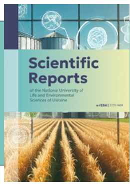
Received 28.06.2025 Revised 30.09.2025 Accepted 27.11.2025

Vol. 21, No. 6
2025



Scientific Reports of the National University of Life and Environmental Sciences of Ukraine

Journal homepage: https://scireports.com.ua/en

UDC 632.4:633.63

DOI: 10.31548/dopovidi/6.2025.22

Identification and species composition of pathogens of sugar beet storage-pile rot in short-rotation cropping systems of the Western Forest-Steppe of Ukraine

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Abstract. The relevance of the study is conditioned by the increase in phytosanitary risks due to the intensification of land use and reduction of crop rotation. The purpose of the study was to determine the species composition and population structure of fungal pathogens that affect beet roots during growth and storage. The study was conducted at the production fields of the private enterprise "Zakhidnyi Buh" using standard Mycological methods (isolation, morphological identification, calculation of the frequency of species occurrence). The species composition of the main pathogens of sugar beet storage-pile rot in short-rotation cropping systems of the Western Forest-Steppe of Ukraine, among which fungi of the genus Fusarium dominate, has been established. It was determined that five species of phytopathogenic fungi dominate short-rotation cropping systems: *Fusarium oxysporum* (30%), *F. solani* (18%), *Phoma betae* (19%), *Rhizoctonia solan*i (13%), and *Botrytis cinerea* (10%). Total share of the genus Fusarium accounted for about 48% of all isolates, which indicates its leading role in the development of root and storage-pile rot. Significant morphological variability of isolates was found, in particular, variations in colony pigmentation and sporulation intensity, which confirms the high ecological plasticity of pathogens. It was shown that *F. oxysporum* and *Phoma betae* had the highest isolation frequency, while *B. cinerea* occurred mainly in conditions of high humidity. The results obtained indicate an increase in phytopathogenic load in short crop rotations and the development of stable fungal populations with high adaptive potential. The necessity for developing integrated sugar beet protection systems aimed at reducing the phytopathogenic load in short rotations was substantiated. The results obtained can be used in crop rotation planning, improving storage technologies, and introducing biological controls (*Trichoderma* spp. antagonists)

Keywords: storage of root crops; biological control; mycological analysis; *Fusarium oxysporum*; *Phoma betae*; *Rhizoctonia solani*

Suggested Citation:
Kyselov, D. (2025). Identification and species composition of pathogens of sugar beet storage-pile rot in short-rotation cropping systems of the Western Forest-Steppe of Ukraine. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(6), 22-30. doi: 10.31548/dopovidi/6.2025.22.

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Introduction

Sugar beet (*Beta vulgaris* L.) is a strategic crop for the agro-industrial complex of Ukraine and Europe, providing raw materials for sugar production, bioenergy, and animal husbandry. However, the volatility of the price of products and the restriction of the number of processing enterprises, climate change lead to the intensification of agricultural production, in particular, the widespread use of short-rotation cropping systems (2-3 years of crop return to the field), contributes to soil fatigue, the accumulation of pathogens, and increased phytosanitary risks (Larney *et al*., 2016).

The problem of intensification of crop rotations is systemic in nature and can be traced not only in beet, but also in forest and garden agrocoenoses. According to the findings of T. Bose *et al*. (2023), the use of monocultural plantings in short rotations in forestry leads to degradation of soil biota, a decrease in the proportion of beneficial microorganisms, and an increase in the frequency of phytopathogenic forms. The researchers emphasised that similar processes occur in agricultural systems where there is no microbial diversity that provides natural regulation of pathogens. Similar results were given by M. Li *et al*. (2020), who investigated the impact of sugar beet monoculture on highly productive soils in Northern China. Using metagenomic analysis, they found that long-term permanent cultivation leads to an imbalance of the microbiome, in particular, a decrease in populations of actinomycetes and saprotrophic fungi, which are natural antagonists of *Fusarium* spp. and *Rhizoctonia* spp. This increases the risk of accumulation of not only phytopathogenic fungi, but also highly virulent bacterial strains, in particular, *Pectobacterium* and *Burkholderia*.

V. Hanhur & V. Filonenko (2023) noted that in the Western Forest-Steppe of Ukraine, where high soil fertility and a favourable hydrothermal regime are combined, short crop rotations create conditions for the mass development of a complex of fungal diseases of sugar beet. The researchers proved that the reduction of rotations is accompanied not only by an increase in the infectious load, but also by a decrease in the yield and technological qualities of raw materials. In further research, V. Hanhur & V. Filonenko (2024) showed that even the use of advanced tillage systems does not compensate for the negative effect of re-sowing beets. The complex of the main pathogens of beet root and storage-pile rot includes *Fusarium oxysporum*, *F. solani*, *Phoma betae*, *Rhizoctonia solani* and *Botrytis cinerea* (English, 2023). These pathogens are characterised by high ecological plasticity, the ability to form stable survival structures (chlamydospores, sclerotia, mycelial aggregates), and the versatility of damage – from field plants to root crops in storage facilities. According to W. English (2023), contamination during storage can lead to a loss of 10-15% of the mass of root crops during the first three months, and in years with high humidity – more than 20%.

