cork borer from actively growing 14-day cultures of each isolate. The

disks were transferred to the center of sterile PDA poured into 100 mm diameter Petri dishes (10 dishes per each isolate). The dishes were incubated in a growth chamber (Versatile Environmental Test Chamber, Sanyo, Japan) at temperatures of 10, 15, 20, and 25 °C in two variants: I) 12h day/12h night, II) no access to light [Nekoduka et al. 2012].

The diameters of the colonies of fungal isolates were measured 28 days after the inoculation. The results were used to calculate the surface area of the colony using the area of ellipse formula:

$$S = \Pi/4 \cdot d \cdot s$$

where: d – colony length, s – colony width.

The experiment was conducted in duplicate. One-way analysis of variance (ANOVA) was performed. The Tukey test (p = 0.05) was used to compare the means. Statistical calculations were made using the Statistica 13.0 program.

Morphology and growth dynamics of selected *V. heterodoxa* isolates cultured on different media and in different incubation conditions

The mentioned above isolates from the highbush blueberry, bilberry, and reference isolates were selected for the morphology studies. The growth dynamic of the fungal colonies was analyzed only in the case of isolates VhVal3A and VhVal4 representing different groups. The morphology and growth dynamics of selected *V. heterodoxa* isolates were analyzed using PDA and WOA (weak oatmeal agar) media supplemented with decoctions of powdered or fresh leaves from selected host plants (highbush blueberry, bilberry, and lily of the valley) placed in Petri dishes (Ø 100 mm).

The media were inoculated using disks overgrown by the mycelial hyphae of the individual isolates as described above. After the inoculation, the Petri dishes were incubated in a growth chamber (Versatile Environmental Test Chamber, Sanyo, Japan) in the following conditions:

– temperature of 19 °C, no light access [Zhao and Shamoun 2006]

– room temperature (20 °C), daylight [Nekoduka et al. 2012]

– temperature of 19/12 °C (day/night), 12-h photoperiod [Vogelgsang and Shamoun 2002].

The morphological features of the pathogen cultures were studied twice on five Petri dishes (Ø 100 mm) per each isolate using the procedure described by Zhao and Shamoun [2006]. The morphology of the fungus colonies was observed four times at 7-day intervals.

In the experiment on the growth dynamics of the *V. heterodoxa* isolates, 10 Petri dishes with each medium were used for each isolate. After 5, 10, and 15 days, measurements of colony diameter were made and the surface area calculated. The rate of the increase in the surface area of the fungal colonies was determined for each of the medium variants with the use of the regression equation:

$$y = a + bx$$

where: y – surface area of mycelium; a – intercept; b – coefficient of the slope of the regression line; x – day of experiment [Krysicki et al. 1994].

Pearson correlation coefficients (r) were calculated. One-way analysis of variance (ANOVA) of the calculated regression coefficients was performed. Homogeneous groups were determined using the Tukey test at the level of p = 0.05. All the statistical calculations were performed using the Statistica 13.0 program.

Formation of *V. heterodoxa* conidia in *in vitro* conditions. The study was conducted using the same set of isolates as mentioned above. The WOA and OA (oatmeal agar) media were inoculated with mycelium-overgrown disks as described above. The experiment was conducted in three variants:

Variant 1. Inoculation of WOA medium on Petri dishes, incubation in a growth chamber, temp. 16 °C, photoperiod 12 h day/12 h night [Nekoduka et al. 2012]

Variant 2. Inoculation of WOA and OA media, incubation in a growth chamber at 17–19/12 °C (day/night), 12 h photoperiod [Vogelgsang and Shamoun 2002, Zhao and Shamoun 2010]

Variant 3. Inoculation of OA medium on Petri dishes, incubation in daylight, temp. approx. 20 °C – modification of conditions developed by the authors of the present study.

Cultures of *V. heterodoxa* isolates growing on the media were observed to detect spore formation on days 7, 14, 21, and 28 after inoculation. The experiments with each of the variants were carried out twice. Three Petri dishes with each medium were allocated for each isolate. The number of conidia of the pathogen formed per day was assessed. The cultures were observed under a SZX16 stereoscopic microscope (Olympus, Tokyo, Japan). The conidia were viewed under a BX50 light microscope (Olympus Tokyo, Japan) and photographed (DP71 camera). The Cell F computer program was used to measure their length and width.

Upon determination of the optimal conditions for the selected isolates to produce spores, the spore formation ability of the other 115 *V. heterodoxa* isolates was analyzed.

Release of *V. heterodoxa* conidia at different heights

The study of the height to which *V. heterodoxa* conidia were released was carried out twice using 2000 ml cylinders. Fresh highbush blueberry and bilberry leaves with visible disease symptoms were rinsed under running water for 2 hours. Next, three leaves of each species were placed on Petri dishes (Ø 50 mm) covered with sterile moistened filter paper. Each dish was covered with a lid with an aluminum cap glued on its upper side. This helped to connect the Petri dish to a threaded rod. The dish with the rod was gently placed on the bottom of a measuring cylinder with the leaves on its underside, and the lid was moved away from the leaf surface at a distance of 5, 10, 15, 20, and 25 cm using the threaded rod as in Redhead and Perrin [1972a, 1972b]. The observations of the Petri dishes placed at the different heights were initiated when conidia produced in the control conditions were visible on the inner surface of the dish lid. A stereoscopic microscope SZ11 (Olympus Tokyo, Japan) was used for the observations.

RESULTS

Occurrence of the pathogen

During our five-year inspection of 18 highbush blueberry plantations and 8 natural stands, the symptoms of valdensia leaf blight were observed several times. In Mazowieckie Voivodeship, the pathogen was noted on highbush blueberry cv. Bluegold and two unknown cultivars (Prażmów plantation). No valdensia leaf blight symptoms caused by *V. heterodoxa* were found in highbush blueberries in the other 17 plantations. In the natural stands, the pathogen was commonly observed each year on wild-growing bilberry shrubs in forests in Kopalino, Karwia, Ostrowo (Pomorskie Voivodeship), Włodawa, Okuninka (Lubelskie Voivodeship), and Zakopane, Witów, Kiry (Małopolskie Voivodeship) and on lingonberry plants growing in the forest in Witów (Małopolskie Voivodeship). The same symptoms were present on blueberry and lingonberry. The disease symptoms were round or oval, brown or almost black circular zonated necrosis outlined by dark-brown borders. On the lower side of the leaves, large star-shaped conidia were observed in the central part of each spot. Additionally, in the material from the forest in Karwia, characteristic disease spots with conidium were detected on the shoots of a young bilberry plant (Fig. 1).

Identification of the pathogen with traditional methods

During our research, 115 *V. heterodoxa* isolates were obtained, including 20 isolates originating from the leaves of highbush blueberry cv. Bluegold, 15 isolates from unknown highbush blueberry cultivars, and 80 isolates from bilberry shrubs. Koch's postulates for the isolates were positive.

