UDC 82.288:631.559 (477)

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To cite this article: Andrianova T. (2025). Hryby, asotsiiovani z *Vaccinium macrocarpon* v Ukraini, ta ekolohichni metody profilaktyky pisliazbyralnoi hnyli plodiv [Fungi associated with *Vaccinium macrocarpon* in Ukraine and ecological methods for the prevention of post-harvest fruit rots]. *Fitoterapiia. Chasopys – Phytotherapy. Journal,* 3, 97–108, doi: https://doi.org/10.32782/2522-9680-2025-3-97

FUNGI ASSOCIATED WITH *VACCINIUM MACROCARPON* IN UKRAINE AND ECOLOGICAL METHODS FOR THE PREVENTION OF POST-HARVEST FRUIT ROTS

Actuality. *In recent years, the commercial cultivation of large cranberry (Vaccinium macrocarpon) has expanded across the depleted peatlands of northwestern Ukraine, particularly in the Rivne and Volyn regions. With yields reaching 5 to 10 metric tons per hectare, the crop demonstrates considerable potential. However, post-harvest fruit rot has become a significant limitation, negatively affecting the storage and marketability of fresh cranberries. The development of effective, environmentally safe methods to reduce fungal contamination is therefore highly relevant to sustainable cranberry production in Ukraine.*

The purpose of the study *was to identify fungal pathogens responsible for cranberry fruit rot and evaluate ecological post-harvest treatments to reduce their incidence.*

Material and methods. *Stored berries were analyzed for fungal colonization by various methods and were checked for fungal invasion caused by species of the genera Allantophomopsis, Alternaria, Botrytis, Cladosporium, Coleophoma, Colletotrichum, Diaporthe, Discosia and Strasseria. Besides, four ecological post-harvest cleaning treatments were tested for stored berries: (a) washing with clean water and drying at 45 °C for 2 minutes, followed by air drying at 20 °C; (b) sieve-showering at 45 °C for 2 minutes, then air drying at 20 °C; (c) washing and drying at 20 °C; and (d) washing followed by two-stage drying at 45 °C. Untreated berries served as a control. All samples were stored at 2–8 °C and 55–65% relative humidity.*

Research results. *An assessment of the degree of fungal damage showed that untreated fruits quickly rotted and were characterized by active development of internal mycelium. By microscopy method and isolation into pure culture, it was identified that large cranberry berries were affected by fungi of the species Alternaria spp., Botrytis cinerea, Cladosporium spp., Coleophoma empetri, Colletotrichum acutatum, and Diaporthe vaccinii. Notably, C. acutatum and D. vaccinii were recorded in Ukraine for the first time. Fungal assessments showed that untreated berries exhibited rapid rot and significant internal mycelial development. Among the tested fruit's treatments, the fourth method – dual-stage thermal drying at 45 °C – proved most effective. It substantially reduced external contamination by peat particles, plant debris, and fungal spores, and also inhibited internal fungal growth in berries. Other methods showed partial efficacy, with variable suppression of rot symptoms.*

Conclusion. *This study is the first to investigate fungi associated with the North American species Vaccinium macrocarpon in Ukraine. For the first time, post-harvest rots of cultivated V. macrocarpon were studied, revealing four pathogenic species – Botrytis cinerea, Coleophoma empetri, Colletotrichum acutatum, and Diaporthe vaccinii – which cause bitter, ripe, viscid, and yellow fruit rots during storage. Notably, C. acutatum and D. vaccinii are new for mycobiota of Ukraine. The dual-stage thermal-air drying treatment at 45 °C proved to be an effective ecological approach for reducing post-harvest fungal deterioration in cranberry fruits. The method has its practical potential as low-impact and eco-friendly strategy for reducing post-harvest fungal infections of cranberries.*

Key words: *cranberries, Ascomycota, biodiversity, post-harvest cleaning, organic berries, Coleophoma empetri, Colletotrichum acutatum, Diaporthe vaccinii.*

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Бібліографічний опис статті: Андріанова Т. (2025). Гриби, асоційовані з *Vaccinium macrocarpon* в Україні, та екологічні методи профілактики післязбиральної гнилі плодів. *Фітотерапія. Часопис,* 3, 97–108, doi: https://doi.org/10.32782/2522-9680-2025-3-97

ГРИБИ, АСОЦІЙОВАНІ З *VACCINIUM MACROCARPON* В УКРАЇНІ, ТА ЕКОЛОГІЧНІ МЕТОДИ ПРОФІЛАКТИКИ ПІСЛЯЗБИРАЛЬНОЇ ГНИЛІ ПЛОДІВ

Актуальність. *Останніми роками промислове вирощування журавлини великоплідної (Vaccinium macrocarpon) активно поширюється на виснажені торфовища північно-західної України, зокрема в Рівненській і Волинській областях. Урожайність культури цієї журавлини сягає 5–10 тон з гектара, що свідчить про її високий потенціал. Водночас суттєвим обмеженням стало післязбиральне загнивання плодів, яке негативно впливає на зберігання та реалізацію свіжої журавлини. Тому розроблення ефективних, екологічно безпечних методів зменшення грибного ураження є вкрай актуальним для сталого розвитку промислового вирощування журавлини в Україні.*

Мета дослідження *– ідентифікувати гриби, які уражують плоди журавлини великоплідної під час зберігання, та підібрати екологічний метод обробки для зменшення ушкодження плодів.*

Матеріал і методи. *Зразки зібраних плодів журавлини аналізували на наявність грибного ураження різними методами, перевіряли на наявність видів таких родів: Allantophomopsis, Alternaria, Botrytis, Cladosporium, Coleophoma, Colletotrichum, Diaporthe, Discosia та Strasseria.*

Окрім того, було випробувано чотири екологічні методи післязбирального очищення плодів журавлини:
а) миття чистою водою і сушіння за 45 °С протягом 2 хвилин із подальшим сушінням на повітрі за температури 20 °С;
б) обробка на ситі водою температурою 45 °С протягом 2 хвилин і досушування за температури 20 °С;
в) миття та сушіння за 20 °С;
г) миття та двоступеневе сушіння за 45 °С.
Необроблені плоди слугували контролем. Усі зразки зберігались за температури 2–8 °С та відносної вологості 55–65%.

Результати дослідження. *Оцінювання ступеня грибного ураження показало, що необроблені плоди швидко загнивали та характеризувались активним розвитком внутрішнього міцелію. Методами мікроскопіювання та виділення в чисту культуру встановлено, що плоди журавлини уражувались грибами Alternaria spp., Botrytis cinerea, Coleophoma empetri, Colletotrichum acutatum і Diaporthe vaccinii. Серед досліджених методів очистки найбільш ефективним виявився четвертий – двоступенева термічна сушка за 45 °С. Він істотно знижував зовнішнє забруднення частками торфу, рослинними рештками та спорами грибів, а також пригнічував розвиток внутрішнього міцелію у плодах. Інші методи виявилися частково ефективними, демонстрували варіативне зменшення проявів гнилі.*

Висновок. *У цьому дослідженні вперше в Україні вивчено гриби, асоційовані з північноамериканським видом Vaccinium macrocarpon. Уперше досліджено післязбиральні гнилі плодів культури V. macrocarpon, виявлено чотири патогенні види – Botrytis cinerea, Coleophoma empetri, Colletotrichum acutatum і Diaporthe vaccinii, які спричиняють гнилі під час зберігання – гірку, перезрілу, липку та жовту. Види C. acutatum та D. vaccinii є новими для мікобіоти України. Метод двоступеневого термічного сушіння плодів повітрям за 45 °С довів свою ефективність як екологічний спосіб зменшення післязбирального ураження журавлини патогенними грибами. Цей метод має практичний потенціал як маловитратна й екологічно безпечна стратегія для зменшення післязбирального ураження журавлини грибними гнилями.*

Ключові слова: *журавлина, Ascomycota, біорізноманіття, післязбиральне очищення, органічні ягоди, Coleophoma empetri, Colletotrichum acutatum, Diaporthe vaccinii.*

Introduction. Actuality. Berry producing plants of the genus *Vaccinium* L. (Ericaceae) are well known and have long been used as a food and medicinal plants. Native for the territory of Ukraine are small-fruited cranberries (*Vaccinium microcarpum* (Turcz. ex Rupr.) Schmalh.), bilberries (*V. myrtillus* L.), marsh cranberries (*V. oxycoccos* L.), blueberries (*V. uliginosum* L.) and lingonberries (*V. vitis-idaea* L.) that are components of coniferous and mixed forests, bog ecosystems.

Most peat bogs serve as ecological niches for cranberries (*Vaccinium oxycoccos* and *V. microcarpum*), primarily located in the Polissia natural zone of Ukraine (Rivne, Volyn, and Zhytomyr regions), as well as at higher elevations in the Carpathian Mountains (Ivano-Frankivsk and Zakarpattia regions). Historically, marsh cranberries have thrived and fruited naturally on sphagnum bogs for thousands of years. During the fruiting period, local populations do not apply pesticides in wild bog habitats, and cranberries continue to grow successfully under these natural conditions (Kozyakov et al., 1974; Vasyuk et al., 2014; Balabak et al., 2016). At the same time, the natural distribution of wild marsh cranberries (*V. oxycoccos*) has declined in recent decades, largely due to intensive peat extraction for agricultural purposes in Ukraine. Concurrently, some *Vaccinium* species are now cultivated on peat bog plantations, including introduced species such as the large-fruited cranberry (*V. macrocarpon* Aiton) from North America.

Approximately 30 species of phytopathogenic fungi from the phyla *Ascomycota* and *Basidiomycota* have been identified in association with *Vaccinium* species in Ukraine. Notable ascomycetous species of fungi include *Coccomyces leptideus* (Fr.) B. Erikss., *Cytospora vaccinii* Died., *Encoeliopsis rhododendri* (Ces. & De Not.) Nannf., *Mycosphaerella stemmatea* (Fr.) Romell, *M. vaccinii* (Cooke) J. Schröt., *Myxothyrium leptideum* (Fr.) Bubák & Kabát, *Phacidium vaccinii* Fr., *Podosphaera myrtillina* Kunze, *Sphaerella myrtillina* Pass., *Sphaerocista schizothecioides* Preuss, *Sporomega degenerans* (Kunze) Corda, and *Terriera cladophila* (Lév.) B. Erikss., as well as basidiomycetous species such as *Exobasidium vaccinii* (Fuckel) Woronin, *E. vaccinii-uliginosi* Boud., and *Naohidemyces vaccinii* (Jørst.) S. Sato, Katsuya & Y. Hirats. ex Vanderweyen & Fraiture, among others (Chmielewski, 1910; Girzitska, 1929; Fungi of Ukraine, 1996; Andrianova & Minter, 2005; Andrianova, 2010; Zykova, 2015; Heluta et al., 2019; Yukhnenko & Andrianova, 2021; Heluta et al., 2024). The fungi that invade marsh cranberries are relatively infrequent. Among the few commonly reported invasions of *Vaccinium oxycoccos* are fungi *Diplodina myrtilli* (Oudem.) Allesch. and *Monilinia oxycocci* (Woronin) Honey, with the latter specifically detected on cranberry berries (Fungi of Ukraine, 1996).