Conidia (macroconidia) of *V. heterodoxa* prepared from the central part of the spots had the shape of a four-pointed and, less often, three-pointed star. This type of conidia is called staurospores. They were large and visible with the naked eye in the central part of the spot on the highbush blueberry and bilberry leaves. The conidia measured in the lingonberry material had smallest length and differed significantly from the length of conidia isolated from the other highbush blueberry shrubs. The conidia isolated from the lingonberry leaves had the smallest width and differed significantly from those measured in the bilberry and highbush blueberry samples (Tab. 1).

The conidia of the fungus present in the central part of the spots on the highbush blueberry, bilberry, and lingonberry leaves had "arms" mostly raised upwards. In turn, the "arms" of conidia located in other areas of the spots were spread out (the conidia resembled

Fig. 1. Bilberry shoot with a visible spot with conidium of *V. heterodoxa* (arrow)

Table 1. Comparison of the conidia size of the *V. heterodoxa* spores isolated from different plant species

Plant species	Length of the arms (µm)		Width of the head (µm)	
	min.–max.	average	min.–max.	average
Lingonberry	205.25–332.01	291.21a*	91.5–152.36	118.38a
Bilberry	187.48–391.25	300.71ab	86.33–172.67	129.59b
Highbush blueberry cv. Bluegold	281.28–365.73	310.75b	105.04–184.00	135.91bc
Highbush blueberry cv. unknown 1	216.85–392.31	313.24b	101.99–198.80	138.21c
Highbush blueberry cv. unknown 2	227.46–384.63	312.30b	105.90–188.45	139.35c

* Letters indicate significant (P = 0.05) differences among means within each column, determined using Tukey test

a star). The macroconidia were formed on short conidiophores and represented the so-called "sessile spores". In the central part of each spore, 60 spherical cells were visible. The cells in the further part were elongated and extended, forming four arms. Younger conidia had strongly hook-shaped arms, while the arms of older conidia were spread more extensively (Fig. 2).

PCR-based identification and characterization of the causal agent of valdensia leaf blight in highbush blueberry

The sequencing of ITS region amplicons yielded 101 sequences of the analyzed isolates with a length of 559 nucleotides (nt) and 14 sequences with a length of 558 nt. The consensus sequences were deposited in the GenBank under numbers PV147766 (559 nt sequence,

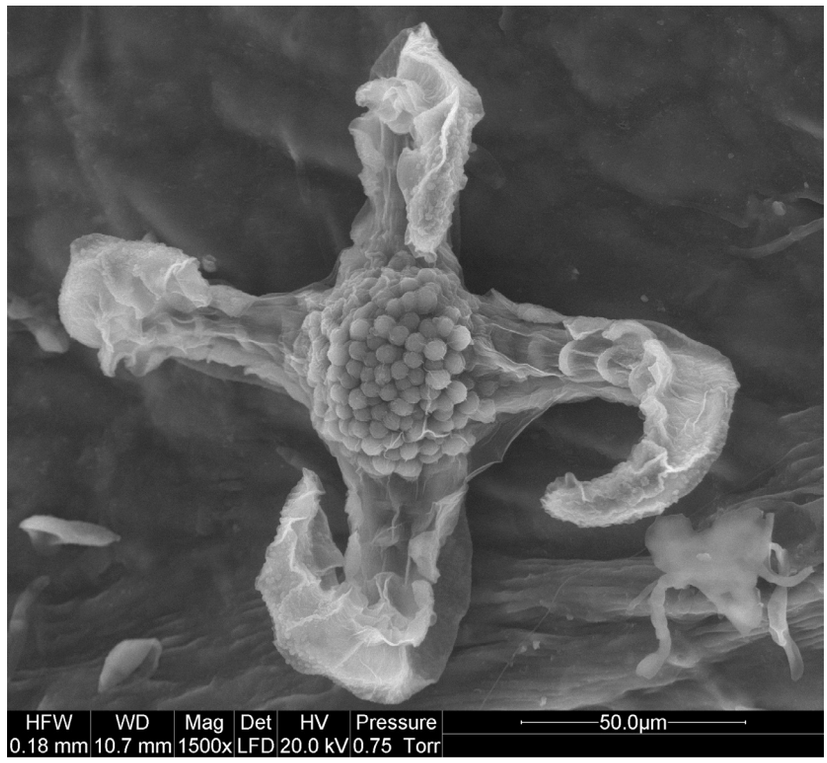

Fig. 2. A macroconidium of *V. heterodoxa* visible under a scanning electron microscope

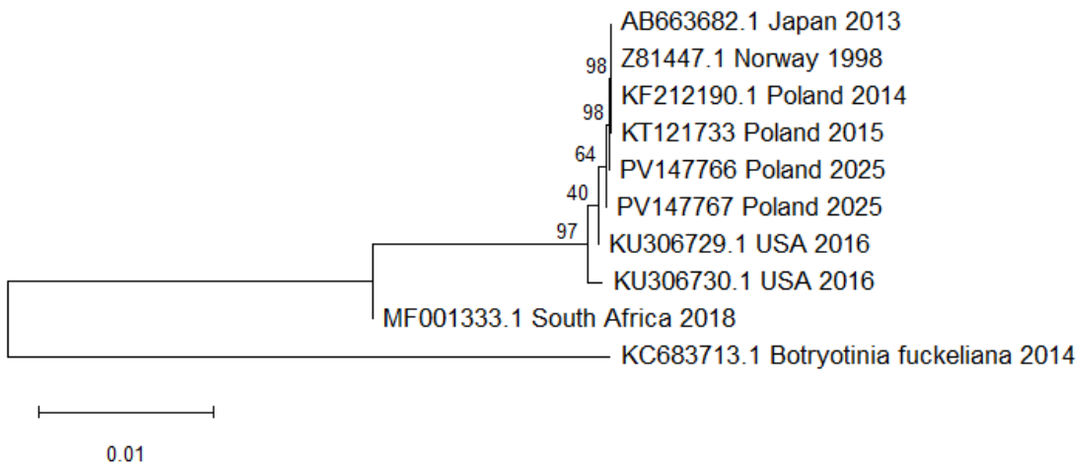

Fig. 3. Comparison of the ITS (ITS1, 5.8 SRNA and ITS2 complete sequence) sequences of the *V. heterodoxa* fungus with the fungal ITS sequences included in the GenBank (Neighbor-Joining method, bootstrap = 1000); the evolutionary distances were computed using the Maximum Composite Likelihood method; low bootstrap support (40%). Tree rooted with the sequence of the *Botryotinia fuckeliana* (KC683713.1) fungus

isolate VhVal3A) and PV147767 (558 nt sequence, isolate VhOstr.2). The differences in the sequences of the isolates from Poland resulted from only a single deletion in the sequences.