Environmental changes also exert a significant influence on cranberry fructification. In particular, direct solar radiation and overheating induce physiological stress in the plants, increasing their vulnerability and susceptibility to fungal infections. This pattern was observed during the anomalously hot summers from 2018 to 2024, which resulted in considerable losses of marsh cranberry (*Vaccinium oxycoccos*) populations growing on swampy hummocks in the Rivne and Volyn regions of Ukraine.

The introduction of new *Vaccinium* species and cultivars into the Ukrainian Polissia may facilitate the migration of phytopathogenic fungi associated with these berry plants, as well as the potential invasion of alien cranberries by native representaties of mycobiota known from other *Vaccinium* hosts. This dynamic raises important concerns regarding plant health and ecosystem stability. Therefore, particular attention should be in studying fungi diseases and post-harvest rots affecting large cranberry (*V. macrocarpon*), which has been recently introduced and is cultivated on plantations established in wetland areas reclaimed after peat extraction in northwestern Ukraine (Polissia region).

Recent studies in Latvia, Poland, and the United States have addressed similar issues regarding fungal diseases and fruit rot in cultivated cranberry (*Vaccinium macrocarpon*). For instance, research by Johnson-Cicalese et al. (2009) emphasized breeding for resistance to fruit rot in cranberries. Additionally, Polish and Latvian studies have documented the diversity and pathogenicity of fungi such *as Diaporthe vaccinii* and *Coleophoma empetri*, and were presented for the first time in Ukraine in this study. These findings underline the global significance of cranberry fruit rot and the importance of developing effective post-harvest solutions.

The purpose of study is to investigate the species composition of fungi associated with fruit rot in cultivated large cranberry (*Vaccinium macrocarpon*) under the conditions of northwestern Ukraine and to evaluate the effectiveness of ecological post-harvest treatment methods for reducing fungal contamination and improving fruit storability. Particular attention is given to identifying newly recorded phytopathogenic fungi in Ukraine and assessing their potential impact on the storage quality of cranberry berries. The study also seeks to determine low-impact, environmentally friendly approaches for managing post-harvest losses in the context of cranberry cultivation on reclaimed peatlands in the Polissia region.

Materials and research methods. The study was conducted on large cranberry (*Vaccinium macrocarpon*) cultivated in experimental plots established in wetland areas reclaimed after peat extraction. The tested cultivars included the early-ripening “Wilcox” and the mid-season cultivars “Bergman” and “Stevens”. The research sites were located near the villages of Verbivka (Sarny district, Rivne Oblast), Sekun’ (Kovel’ district, Volyn Oblast), Galuzia (Kamin’-Kashyrskyi district, Volyn Oblast) during 2019–2020, and near the settlement of Manevychy (Kamin’-Kashyrskyi district, Volyn Oblast) in 2024. The cranberries were cultivated on plots without winter flooding.

Berries of the “Wilcox” cultivar, selected for their higher commercial potential, were chosen for further investigation of fungal invasion presence and development. Harvesting was performed manually. The collected berries were cleaned of leaves and dry debris by air winnowing near the plantation, then packed in polyethylene-lined bags (10–12 kg each) and transported to a storage facility. There, samples were taken to assess the effects of post-harvest cleaning on long-term storage performance and development of fungi.

Post-harvest treatment of fruits. Two separate experiments were conducted to evaluate the effects of different post-harvest treatments on the development of fruit rots in large cranberry (*Vaccinium macrocarpon*) during storage.

Experiment 1 was started in late October 2019 and continued up to March 2020. It was four-month experiment based on fresh berries harvested in autumn 2019. From each 10 kg box of cranberries, 1 kg samples were taken (approximately 1000 fruits per sample, with an average fruit weight of 1 g; range: 0,8–1,3 g). Sampled berries were washed with clean water and air-dried at 45 °C for 2 minutes, then stored in plastic containers at a temperature of 2–8 °C. The quality of stored berries was assessed monthly, and the number of rot-affected fruits was recorded.

Experiment 2 focused on berries harvested in January 2020 and involved various post-harvest cleaning methods. The treatments were as follows:

1. Treatment 1 – Berries washed with clean water, air-dried at 45 °C for 2 minutes, then further air-dried at 20 °C.
2. Treatment 2 – Berries placed in a sieve layer (5–7 cm thick), rinsed with a water shower at 45 °C for 2 minutes, then air-dried at 20 °C.
3. Treatment 3 – Berries washed with 20 °C water for 2 minutes, then air-dried at 20 °C.
4. Treatment 4 – Berries washed with clean water, air-dried at 45 °C for 2 minutes, followed by a second air-drying step at 45 °C (fig. 1-A).
5. Control – Berries not subjected to any post-harvest cleaning.

Fig. 1. Storage of *Vaccinium macrocarpon* fruits after post-harvest treatment at 2–8 °C.
A – view of large cranberry berries one month after four different variants of cleaning treatments (see text for details). B – storage conditions for the samples: 2–8 °C and 55–65% relative humidity

All treated and control samples were stored at 2–8 °C with relative humidity maintained between 55–65% (fig. 1-B). Assessments were carried out monthly. The presence of rot symptoms was recorded, and primary fungal pathogens were identified from symptomatic fruits. The effectiveness of each treatment was evaluated based on the incidence of visible rot symptoms, the average infection rate by major fungal pathogens, and the extent of fungal mycelial development within fruit tissues, following the procedures (Kozyakov et al., 1974; Khomich, Tkach, 2009).

Fungi identification and cultivation. Fungi associated with cranberry (*V. macrocarpon*) berries were identified using two complementary methods. The post-harvest treatment protocols and fungal isolation techniques used in this study were partially adapted from standard procedures previously described by (Kozyakov et al., 1974) and (Khomych & Tkach, 2009), with modifications developed by the author. These methods were selected due to their applicability to field-collected cranberry fruits and relevance to ecological storage practices. Alternative methods such as chemical disinfection or fungicide application were not considered due to the organic status of the experimental plots.