These sequences were aligned with very high similarity (99–100%) to the nucleotide sequences of the *V. heterodoxa* species deposited in the GenBank under numbers Z81447.1, AB663682.1, KF212190.1, KT121733 and KU306730.1. The comparison of the ITS sequences of the fungus *V. heterodoxa* obtained in the present study with the sequences of fungi available in the GenBank is presented in the dendrogram (Fig. 3).

Characteristics of *V. heterodoxa* isolates obtained with classical methods

Effect of temperature and light on the growth of selected *V. heterodoxa* cultures. The cultures of tested isolates exhibited the highest growth rate at 20 °C with the 12-hour photoperiod. The statistical analysis showed a significant effect of this temperature on the growth of most of the isolates tested (Tab. 2).

Morphology and growth dynamics of selected *V. heterodoxa* isolates on different media and in different incubation conditions. Given the differences in the morphology of the colonies of the isolates on PDA, the isolates were divided into two groups. The first group comprised 55 isolates whose mycelium surface was corrugated, and some part of the leathery and velvety colony was raised above the medium surface. The color of the upper part of the colony was uniformly dark brown, light cream or speckled. The reverse side of the colony was dark brown or black. The second group comprised 60 isolates producing light cream mycelium with a pink and grayish shade. In the central part, the velvety and leathery mycelium of these isolates was strongly corrugated. The reverse side of the isolates from this group was cream colored. The reference isolates also differed in the appearance of their mycelium. The morphology of the culture of the HBI0401 reference isolate (Japan) was typical of group I isolates, while the morphology of the DAOMC186993 reference isolate (Canada) was characteristic of group II.

The selected fungal isolates, i.e. VhVal3A and VhVal4, as well as VhKir1 and VhK26/2a, grew most efficiently on the PDA medium enriched with highbush blueberry leaves (PDA-Bor) at daylight and 20 °C ambient temperature. The mycelium of both isolates was very well developed, covering practically the entire available surface of the medium in the Petri dishes. The morphology of the fungal colonies differed on the individual plates, depending on the supplementation of the medium (Fig. 4).

Table 2. Average sizes (mm^2) of the cultures incubated under different temperature conditions on the PDA medium

Isolate	Incubation temperature			
	10 °C	15 °C	20 °C	25 °C
VhVal3A	611.20aA*	2984.57bA	6158.17dA	5735.69cB
VhVal4	648.88aA	2369.68bA	6973.47dB	6258.02cC
VhBg/1z/16	695.20aA	3620.34bB	6935.16dB	6258.81cC
VhDM1	796.46aAB	3184.67bA	6125.98cA	5779.64cB
VhKir1	923.16aB	2826.55bA	6954.39dB	5042.13cAB
VhK26/2a	987.84aB	2948.77bA	6373.73dAB	4176.44cA
HBI0401	1144.61aC	3500.32bB	7039.33dB	4863.08cA
DAOMC	1078.59aC	3491.68bB	6302.29dAB	5026.04cAB

* Homogeneous groups according to Tukey test, p = 0.05
Values in rows marked with the same lowercase letter do not differ significantly
Values in columns marked with the same capital letter do not differ significantly

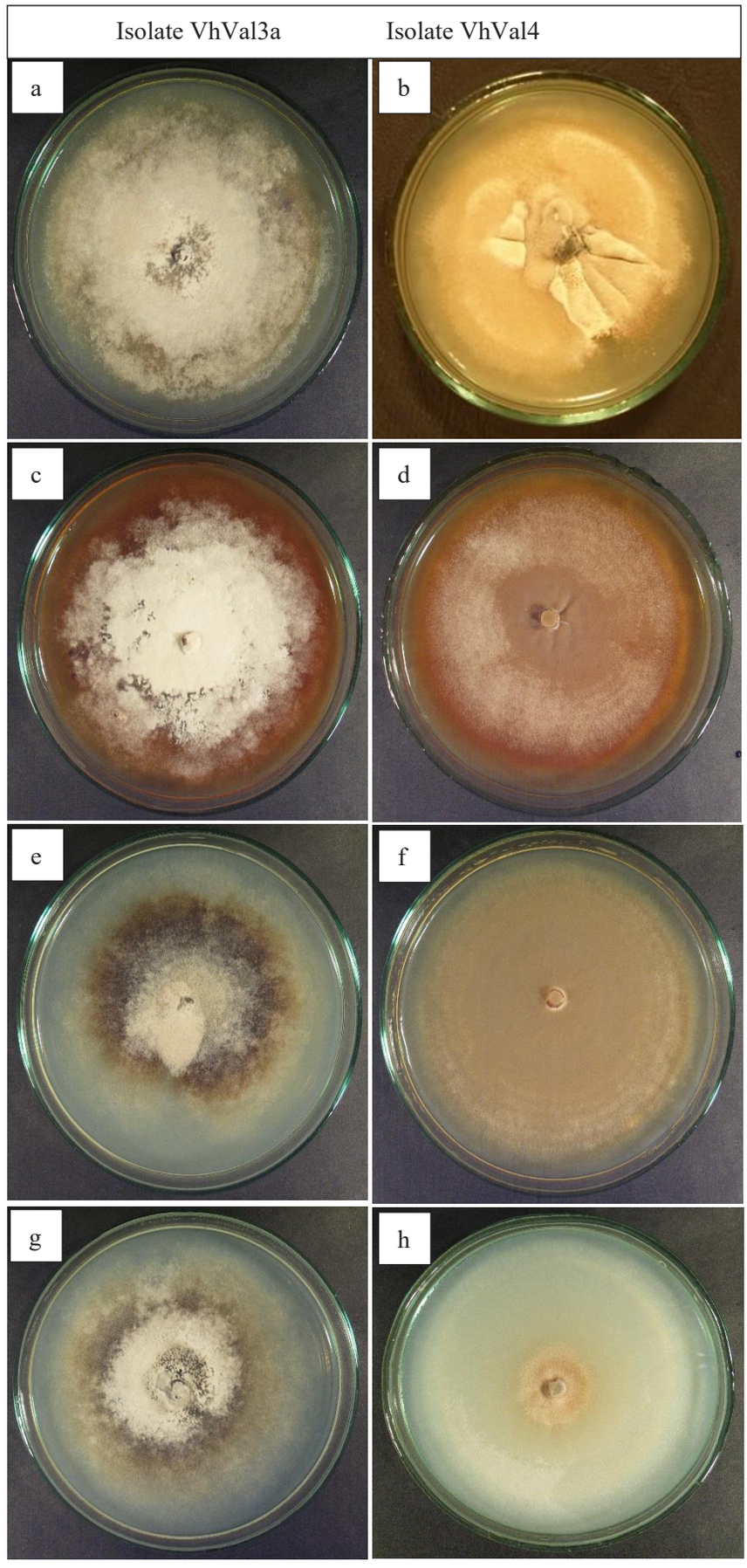

Fig. 4. Variation in the morphology of the fungal cultures of the VhVal3A and VhVal4 *V. heterodoxa* isolates on different media: PDA (a, b), Bor-PDA (c, d), Jag-PDA (e, f), Kon-PDA (g, h)

The cultures of isolates VhVal3A and VhVal4 growing on the WOA medium produced delicate white-cream aerial mycelium on the surface of the medium. At 6 weeks after the inoculation, initially black and fleshy sclerotia, becoming compact and hard with time, were observed on the surface of the medium. The appearance of the colonies growing on the other media modified with WOA (Bor-WOA, Jag-WOA, Kon-WOA) was similar to that of isolate VhVal3A cultured on the WOA medium.

The statistical analysis of the growth dynamics of isolates VhVal3A and VhVal4 showed significant differences in the culture growth on the eight media in each of the incubation conditions (Tab. 3).

Production of *V. heterodoxa* conidia in *in vitro* conditions. The production of *V. heterodoxa* spores was only observed in the diffuse daylight conditions at approx. 20 °C on the OA substrate (variant III). Conidia appeared after 8 days of the experiment. They were visible on the medium surface as initially deli-

Table 3. Comparison of the growth dynamics (mm^2/day) of the *V. heterodoxa* cultures growing on selected media under different lighting conditions (regression line slope coefficient b)

Conditions	Medium							
	Bor-WOA	WOA	Jag-WOA	Kon-WOA	PDA	Bor-PDA	Kon-PDA	Jag-PDA
				Isolate VhVal3A				
Darkness, 19 °C	5.2aA*	5.0aA	6.9abA	7.7abA	8.4abA	9.0bcA	12.1cdA	13.2dA
Photoperiod 12h, 19/12 °C	315.5abB	296.2aB	304.0abB	285.6aB	330.7abB	413.6cB	403.3cC	366.3bcB
Day, 20 °C	306.4aB	307.7aB	311.4abB	310.5abC	363.9bcB	437.9dB	328.3abcB	377.1cB
				Isolate VhVal4				
Darkness, 19 °C	8.4aA	5.3aA	7.2aA	5.6aA	51.7bA	55.2bA	51.8bA	53.6bA
Photoperiod 12h, 19/12 °C	288.5aB	289.9aB	293.6aB	293aB	345.2bcB	420.0dB	310.1abB	358.0cB
Day, 20 °C	313.5aB	299.2aB	310.4abB	282.3abB	360.5bcdB	415.1dB	338.1abcB	380.6cdB

*Homogeneous group according to the Tukey test, p = 0.05
Values in the rows marked with the same lowercase letter do not differ significantly
Values in the columns marked with the same uppercase letter do not differ significantly

Table 4. The number of spores released from the leaf surface of highbush blueberry and bilberry depending on the height of the trapping plate

Distance from the leaf surface (cm)	Number of spores after 4 (pcs)	Number of spores after 9 days (pcs)
1	4	12
5	4	14
10	3	8
15	3	8
20	1	1
25	0	0

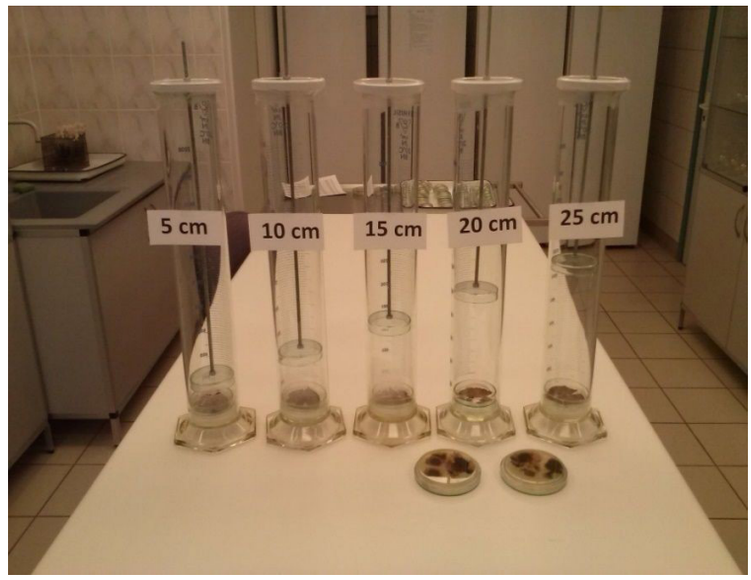

Fig. 5. The release of conidial spores of the fungus *V. heterodoxa* at different heights directly from the leaves of highbush blueberry and bilberry

cate, whitish, aerial mycelium limited by a brown ring composed of interlaced mycelial hyphae.

The conidia produced on the mycelium growing on the OA substrate had a typical appearance for the species *V. heterodoxa*. They were large, 4.5-armed, star-shaped, 200.32–327.77 µm long, and 94.68–158.52 µm wide. The mean dimensions measured in 100 spores were 274.26 (±26.3) µm × 127.29 (±13.87) µm. On day 10 of the experiment, there were 120 spores per plate, and they had a diameter of 100 mm. Isolates VhKir1 and VhK26/2a (bilberry) were found to produce conidia. In turn, isolates VhVal3A, VhVal4, and VhBg/1z/16 (highbush blueberry), isolate VhDM1 (bilberry), and the reference isolates (HBI0401 and DAOMC) did not produce spores in any of the variants tested in this study. In total, 65 out of the 115 *V. heterodoxa* isolates produced conidia in optimal conditions for fungal spore formation.

Release of *V. heterodoxa* conidia at different heights

In the cylinders, single conidia (from 1 to 4) were found on the plates placed at the height of 1, 5, 10, and 15 cm after only 4 days of the experiment. After another 5 days, the number of conidia increased to 14. Their precise number is shown in Table 4.

In the cylinder in which the plate was suspended at a height of 20 cm, a single conidium was found on the cylinder wall at the height of approx. 18 cm (Fig. 5). In turn, no spores were observed in the cylinder with the plate suspended at a height of 25 cm.

DISCUSSION

The fungus *Valdensinia heterodoxa* occurs commonly in Polish forests and is the cause of valdensia leaf blight, a disease of bilberry plants [Siemaszko 1929, Siemaszko 1934, Mułenko and Woodward 1996, Kukuła et al. 2017]. This may be associated with the specific habitat requirements of this pathogen and with the high content of organic matter, which is a source of nitrogen for plants. In bilberry plants growing in the boreal forests in Sweden, the high susceptibility to this disease was correlated with increased concentrations of amino acids, especially glutamine, in host plant leaves [Nordin et al. 1998, Strengbom et al. 2002]. In the present study, a high prevalence of the pathogen was noted every year, mainly in the forests growing in coastal and submontane areas. The conditions in these regions, primarily the high relative air humidity and the large amount of light reaching plants growing in

the lowest parts of the forests, were most probably favourable for the growth of the fungus. *V. heterodoxa* was found to infect bilberry shrubs in Pomorskie (Kopalino, Karwia, Ostrowo) and Małopolskie Voivodeship (Zakopane, Witów, Kiry). The possibility of occurrence of the pathogen in highly humid conditions was also evidenced by its presence in the village of Okuninka (Lubelskie Voivodeship), where infected plants were found in a forest near the lake. High air humidity is one of the main determinants of spore formation and fungal growth [Aamlid 2000, Vogelgsang and Shamoun 2002, 2004, Wilkin 2004, Wilkin et al. 2005a, Wilkin et al. 2005b, Zhao and Shamoun 2006, Annis and Yarborough 2009, Zhao and Shamoun 2010, Abbasi et al. 2023].