The first method involved direct microscopic examination of fungal structures in damaged berry tissues. This included observation under a dissecting microscope and standard light microscopy of specimens finely sectioned and mounted in water or 5% aqueous lactic acid solution, or 1% cotton blue in lactophenol. The names of fungal species used in this study follow the nomenclature standards established by the MycoBank database (http://www.mycobank.org).

Following microscopic identification, attempts were made to isolate the observed fungi in pure culture using standard potato dextrose agar (PDA) medium. Large tissue segments or entire affected berries were surface-sterilized with 70% ethanol followed by a 40% aqueous formaldehyde solution, rinsed with sterile water, and placed on PDA in Petri dishes. Noticeable mycelial growth typically developed from the treated berries within 3–5 days of incubation at 22–25 °C, in the dark. Inoculum from distinct fungal colonies was then subcultured onto fresh PDA plates and incubated at 25 °C in darkness for 7–14 days.

The second method focused on assessing the incidence of fungi development on berry surfaces following post-harvest cleaning treatments, without dissection or surface sterilization. Fungi were cultivated directly on PDA under dark conditions at 25 °C for 14–20 days. Identification was based on colony and spore morphology, type of sporulation.

Research results and their discussion. Cultivation of the large cranberry (*Vaccinium macrocarpon*) was initiated in Ukraine in the mid-1980 years (Konovalchuk, 2002), though still is not much developed and stands do not occupy large areas. Yield assessments of the studied *V. macrocarpon* cultivars indicated that their productivity on experimental plots in the Rivne and Volyn regions ranged from 470 to 960 g/m² in 2019–2020. In contrast, the productivity of wild native cranberry species (*V. oxycoccos* and *V. microcarpum*), which grow in natural peat bogs of the same regions, is significantly lower – reaching up to only 70 g/m². Currently, wetlands reclaimed after peat extraction cover thousands of hectares in the Rivne, Volyn, and Zhytomyr regions, and are considered promising areas for the large-scale cultivation of *V. macrocarpon* in Ukraine (Lavrenyuk & Konovalchuk, 2015).

Post-harvest losses were recorded during berry storage, primarily due to infections caused by pathogenic fungi. Cranberry fruit rot was observed in harvests from multiple years (2019, 2020, and 2024) and throughout the storage period. The initial experiment was conducted using berries of the “Wilcox” cultivar manually harvested in October 2019. These samples underwent post-harvest treatment using the first method (Experiment 1), while the control group consisted of berries that were air-cleaned by blowing, without additional treatment. All samples were stored at 2–8 °C with 55–65% relative humidity. After one month of storage, only 4,3% of the treated berries showed signs of fruit rot. By the end of the third month, the proportion of affected berries increased to 21,1%. In contrast, a significantly higher incidence of infection was observed in the control group, with 8,3% of berries affected after one month and 26,8% after three months of storage (table 1).

It should be noted that in all treated samples, a subset of berries exhibited black discoloration and signs of rot during the storage. These symptoms closely resembled black fruit rot, a known postharvest disease caused by the fungi *Allantophomopsis cytisporea* (Fr.) Petr. and *Strasseria geniculata* (Berk. & Broome) Höhn. (Polashock et al., 2017). Blackened berries (Fig. 2-C) were observed in low quantities – approximately 0,8% in treated samples and 1,9% in the control – after one month of storage, increasing to 3,5% after three months. However, the above-mentioned pathogens were not conclusively identified in these cases, and no other fungi produced visible fruiting bodies on or within the tissues of the blackened berries. Detailed examination of the infected fruits under a light microscope, combined with isolation of the fungi on potato dextrose agar (PDA), revealed the presence of a pathogenic fungus exhibiting morphological and symptomatic characters consistent with *Colletotrichum acutatum* J.H. Simmonds, a known causative agents of cranberry fruit bitter rot (fig. 2-C).

Fig. 2. Symptoms of fruit rots in *Vaccinium macrocarpon* berries that developed after post-harvest treatment and subsequent three-month storage:

A – ripe rot of *Coleophoma empetri,* with small black spots, later black; B – viscid rot of *Diaporthe vaccinii*; C – bitter rot of *Colletotrichum acutatum*; D – yellow rot of *Botrytis cinerea* and other fungi

In addition to bitter fruit rot, some berries exhibited symptoms of viscid fruit rot and yellow fruit rot. Fur-

Table 1

Incidence of *V. macrocarpon* fruit rot during storage at 2–8 °C and 55–65% relative humidity, depending on post-harvest treatment

Treatment method	**Storage duration**	Healthy, unaffected berries¹ **(number / %)**	**Infected berries (number / %)**	**Total berries (number / %)**
Cleaned with pure water, air-dried at 45 °C (2 min)	1 month	957 / 95,7%	43 / 4,3%	1 000 / 100%
Control (air-cleaned only)	1 month	917 / 91,7%	83 / 8,3%	1 000 / 100%
Cleaned with pure water, air-dried at 45 °C (2 min)	additional 3 months	789 / 78,9%	211 / 21,1%	1 000 / 100%
Control (air-cleaned only)	additional 3 months	732 / 73,2%	268 / 26,8%	1 000 / 100%

Healthy, unaffected by fungi, berries included only those that met the Ukrainian state standard requirements as defined in DSTU 5035:2008 – Cranberry Fresh (2008).

ther examination during storage period helped to reveal in berries the presence of the fungi *Diaporthe vaccinii* Shear (fig. 2-B) and *Botrytis cinerea* Pers. (fig. 2-D) as the causal agents of viscid and yellow fruit rots, respectively. During the development of viscid fruit rot caused by *D. vaccinii*, spores of *Alternaria* sp. were occasionally observed on affected berries; however, following surface sterilization, the fungus did not produce mycelium in culture, suggesting it was not actively involved in the infection process. Some rotten berries exhibited softened lesions beneath the epidermal tissue layer, with immersed conidiomata of *Coleophoma empetri* (Rostr.) Petr., which caused fruit ripe rot (fig. 2-A).