To date, the national literature has provided little information on the biology and harmful effects of *V. heterodoxa* and the epidemiology of valdensia leaf blight, probably because the pathogen was found to infect economically unimportant host plant species, e.g. bilberry, lingonberry, or lowbush blueberry. A cue to undertake investigations of the biology and detrimental effects of *V. heterodoxa* and the epidemiology of the disease was the detection of the fungus in the highbush blueberry cv. Jersey in Japan [Nekoduka et al. 2012] and in the highbush blueberry cv. Bluecrop cultivated in a nursery [Dzięcioł et al. 2014] or in the Bluegold cultivar growing in a commercial plantation in Poland [Kukuła et al. 2017].

On the one hand, the present study is a continuation of previous investigations, and on the other hand, the results enlarge the knowledge provided by Kukuła et al. [2017] on the biology of *V. heterodoxa* and the epidemiology of the disease. The research conducted in 18 commercial plantations: eight plantations in Mazowieckie Voivodeship (Prażmów, Piskórka, Wola Żyrowska, Żyrów, Błędów, Huta Błędowska), five in Łódzkie Voivodeship (Paprotnia, Jajkowice, Kłopoczyn), three in Podlaskie Voivodeship (Białousy – two plantations, Sokółka), and two in Lubelskie Voivodeship (Matcze, Horodło) confirmed the presence of *V. heterodoxa* in one of the commercial cultivation of highbush blueberry plants (Prażmów) with high humidity and without chemical control. The commercial plantations selected for the study differed in the location, soil conditions, microclimate, and the age of cultivated plants. The central part of the country is flat or, less frequently, has a southern slope. The soil structure in these plantations was dominated by permeable sandy-peat soils with pH 4–4.5. The soils represented quality class V and/or VI. The planting material consisted of plants purchased in licensed Polish nurseries of fruit trees and shrubs. Plantations with shrubs aged 5–10 years dominated. They were located close to forests or other woodland areas, and one or two of their sides were most often directly adjacent to the forests or were located in forest centers. This suggests that infected bilberry plants may have been a potential source of *V. heterodoxa* infecting the highbush blueberry plants. This hypothesis was verified, as the plants in these plantations had characteristic concentric necrotic spots on their leaves similar to those observed in Japan in a commercial plantation of blueberry cv. Jersey described by Nekoduka et al. [2012] and on blueberry cv. Bluecrop in a planting material nursery reported by Dzięcioł et al. [2014].

In the areas surrounding the commercial highbush blueberry plantations, the occurrence of *V. heterodoxa* was also recorded on lingonberry leaves and non-woody bilberry shoots. Similar findings were reported by Hildebrand and Renderos [2007, 2010, 2012], Hildebrand et al. [2011, 2016], Nekoduka et al. [2012], Lyon [2015], and Abbasi et al. [2023], who found that the fungus infected all green parts of plants, including shoots and unripe berries. In turn, our observations of other plants regarded by other researchers as host plants of the pathogen [Siemaszko 1929, Siemaszko 1934, Bavendamm 1944, Melnik 1981, Norvell and Redhead 1994, Mułenko and Woodward 1996, Melnik 2004, Melnik et al. 2007], i.e. bigleaf maple, downy birch, common hazel, common beech, pedunculate oak, sessile oak, common rowan, wild raspberries, and blackberries as well as such perennials as lily of the valley, wood sorrel, or polygonatum, did not confirm the presence of this fungus on any of these species.

In natural conditions, *V. heterodoxa* conidia are large, macroscopically visible, and resemble 3–4-pointed or sometimes even 5-pointed stars with a size of 400–600 × 100–150 µm [Peyronel 1923, Bavendamm 1944, Peyronel 1953, Redhead and Perrin 1972a, Redhead and Perrin 1972b, Mułenko and Woodward 1996]. In this study, the conidia isolated from the leaves of infected plants (lingonberry, bilberry and highbush blueberry cv. Bluegold) were smaller,

in some cases even by 50%, than those described in the literature. Moreover, the size of the conidia was specific to the host plant species, and the statistical analysis confirmed the significance of these size differences. Fungi from the phylum *Ascomycota* are spread by ascospores and conidia [Kirk et al. 2008]. In many cases, the processes of generation of ascospores and conidia are separated in time. Nevertheless, in some cases, only the imperfect stage occurs commonly in nature, with conidia as the main source of the fungus spread [Marcinkowska 2012], which is the case of *V. heterodoxa*. In the present study, regardless of the host plant, conidia of this fungus on short conidiophores were visible within the spots on the infected leaves, as in the studies conducted by Peyronel [1923], Redhead and Perrin [1972b], Nekoduka et al. [2012], Zhao and Shamoun [2010], and Abbasi et al. [2023]. The unique way of releasing these spores, i.e. through pressure exerted by folding arms on the leaf surface, is considered highly interesting. As suggested by Redhead and Perrin [1972b], this process contributes to the upward ejection of spores to a height of approx. 20 cm. This mechanism has never been recorded, but the ability of conidia to "detach" from the leaf surface was used in this study to acquire single-spore isolates. It was also observed that the placement of the leaves on the Petri dish, with the upper side of the leaf blade facing up, exerted a considerable impact on the frequency and number of produced conidia. They were generated in the characteristic spots formed during the pathogen infection on the upper side of the leaves. During their release, their heads were attached to the medium. The results of the present study indicate that conidia can be released by upward ejection to a height of approximately 18 cm. The active release of spores seems to be a highly effective way of pathogen transmission, which can explain the rapid spread of the disease onto plants.

The development of the disease in natural conditions depends on the weather prevailing in the growing season and the plant vegetation stage. This is closely related to the growth and development of the fungus *V. heterodoxa*. In Canada, the first conidia appeared after overwintering on leaves infected in the previous year; they were formed within sclerotia at high humidity persisting for three consecutive days [Hildebrand and Renderos 2010]. Conidia transferred onto young 3-week-old leaves infected and established parasitic contact with the plant already after 6 hours. The first weak symptoms were observed already in late May or early June, especially when the leaves remained wet for 6–10 hours [Hildebrand and Renderos 2012]. In addition to high humidity, an optimal temperature is required for the growth and development of the pathogen and for colonization of new plants. Conidia infect plant leaves especially quickly in the temperature range of 15–25 °C [Hildebrand and Renderos 2010, Abbasi et al. 2023]. During our observations, the first spots were found on the leaves after abundant rainfall recorded at the beginning of June (at the beginning of the season), and they developed gradually in July.