A substantial number of both historical and recent publications have addressed fungi associated with *Vaccinium* species and the various rots and other plant diseases they cause, often presenting differing viewpoints interpretations among research groups. Numerous studies have investigated pathogenic fungi affecting large cranberry during growth and storage across various countries in the Northern Hemisphere, particularly in Canada and the United States, where the plant is native. To date, approximately 20 fungal species have been identified as causal agents of storage-related diseases in *V. macrocarpon* (Olatinwo et al., 2003; Vilka et al., 2009b; Vilka & Bankina, 2013; Polashock et al., 2017).

In Europe, research on the pathogenic fungi causing fruit rot complex of cultivated large cranberry started in Latvia in 2006. The list of identified fungi includes *Allantophomopsis cytisporea*, *Botrytis cinerea*, *Coleophoma empetri*, *Diaporthe vaccinii* (syn. *Phomopsis vaccinii* Shear), *Discosia artocreas* (Tode) Fr., *Godronia cassandrae* Peck (syn. *Fusicoccum putrefaciens* Shear), *Pestalotia vaccinii* (Shear) Guba, *Phyllosticta elongata* Weid., and *Physalospora vaccinii* (Shear) Arx & E. Müll. (syn. *Acanthorhynchus vaccinii* Shear) (Vilka et al., 2009a; Vilka & Bankina, 2013). In Poland, during the 2013–2014 growing seasons, a type of viscid fruit rot was observed. Subsequent fungal isolation and ITS rDNA analysis revealed a complex infection caused by *Diaporthe vaccinii* and *D. eres* Nitschke (Michalecka et al., 2017). More recent molecular identification of fungi from *V. macrocarpon* plantations in Poland indicated the potential presence in fruits of large cranberry such fungal species as *Aspergillus creber* Jurjević, S.W. Peterson & B.W. Horn, *A. versicolor* (Vuill.) Tirab., *Penicillium chrysogenum* Thom, *P. corylophilum* Dierckx, *Pestalotiopsis scoparia* Maharachch., K.D. Hyde & Crous, *P. unicolor* Maharachch. & K.D. Hyde, *Physalospora vaccinii*, and *Simplicillium aogashimaense* Nonaka, Kaifuchi & Masuma. Leaves of large cranberry harbor a more diverse mycobiota. One-year-old leaf tissues were found to contain *Apiospora guangdongensis* C.F. Liao & Doilom, *Didymella prosopidis* (Crous & A.R. Wood) L.W. Hou, L. Cai & Crous, *Epicoccum layuense* Qian Chen, Crous & L. Cai, *E. tritici* Henn., *Penicillium onobense* C. Ramírez & A.T. Martínez, as well as unidentified species of *Discosia* and *Neopestalotiopsis*. In two-year-old plants, *Diaporthe vaccinii*, possibly *D. eres*, *Alternaria geophila* Dasz., *A. senecionicola* E.G. Simmons & C.F. Hill, *Aspergillus aflatoxiformans* Frisvad, Ezekiel, Samson & Houbraken, *A. flavus* Link, *A. fumigatus* Fresen., *A. pipericola* Frisvad, Samson & Houbraken, and *A. sydowii* (Bainier & Sartory) Thom & Church were detected. Three-year-old plants exhibited ITS regions closely matching *Aspergillus aflatoxiformans, A. flavus, A. fumigatus, A. pipericola, Diaporthe eres, Penicillium corylophilum, Pestalotiopsis scoparia, P. unicolor*, and *Physalospora vaccinii* (Oksińska et al., 2024)..

The natural conditions in Ukraine are comparatively drier and more continental than in other European regions where *Vaccinium macrocarpon* plantations are located, which may influence the diversity of associated fungal species. As this is the first study on fungi associated with *V. macrocarpon* in Ukraine, a preliminary list of the main identified species is presented below, along with their morphological characteristics and growth features in culture.

1. ***Botrytis cinerea*** **Pers.,** ***Synopsis Methodica Fungorum*** **(Göttingen). 2: 690 (1801).**

Conidiophores branched, smooth, pale brown, 15–18 µm thick, various length, with a stipe and branches. Conidia colourless, dry, obovoid to ellipsoid, smooth, 6–14 (16) x 4–6 µm, with protuberant hila.

In culture on PDA, colonies were grey to dark grey, cottony at 22–25 °C, in the dark. The strain was not forming sclerotia.

Distribution in Ukraine. The species is widely distributed across Ukraine.

Occurrence on *V. macrocarpon*: Rivne oblast, Sarny district, Verbivka village, 08.10.2019; Volyn oblast, Kamin-Kashyrsky district, Manevichy village, 02.10.2024.

Notes. The fungus is commonly found on various substrates and host plants. Its presence on *Vaccinium* berries has also been reported in neighboring European countries, including Belarus, Latvia, and Russia (Vilka et al., 2009b).

2. ***Coleophoma empetri*** **(Rostr.) Petr.,** ***Annales Mycologici*****. 27 (5/6): 331 (1929).** – *Septoria empetri* Rostr., *Meddr Grønland*, Biosc. 3: 574 (1888). – *Rhabdospora empetri* (Rostr.) Kuntze, *Revis. gen. pl.* (Leipzig) 3 (3): 511 (1898). – *Rhabdostromina empetri* (Rostr.) Died., *Annls mycol.* 19 (5–6): 297 (1921). –

Sporonema oxycocci Shear, *Bull. Torrey bot. Club*. 34 (6): 308 (1907).

Conidiomata immersed, subepidermal, pycnidial, flattened, brown, about 100 µm in diam, with uneven thickening of the dark brown wall in basal part, unilocular, ostiolate. Conidiophores lageniform, slightly colored, brownish. Paraphyses cylindrical to obclavate, among conidiophores. Conidiogenous cells small, 4–5 x 3,0–3,5 µm, enteroblastic, phialidic. Conidia colourless, cylindrical, unicellular, 10–12 (14) x 2,0–2,5 (3,0) µm, with obtuse apex, acute base, smooth, straight.

In culture on PDA, colonies were initially whitish, later becoming dark grey, thick, slightly raised, and cottony in texture at 22–25 °C, in the dark.

Distribution in Ukraine. Polissya, Crimea.

Occurrence on *V. macrocarpon*: Rivne oblast, Sarny district, Verbivka village, 08.10.2019.

Notes. The fungus is widespread and occurs in both the Northern and Southern Hemisphere, with a broad host range. It has been reported in Ukraine and neighboring countries on different host plants as well (Sutton, 1980; Dudka et al., 2004; Polashock et al., 2009, 2017; Farr, Rossman, 2020). Incidence of *C. empetri* varied between years in Latvia and reached 1,2–21,0% in storage (Vilka, Bankina, 2013).

3. ***Colletotrichum acutatum*** **J.H. Simmonds**, ***Queensland Journal of Agricultural and Animal Science*****. 25: 178A (1968).** – *Colletotrichum acutatum* J.H. Simmonds, *Queensland Journal of Agricultural and Animal Science*. 22: 458 (1965), nom. inval. – *Colletotrichum acutatum* f. *pineum* Arx, *The genera of fungi sporulating in pure culture*: 222 (1981), nom. inval. – *Glomerella acutata* Guerber & J.C. Correll, *Mycologia*. 93 (1): 225 (2001).

Conidiomata not clearly detectable, pale brown, acervular, subcuticular, dehiscence irregular. Conidiophores in aggregations, immersed, lageniform, colorless, septate, smooth. Conidiogenous cells not clearly separated from other cells, enteroblastic, phialidic. Conidia fusiform, colorless, unicellular, 6,0–9,0 (10,0) x 3,0–3,5 (4,5) µm, with acute apex and base, smooth, straight. Appressoria present, pale brown.

In culture on PDA, colonies exhibited pale grey-yellowish initial mycelium with a slightly orange reverse side, effuse; later turned greenish-grey, with limited aerial mycelium, eventually becoming blackish-grey when incubated at 22–25 °C, in the dark (Fig. 3-B).

Distribution in Ukraine. Occurrence on *V. macrocarpon*: Volyn oblast, Kamin-Kashyrsky district, Galuziya village, 14.10.2019; Manevichy village, 02.10.2024.

Notes. The fungus clearly belongs to the *Colletotrichum acutatum* species complex (Damm et al., 2012). The species *C. acutatum* s. str. is more often registered in Canada and the USA on species of *Vaccinium,* though it is known in the Netherlands, Norway and Spain on the same hosts (Oudemans et al., 1998; Olatinwo et al., 2003; Damm et al., 2012; Farr, Rossman, 2020). Another member of the complex, *Colletotrichum fioriniae* (Marcelino & Gouli) Pennycook, is associated in its development with *Vaccinium* and other host plants in temperate regions. Several *C. acutatum* strains, isolated from *Vaccinium* species of different geographical origin, have been reidentified as *C. fioriniae* upon further analysis. The fungus *C. fioriniae* was collected on *Vaccinium* species in Europe, including Italy and Poland (Damm et al., 2012). These findings suggest that additional *Colletotrichum* species may be present on cranberries in Ukraine.

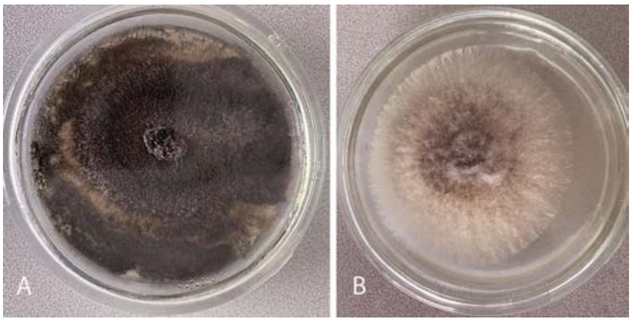

Fig. 3. Cultures of fungi isolated from fruit rots of *Vaccinium macrocarpon* on potato dextrose agar (PDA) at 25 °C.

A – *Diaporthe vaccinii;* B – *Colletotrichum acutatum*

4. ***Diaporthe vaccinii*** **Shear, in Shear, Stevens & Bain, *Technical Bulletin of the United States Department of Agriculture.* 258: 1 (1931).** – *Phomopsis vaccinii* Shear, N.E. Stevens & H.F. Bain, *Technical Bulletin of the United States Department of Agriculture.* 258: 7 (1931).

Conidiomata immersed, aggregated, eustromatic, brown, pycnidial, globose, 300-400 µm in diam, unilocular, ostiolate. Conidiophores lageniform, cylindrical, colorless, 10–12 µm. Conidiogenous cells enteroblastic, phialidic. Alpha conidia fusiform, elliptical, colorless, unicellular, 5,5–8,0 (9,0) x 2,5–3,5 (4,0) µm, smooth, straight, guttulate. Beta conidia filiform, colorless, unicellular, 7,5–11,0 x 0,5–0,8 µm, curved or hook-shaped (hamate).