In their studies, Redhead and Perrin [1972a] found that mycelium growth and pathogen spore formation on artificial media were mainly influenced by the type of media, temperature, and lighting conditions in which Petri dishes with the inoculated pathogen were incubated. Fungal colonies produced spores most efficiently on CM (Cornmeal Agar, Difco) and WOA media incubated at 15 °C for 4–5 days. Vogelgsang and Shamoun [2002] confirmed the strict dependence of fungal spore formation on the temperature and photoperiod. At temperatures <10 °C and without additional lighting (0-hour photoperiod), the growth and development of pathogen colonies were inhibited. In turn, Magnussen et al. [2004] observed the most abundant spore formation at a 12 h day/12 h night photoperiod and a temperature of 16–19 °C. Zhao and Shamoun [2010] reported the most efficient growth and spore formation in a fungus colony on SPDA (Salal-PDA), SOA (Salal-Oatmeal Agar), and WOA media at a temperature of 19 °C (day) and 12 °C (night) and a 12 h day/12 h night photoperiod. The present results indicate that 15–20 °C is the optimum temperature for mycelium growth in laboratory conditions, which is consistent with the findings reported by Hildebrand and Renderos [2012]. Our subsequent analyses showed inhibition of the growth of *V. heterodoxa* colonies on media incubated without access to light, which was also observed by Zhao and Shamoun [2006]. A positive effect was observed when decoctions from highbush blueberry, bilberry, and lily of the valley leaves were added to the media. The analysis of the dynamics of growth of the pathogen colonies on various media and in various light and temperature conditions indi-

cated that, in most cases, both *V. heterodoxa* isolates grew most efficiently on the PDA medium supplemented with leaves from different plants (temp. 20 °C, daylight, and temperature of 19 °C during the day and 12 °C at night with 12-h illumination). The correlation coefficients (r) determined for these isolates were high (above 0.9), which indicates a very strong positive correlation between the size of the fungal colony and the measurement time point in the different incubation conditions.

In the *in vitro* conditions, the colonies of the Polish *V. heterodoxa* isolates exhibited the highest spore production rate on the OA medium with incubation at daylight at 20 °C. The conidia formed in these conditions were smaller than those isolated from the surface of infected leaves collected in natural conditions and conidia obtained by Zhao and Shamoun [2010] on solid media from wheat and rice grains.

Currently, the identification of fungi should be based not only on classical methods but also on molecular techniques, e.g. analyses of species-specific gene sequences or regions, such as the non-coding regions of nuclear DNA – ITS (*Internal Transcribed Spacer*) fragments, which retain polymorphic traits [White et al.1990, Larena et al.1999]. Phylogenetic analyses based on the large subunit rDNA sequence have shown that *V. heterodoxa* forms a separate monophyletic group within the family *Sclerotiniaceae* [Holst-Jensen et al. 1997]. In the present study, the fungal isolates were identified using primers of the ITS region, and a 559-nucleotide product was obtained in the PCR reaction. The BLASTn algorithm-based comparison of the sequence obtained in this study to those available in the GenBank showed its 100% similarity to the sequences deposited under numbers KU306729.1, KF212190.1, AB663682.1, Z81447.1, and KT121733.1, which allowed unambiguous assignment of the studied isolates to the species *Valdensinia heterodoxa*. Based on the nucleotide sequence, a group of 14 isolates from the bilberry shrubs (northern Poland) with a deletion of nucleotide 12 was distinguished.

Valdensia leaf blight caused by the fungus *V. heterodoxa*, initially observed only in New Brunswick province (Canada), is now recorded in neighboring provinces and some US states. Its harmful effects on lowbush blueberry plantations are reflected in a 20 to 60% decrease in yields due to premature defoliation [Hildebrand and Renderos 2010] and disruption of processes responsible for the formation of flower buds for the subsequent season [Ali et al. 2021]. One of the most effective measures to protect plants against this disease in Canada is prevention of pathogen transmission on shoes, clothing, or agricultural machinery wheels [Cornel and Percival 2023]. In Canada, *V. heterodoxa* develops at the same time as other pathogens, e.g. *Botrytis cinerea* [Hildebrand et al. 2001], *Sphaerulina vaccinii* S. Ali, P.D. Hildebrand & P.A. Abbasi, and *Botryosphaeria corticis* (Demaree & Wilcox) Arx & E. Müll. [Ali et al. 2021]. Therefore, to limit its growth, agents used to combat other diseases that pose a high threat to highbush blueberries in Poland, such as anthracnose or gray mold, may prove effective [Bryk et al. 2020].

The presented investigation of the occurrence of *V. heterodoxa* in highbush blueberry plantations in Poland, the study of the dynamics of growth of Polish isolates of the pathogen, the determination of the conditions of conidia production and the height at which staurospores are released, and the molecular characterization of the pathogen isolates are innovative on a national scale and provide more detailed insight into the biology of *V. heterodoxa* and the epidemiology of valdensia leaf blight. The present findings help to be prepared in case of an epidemic of the disease through development and improvement of methods for protection of highbush blueberry plants against the pathogen. Assessing the threat from various diseases and minimizing ecological damage when selecting protection methods is enabled only by thorough knowledge of pathogen biology.

CONCLUSIONS

The following conclusions can be formulated in the present study:

1. Plants belonging to the family *Ericaceae*, including highbush blueberry, are particularly susceptible to *V. heterodoxa* infection.

2. The *V. heterodoxa* isolates originating from the highbush blueberry plants were found to be less capable of spore formation than the isolates obtained from the bilberry plants.

3. In Poland, *V. heterodoxa* is a common pathogen on plants of the genus *Vaccinium*.

4. The fungus exhibits characteristics of a mesophilic and eurythermic species.

5. In axenic cultures the vegetative growth and conidiation of *V. heterodoxa* are strongly influenced by light conditions and the carbon sources provided in the growth medium.

SOURCE OF FUNDING

This study was not supported by external funding.

REFERENCES

Aamlid, D. (2000). Infections of *Valdensinia heterodoxa* and *Pucciniastrum vaccinii* on bilberry (*Vaccinium myrtillus*). For. Pathol., 30, 135–139. https://doi.org/10.1046/j.1439-0329.2000.00194.x

Abbasi, P.A., Hildebrand, P.D., Ali, S., Wright, H., Franklin, J., Renderos W.E. (2023). Effects of temperature and wetness on components of the infection cycle of *Valdensia heterodoxa* in lowbush blueberry. Plant Dis., 107, 167–176. https://doi.org/10.1094/PDIS-10-21-2248-RE

Ali, S., Hildebrand, P.D., Renderos, W.E., Abbasi, P.A. (2021). Identification and characterization of *Sphaerulina vaccinia* sp. nov. as the cause of leaf spot and stem canker in lowbush blueberry and its epidemiology. Phytopathology, 111(9), 1560–1570. https://doi.org/10.1094/PHYTO-04-20-0143-R

Annis, S., Yarborough, D. (2009). Valdensinia leaf spot disease. The University of Mine–Cooperative Extension: Maine Wild Blueberries. Available online: http://umaine.edu/blueberries/factsheets/disease/valdensinia–leaf–spot–disease [date of access: 24.03.2014].

Bavendamm, W. (1944). *Valdensia heterodoxa*, ein neuer Buchenschädling. Forstwissenschaftliches Centralblatt, 66, 54–60.

Bryk, H., Michalecka, M., Poniatowska, A., Kałużna, M., Cieślińska, M. (2020). Integrowane metody ograniczania chorób [Integrated disease control methods]. In: H., Bryk (eds), Metodyka integrowanej ochrony borówki wysokiej [Methodology for integrated protection of highbush blueberries]. Instytut Ogrodnictwa, Skierniewice, 18–26.

Cieślińska, M. (2020). First report of Blueberry mosaic associated virus in highbush blueberry in Poland. Plant Dis., 104(10), 2743. https://doi.org/10.1094/PDIS-03-20-0574-PDN

Cornel, A., Percival, D. (2023). Evaluating fungicide efficacy for wild blueberry foliar disease complex management in eastern Canada. Acta Hortic., 1378, 113–120. https://doi.org/10.17660/ActaHortic.2023.1378.15 [date of access: 6.06.2024]

Doyle, J.J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19, 11–15.

Dzięcioł, R., Mirzwa-Mróz, E., Zielińska, E., Wińska-Krysiak, M., Wakuliński, W. (2014). *Valdensinia heterodoxa* Peyronel as a new pathogen of blueberry in Poland. Plant Dis., 98(5), 688. https://doi.org/10.1094/PDIS-06-13-0644-PDN

Farr, D.F., Rossman, A.Y. (2013). Fungal databases, systematic mycology and microbiology laboratory ARS, USDA. Available online: http://nt.ars-grin.gov/fungaldatabases/ [date of access: 6.06.2015].

Gams, W., Van Der Aa, H.A., Van Der Plaats-Niterink, A.J., Samson, R.A., Stalpers, J.A. (1987). CBS Course of mycology, 3th ed. Centraalbureau Voor Schimmelcultures, Baarn, p. 105.

Gardes, M., Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol., 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hildebrand, P.D., McRae K.B., Lu X. (2001). Factors affecting flower infection and disease severity of lowbush blueberry by *Botrytis cinerea*. Can. J. Plant Pathol., 23, 364–370. https://doi.org/10.1080/07060660109506957

Hildebrand, P.D., Renderos, W.E. (2007). Valdensinia leaf spot (*Valdensinia heterodoxa*) of commercial lowbush blueberry in Atlantic Canada: an emerging new threat. Can J. Plant Pathol., 29, 90.

Hildebrand, P.D., Renderos, W.E. (2010). Valdensinia leaf spot of lowbush blueberry. Agriculture and Agri-Food Canada.Available online:www.gov.pe.ca/photos/original/af fact Valden.pdf. [date of access: 11.03.2015].

Hildebrand, P.D., Renderos, W.E. (2012). Seasonal release of conidia of *Valdensia heterodoxa* and disease progress in lowbush blueberry. Can. J. Plant Pathol., 35, 273–278.

Hildebrand, P.D., Renderos, W.E., Delbridge, R.W. (2016). Diseases of lowbush blueberry and their identification. Agriculture and Agri-Food Canada, Ottawa, p. 44. Available online: https://publications.gc.ca/collections/collection_2016/aac-aafc/A59-37-2016-eng.pdf [date of access: 13.05.2024].

Hildebrand, P.D., Renderos, W.E., Fillmore, S.A., Walker, B.A. (2011). Susceptibility of lowbush blueberry in the sprout and crop phase to Valdensinia leaf spot. Can. J. Plant Pathol., 33, 568.

Holst-Jensen, A., Kohn, L.M., Schumacher, T. (1997). Nuclear rDNA phylogeny of the *Sclerotiniaceae*. Mycologia, 89, 885–899. https://doi.org/10.1080/00275514.1997.12026859

https://blast.ncbi.nlm.nih.gov/Blast.cgi

Kałużna, M., Pothier, J.F. (2022). Complete genome sequence data of two *Xanthomonas arboricola* strains

isolated from blueberry plants displaying bacterial leaf blight in Poland. Phytopathology, 112,8, 1814–1818. https://doi.org/10.1094/PHYTO-11-21-0484-A

Kałużna, M., Puławska, J., Meszka, B. (2013). A new bacterial disease on blueberry (*Vaccinium corymbosum*) caused by *Pseudomonas* spp. J. Plant Prot. Res., 53(1), 32–36.

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A. (2008). Ainsworth & Bisby's dictionary of the fungi. 10th ed. CABI Europe–UK, Egham and CAB International, Wallingford United Kingdom, 623–624, 719.

Krysicki W., Bartos J., Dyczka, W., Królikowska, K., Wasilewski, M. (1994). Rachunek prawdopodobieństwa i statystyka matematyczna w zadaniach. Cz. II. Statystyka matematyczna [Probability theory and mathematical statistics in tasks. Part II. Mathematical statistics]. Wydawnictwo Naukowe PWN, Warszawa, p. 325.

Kukuła, W., Mirzwa-Mróz, E., Wakuliński, W., Paduch-Cichal, E. (2017). Characteristics of *Valdensinia heterodoxa* Peyr. as an *Ericaceae* pathogen in Poland. Acta Sci. Pol. Hortorum Cultus, 16(4), 101–112. https://doi.org/10.24326/asphc.2017.4.11

Larena, I., Salazar, O., Gonzales, V., Julian, M.C., Rubio, V. (1999). Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. J. Biotech., 75, 187–194.

Lyon, E. (2015). Dispersal and establishment of the fungal pathogen, *Valdensia heterodoxa*, in Maine [M. Sc Thesis]. The University of Maine, Orono, ME. Available online: https://digitalcommons.library.umaine.edu/etd/2382 [date of access: 10.06.2024].

Magnussen, S., Vogelgsang, S., Shamoun, S.F. (2004). Nonlinear mixed models for repeated data assessment of time and temperature effects on conidia production in the fungus *Valdensinia heterodoxa*. Biocontrol, 49, 47–62. https://doi.org/10.1023/B:BICO.0000009376.53688.8e

Marcinkowska, J. (2012). Oznaczanie rodzajów grzybów sensu lato ważnych w fitopatologii [Identification of fungal species important in plant pathology]. Wydawnictwo Rolnicze i Leśne PWRiL, Warsaw, Poland, p. 508.