In culture on PDA, colonies were initially whitish, later becoming blackish-grey, thick, prostrate, cottony in texture, with a zonate appearance and some immersed sporulation (Fig. 3-A).

Distribution in Ukraine. Occurrence on *V. macrocarpon*: Rivne oblast, Sarny district, Verbivka village, 08.10.2019.

Notes. Diaporthe vaccinii is a fungus with a temperate distribution in the Northern Hemisphere. Its occurrence on *Vaccinium* species has been reported in several European countries, including Belarus, Germany, Latvia, Lithuania, the Netherlands, Poland, Romania, Russia, UK (Wilcox, Falconer, 1961; Teodorescu et al., 1985; Farr et al., 2002; Kačergius et al., 2004; Vilka et al., 2009a; Vilka, Bankina, 2013; Narouei-Khandan et al., 2017).

Large cranberry fruit rot is a complex disease caused by different groups of fungi. One group initiates visible damage in the field, known as field rots, while another group causes deterioration during storage, referred to as storage rots. Additionally, a group of fungi with uncertain pathogenicity are frequently isolated from rotting berries (Oudemans et al., 1998). The full list of fungi affecting cranberry fruit includes approximately 35 species, which ecologically fall into the categories of hemibiotrophs, saprotrophs, and necrotrophs (McManus et al., 2003; Lombard et al., 2014; Polashock et al., 2017; Oksińska et al., 2024). However, fruit rots are most commonly associated with around 15 species of ascomycetous fungi (Oudemans et al., 1998; McManus et al., 2003; Aghel et al., 2023). These rots significantly reduce crop yield and shorten the shelf life of cranberries. The composition of pathogenic fungi species complexes varies among cranberry-growing regions, and fungi have been isolated from both visibly rotted and apparently healthy berries. Advances in molecular identification methods have expanded the known diversity of associated fungal species (Lombard et al., 2014; Oksińska et al., 2024).

In Ukrainian samples of cultivated large cranberry, the identified fungi were primarily phytopathogens associated with field rots, including *Coleophoma empetri, Colletotrichum acutatum,* and *Diaporthe vaccinii.* Among these, only *C. empetri* is typically linked to storage rot. The collected data showed that the proportion of visibly damaged berries remained relatively low (4,3% – cleaned, and 8,3% – control, Table 1) after one month of storage. However, symptoms increased with extended storage duration, accompanied by secondary fungal infections – most notably *Botrytis cinerea,* which can be associated with mechanical injury to berries (Polashock et al., 2017), as well as fungi of uncertain pathogenicity such as species of *Alternaria, Cladosporium,* and *Penicillium.*

The second pilot study was conducted from January to March 2020. It was based on fresh berries that had been manually harvested on January 12, 2020, as well as stored berries from the harvest of October 7–15, 2019. Samples were taken from healthy, sorted berries to assess the effect of different post-harvest cleaning methods on fungal infestation and the frequency of fungal colony development in agar medium. For each treatment, 50 fruits were used. Whole berries of *V. macrocarpon*, cleaned using the four previously described methods, were plated directly onto Petri plates with PDA and incubated for 20 days at 25 °C in dark, without fruit dissection or additional surface sterilization. The number and type of fungal colonies isolated in pure culture served as indicators of the effectiveness of the cleaning methods.

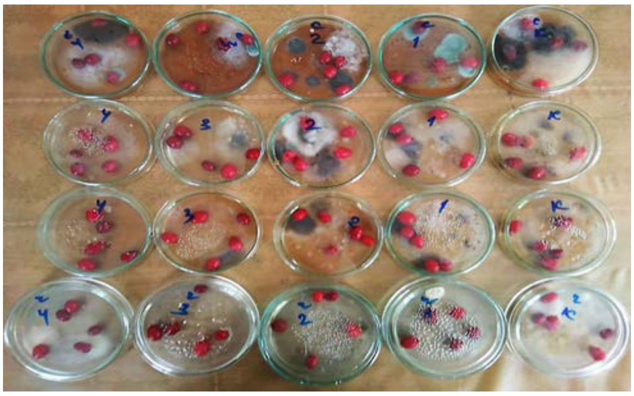

Fig. 4. Development of fungi colonies from the surfaces of post-harvest cleaned *Vaccinium macrocarpon* berries subjected to various experimental treatments, cultured on PDA at 25 °C.
Petri dish labels indicate the treatment variants listed in table 2

The incidence of fruit rots and fungal development in pure culture 20 days after post-harvest treatment of fresh berries is presented in table 2 and fig. 4. In addition to assessing the effects of the four primary treatments (variants 1–4) and the control – based on berries harvested in January 2020 – treatments 1 and 2 were also applied to berries harvested in October 2019. Furthermore, berries with early signs of rot were included as variant 6.

Assessment of fungi rot symptoms on *V. macrocarpon* berries during storage revealed that the lowest incidence of fungal infestation occurred under treatment variant 4, which involved washing the berries with pure water followed by air-drying at 45 °C until surface moisture evaporated. Under this method, only about 2% of berries showed visible development of fungal infection. In contrast, the highest rates of injured berries were observed in the untreated control (24%) and in berries with initial signs of rot (48%, variant 6). The mean incidence of fungi isolation in culture, along with the representation of dominant genera of fungi that germinated on agar medium (table 3), supports the conclusion that post-harvest cleaning methods could significantly reduce the development of fungal infections. Fungi were far more prevalent in untreated berries and those subjected to prolonged storage. Thus, it can be concluded that post-harvest cleaning followed by drying at 45 °C (variant 4) provides effective protection against fungal deterioration during storage (table 2).

Plant material cleaning with water at various temperatures effectively removes exogenous contaminants such as peat, soil, and decaying plant debris, which could serve as nutrient sources for fungal spore germination and mycelial growth. Subsequent air-drying of the harvested berries at different temperatures can further inhibit mycelium growth and fungal spore germination, which is typically more multiple in wet-harvested samples (McManus et al., 2003), thereby enhancing the storability of fresh fruits. Fruit rots in large cranberry (*V. macrocarpon*) are likely the result of multiple interacting factors, including invasion of natural fungal pathogens, mechanical damage, unfavorable storage conditions, and colonization by hemibiotrophic and saprotrophic fungi with broad substrate specificity. However, not all fungi isolated from berry surfaces during the study were capable of invading the undamaged fruit tissues during the indoor storage. This is likely to the antifungal properties of organic acids naturally present in cranberries, which inhibit fungal growth under standard storage conditions – characterized by limited nutrient availability and the absence of excessive moisture. Consequently, visible symptoms of fungal rot do not always manifest on the fruit surfaces.

Conclusions. In conclusion, harvested large cranberry, *Vaccinium macrocarpon*, on depleted peatlands in the Rivne and Volyn regions has exhibited post-harvest fruit rots associated with various invasive fungi previously unstudied on this host plant in Ukraine. Previously unreported four main fruit-associated fungi were identified during storage of *V. macrocarpon* berries, including *Botrytis cinerea, Coleophoma empetri, Colletotrichum acutatum,* and *Diaporthe vaccinii.* Among these, *C. empetri, C. acutatum,* and *D. vaccinii* **are recognized as field pathogens responsible for fruit**

Table 2

Percentage of *Vaccinium macrocarpon* berries exhibiting symptoms of fungal invasion and frequency of fungal colony development in berry samples subjected to different post-harvest cleaning methods (after 3 months of storage)

Treatment Method	Petri plate indicator	Infected berries (per 50) / %	Mean incidence of fungal colony development (4 replicates)
1 – Cleaned with pure water, air-dried at 45 °C for 2 min, then at 20 °C; sampled 12.01.2020	4	3 / 6%	5
2 – Cleaned with 45 °C water for 2 min, air-dried at 20 °C; sampled 12.01.2020	3	4 / 8%	7
3 – Cleaned with 20 °C water for 2 min, air-dried at 20 °C; sampled 12.01.2020	2	2 / 4%	12
4 – Cleaned with pure water, air-dried at 45 °C until water evaporated; sampled 12.01.2020	1	1 / 2%	11
5 – Control (no treatment); sampled 12.01.2020	к	12 / 24%	11
1a – Cleaned with pure water, air-dried at 45 °C for 2 min, then at 20 °C; sampled 15.10.2019	1c	7 / 14%	50
2a – Cleaned with 45 °C water for 2 min, air-dried at 20 °C; sampled 15.10.2019	2c	8 / 16%	31
6 – With visible fungal rot symptoms, no treatment; sampled 12.01.2020	0	24 / 48%	50

Table 3

Dominant fungal genera of uncertain pathogenicity isolated from post-harvest cleaned berries of large cranberry (*Vaccinium macrocarpon*) in different experimental variants

Post-Harvest Treatment Variant (according table 2)	Dominant Fungal Genera Isolated from Berry Surfaces
1	*Aspergillus*, *Penicillium*
2	*Aspergillus*, *Penicillium*
3	*Alternaria*, *Cladosporium*
4	*Cladosporium*, *Penicillium*
5	*Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Penicillium*
1a	*Alternaria*, *Aspergillus*, *Penicillium*
2a	*Alternaria*, *Aspergillus*, *Penicillium*
6	*Aspergillus*, *Alternaria*, *Cladosporium*, *Epicoccum*, *Penicillium*, etc.

rots (ripe, bitter and viscid accordingly) under natural growing conditions. Fungus *C. empetri* is only associated with a storage-related rots in Ukraine. *B. cinerea* caused yellow rot of the damaged berries. Overall, the incidence of fungal invasion during storage was relatively low. Notably, *C. acutatum* and *D. vaccinii* – both ascomycetous fungi – were reported for the first time in Ukraine for its mycobiota, based on microscopic identification and study of the cultures isolated from the studied berries. Fruit rot dynamics and post-harvest treatment efficacy studied for the "Wilcox" cultivar cultivated in the Ukrainian Polissya. The incidence of fruit rots damage in *V. macrocarpon* during the storage at temperature 2–8 °C was 8,3% after one month and increased to 26,8% after three months. The post-harvest additional fruit cleaning significantly improved storability: berries washed with water and air-dried at 45 °C showed a lower incidence of rot – 4,3% after one month period and 8,3% after three months. Further experiments involving fungal isolation in pure culture demonstrated that cleaning berries with water jets at 45 °C followed by drying with warm air at the same temperature significantly reduced the incidence of fruit pathogens.

Prospect of further research: Natural bogs hosting wild marsh cranberry (*V. oxycoccos*) may serve as reservoirs for pathogenic fungi potentially affecting *V. macrocarpon* in cultivated settings. Considering the recent trend of higher average temperatures, further detailed studies of the fungal communities associated with *V. oxycoccos* are necessary. Such investigations may clarify the further risks and prospects of expanding large cranberry cultivation under changing climatic conditions.

Acknowledgements. The author expresses sincere gratitude to Dr. Valeriy Konovalchuk, former scientist of the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, for his generous contribution of multiple fruit samples and the help in performing field study. The author would like to thank Dr. Olena Bashta, assoc. prof. of the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, for help in organization of experiment on berries contamination by *Aspergillus* and *Penicillium* fungi.

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Стаття надійшла до редакції 12.05.2025
Стаття прийнята до друку 19.07.2025
Опубліковано: 15.10.2025

Conflict of interests: There is no conflict of interests related to this research study.
Author's contribution:
Andrianova T.V. – data collection, literature analysis, research, writing of the article, conclusions, abstracts.

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