Melnik, V.A. (1981). *Valdensia heterodoxa* Peyronel, an unusual but very common *Hyphomycetes* [Fungi]. Novosti sistematiki nizshikh rastenii – Akademiia Nauk SSSR, Botanicheskii Institut Petersburg, Russia, 18, 96–99.

Melnik, V.A. (2004). Parasitica hyphomycete *Valdensia heterodoxa* on *Rubus saxatilis* in Leningrad oblast. Mikol. Fitopatol., 28, 43–45.

Melnik, V.A., Popov, E.S., Shabunin, D.A. (2007). Contributions to the studies of mycobiota in Novgorod and Pskov regions. Hyphomycetes. Mikol Fitopatol., 41, 515–525.

Meszka, B., Bielenin, A. (2012). Antraknoza borówki wysokiej, występowanie, szkodliwość i możliwości zwalczania [Blueberry anthracnose, occurrence, harmfulness and control possibilities]. Prog. Plant Prot., 52(1), 88–91.

Mirzwa-Mróz, E., Szyndel, M.S., Wdowiak, M., Wit, M., Paduch-Cichal, E., Wilkos, A., Felczak-Konarska, K., Wakuliński W. (2023). Phenotypic characterization and phylogeny of *Godronia myrtilli* (Anamorph: *Topospora myrtilli*) – causal agent of Godronia canker on highbush blueberry. Pathogens, 12(5), 1–11. https://doi.org/10.3390/pathogens12050642.

Mułenko, W., Woodward, S. (1996). Plant parasitic hyphomycetes new to Britain. Mycologist, 10, 69–72.

Nekoduka, S., Kanematsu, S., Tanaka, K., Harada, Y., Sano, T. (2012). Valdensinia leaf blight of blueberry caused by *Valdensinia heterodoxa*, a new fungal disease in Japan. J. Gen. Plant. Pathol., 78(3), 151–159. https://doi.org/10.1007/s10327-012-0373-y

Nordin, A., Nasholm, T., Ericson, L. (1998). Effects of simulated N deposition on understory vegetation of a boreal coniferous forest. Ecology, 12, 691–699. https://doi.org/10.1046/j.1365-2435.1998.00240.x

Norvell, L.L., Redhead, S.A. (1994). *Valdensinia heterodoxa* (*Sclerotiniaceae*) in the United States. Can. J. For. Res., 24, 1981–1983. https://doi.org/10.1139/x94-253

Paduch-Cichal, E., Kalinowska, E., Chodorska, M., Sala-Rejczak, K., Nowak, B. (2011). Detection and identification of viruses of highbush blueberry and cranberry using serological ELISA test and PCR technique. Acta Sci. Pol., Hortorum Cultus, 10(4), 201–215.

Peyronel, B. (1923). Sopra un singolare parassita polifago: *Valdensia heterodoxa* n. gen et n. sp. [On a singular polyphagous parasite: *Valdensia heterodoxa* n. gen. et n. sp.]. Stazione Sperimentale di Agrumicoltura Italiane, 56, 521–538.

Peyronel, B. (1953). La forma ascofora di *Valdensia heterodoxa* Peyronel, tipo di un nuovo genere di *Sclerotiniacee*: *Valdensinia*. Nuovo Giornale Botanico Italiane, 59, 181–185.

Redhead, S.A., Perrin, P.W. (1972a). *Asterobolus*: a new parasitic hyphomycete with a novel dispersal mechanism. Can. J. Bot., 50, 409–412.

Redhead, S.A., Perrin, P.W. (1972b). *Asterobolus*: a synonym of *Valdensia*. Can. J. Bot., 50, 2083–2084.

Siemaszko, W. (1929). Phytopathologische Beobachtungen in Polen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg., 78, 113–116.

Siemaszko, W. (1934). Zagadnienia zasięgów geograficznych chorób roślin uprawnych [Issues regarding the geographical range of crop diseases]. Rocz. Nauk Ogrod., 1, 163–170.

Strengbom, J., Nordin, A., Nasholm, T., Ericson, L. (2002). Parasitic fungus mediates change in nitrogen-exposed

boreal forest vegetation. J. Ecol., 90, 61–67. https://doi.org/10.1046/j.0022-0477.2001.00629.x

Szmagara, M. (2009). Biodiversity of fungi inhabiting the highbush blueberry stems. Acta Sci. Pol. Hortorum Cultus, 8(1), 37–50.

Vogelgsang, S., Shamoun, S.F. (2002). Growth, sporulation, and conidia discharge of *Valdensinia heterodoxa*, a foliar pathogen of salal, as influenced by temperature and photoperiod *in vitro*. Mycol. Res., 106, 480–490. https://doi.org/10.1017/S0953756202005737

Vogelgsang, S., Shamoun, S.F. (2004). Evaluation of an inoculum production and delivery technique for *Valdensinia heterodoxa*, a potential biological control agent for salal. Biocontrol Sci. Techn., 14, 747–756. https://doi.org/10.1080/09583150410001720725

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M.A., Innis, D.H., Gelfand, J.J. Sninsky, T.J. White (eds), PCR Protocols: a guide to methods and applications. Academic Press Inc., 315–322.

Wilkin, J.E. (2004). Genetic diversity and population structure of the potential biocontrol agent, *Valdensinia heterodoxa*, and its host *Gaultheria shallon* (salal) [M.Sc. Thesis]. Department of Forest Sciences, The University of British Columbia, Vancouver, B.C., Canada, 6–7.

Wilkin, J.E., Shamoun, S.F., Ritland, C., Ritland, K., El-Kassaby, Y.A. (2005a). Population genetics of *Gaultheria shallon* in British Columbia and the implications for management using biocontrol. Can. J. Bot., 83, 501–509. https://doi.org/10.1139/b05-039

Wilkin, J.E., Ritland, C., Ritland, K., El-Kassaby, Y.A. (2005b). Genetic diversity and population structure of *Valdensinia heterodoxa*, a potential biocontrol agent for salal in coastal British Columbia. Can. J. Plant Pathol., 27, 559–571. https://doi.org/10.1080/07060660509507257

Zalewska, E., Król, E., Machowicz-Stefaniak, Z. (2007). Grzyby zasiedlające owoce borówki wysokiej (*Vaccinium corymbosum* L.) uprawianej w południowo-wschodniej Polsce [Fungi colonizing the fruits of blueberry (*Vaccinium corymbosum* L.) cultivated in south-eastern Poland]. Prog. Plant Prot., 47(2), 380–384.

Zhao, S., Shamoun, S.F. (2006). The effects of culture media, solid substrates, and relative humidity on growth, sporulation and conidial discharge of *Valdensinia heterodoxa*. Mycol. Res., 110, 1340–1346. https://doi.org/10.1016/j.mycres.2006.08.001

Zhao, S., Shamoun, S.F. (2010). Conidial morphology, structure and development in *Valdensinia heterodoxa*. Mycology, 1, 113–120. https://doi.org/10.1080/21501201003720665