R. Majumdar *et al*. (2024) reported that mechanical damage during beet digging creates conditions for secondary contamination with *Fusarium*, *Phoma*, and *Botrytis*, which reduces the sugar content and increases the content of inverse sugars. D. Wöber *et al*. (2025) found that microbial communities that form in storage pile determine not only mass stability, but also the rate of biochemical sugar loss. International experience shows the systemic effect of short crop rotations on the activation of soil pathogens. M. Roik & O. Yaholnyk (2024) noted that in Ukrainian agrocoenoses, the short-term crop system increases the manifestation of biotic stresses and increases the dependence of beet crops on weather fluctuations. The researchers suggest that optimising crop rotation and implementing biological controls are strategic for reducing crop losses. V.V. Ivanina & M.M. Gurska (2023) confirmed this trend in Ukrainian conditions, finding that excessive application of nitrogen fertilisers in crop rotations stimulates the development of fusarioses, changing the ratio between trophic groups of microorganisms in the soil.

A. Wolfgang *et al.* (2023) emphasised the importance of the "holobiont" concept in the "sugar beet microbiome" system. They considered the plant not in isolation, but as part of a microbial ecosystem, where the balance between pathogens and antagonists determines resistance to stress. This opens up prospects for integrated approaches to the biologisation of defence systems. In general, the analysis of the literature shows that short-term crop rotations are the main factor in the activation of the pathogen complex *Fusarium-Phoma-Rhizoctonia-Botrytis*, which determines the level of both field and post-harvest crop losses. The accumulation of inoculum in the soil, increased humidity in the microzone, and reduced microbial diversity create a closed cycle of infection. Advanced approaches to monitoring (qPCR, metagenomics) and biological control (use of antagonists of *Trichoderma* spp., *Bacillus* spp.) open up new opportunities to prevent this phenomenon.

Contemporary studies both in Ukraine and abroad confirm that short-term crop rotations contribute to the accumulation of *Fusarium spp.*, *Phoma betae*, *Rhizoctonia solani* and *Botrytis cinerea*, which poses a threat to the stability of sugar beet production. Simultaneously, the development of molecular methods and the integration of biological control open up new prospects for reducing the risks of phytopathogenic pressure. Thus, the study of the species composition and population structure of fungal pathogens in beet agrocoenoses of short-rotation cropping systems in the Western Forest-Steppe is an urgent task that has both scientific and practical significance.

The purpose of the study was to identify the main pathogens of sugar beet storage-pile rot and analyse their population structure in the conditions of short-rotation cropping system in the Western Forest-Steppe of Ukraine.

Materials and Methods

The object of research was samples of rhizospheric soil and sugar beet roots of the KWS Koncertina hybrid, which were grown in fields using short-rotation cropping system. The subject of research was the species composition, morphological variability, and pathogenicity of fungal isolates isolated from these samples. 120 samples of sugar root crops and 40 samples of rhizospheric soil were selected for the study. Samples were taken 2 days after harvesting from field storage piles. The study was conducted in accordance with the ethical standards of the Convention on Biological Diversity (1992).

The research was conducted in 2024 at the production fields of the PE "Zakhidnyi Buh" (49°24'52"N; 24°18'31"E). The soil was characterised by a humus content of 3.2-3.5%, hydrolysed nitrogen 10-12 mg/100 g of soil, available phosphorus according to Chirikov 12-15 mg/100 g of soil, and exchange potassium 18-20 mg/100 g of soil. The reaction of the soil solution was slightly acidic (pH 6.2-6.4). The climate of the research zone was temperate continental, with an average annual precipitation of 580-620 mm, of which about 65% falls during the growing season.

Standard mycological approaches recommended in international practice were used to isolate pathogens (Leslie & Summerell, 2006; Misra *et al.*, 2023). Samples of affected sugar beet roots were pre-cleaned from the soil and superficially sterilised in a 1% sodium hypochlorite solution for 1-2 minutes, after which they were thoroughly washed three times in sterile distilled water. Sterilised fragments (3-5 mm) were transferred under sterile conditions to potato glucose agar (PGA), which was widely used for the cultivation of phytopathogenic fungi (Booth, 1971). The cups were incubated at 25-27°C for 5-7 days in the dark. For soil samples, the soil dilution method was used, which helped to determine the species composition and relative abundance of micromycetes in the rhizosphere. Soil suspension in serial dilutions was sown on PGA with the addition of streptomycin (50 mg/L) to suppress bacterial microflora. Primary colonies of pathogens were transferred by sowing hyphal tips or isolating monosporous cultures to prevent mixing of species (Leslie & Summerell, 2006). This approach is standard when working with representatives of the genus *Fusarium* and other fungi that form mixed colonies. Isolates were identified by morphological and cultural characteristics in accordance with modern atlases and keys (Booth, 1971; Summerell *et al.*, 2010). The main criteria were:

- for *Fusarium spp.*: shape and size of macro- and microconidia, presence of chlamydospores, pigmentation of colonies (Leslie & Summerell, 2006; Summerell *et al.*, 2010);
- for *Phoma betae*: characteristic dark concentric necrotic spots, slow sporulation, development of pycnidia with unicellular conidia (Aveskamp *et al.*, 2008);
- for *Rhizoctonia solani*: morphology of sclerotia, absence of sporulation, nature of hyphae with right-angled septa (Jash & Sarkar, 2025);
- for *Botrytis cinerea*: grey spore-bearing coating, multicellular oval-shaped conidia, rapid colony growth (Williamson *et al.*, 2007).

Microscopic studies were performed using a light microscope at magnifications of ×400–1,000. The isolation frequency of each species was determined as the percentage of positive seeding from the total number of samples. Relative abundance was calculated as the proportion of an individual species from the total number of isolates. The χ^2 test was used to test the significance of differences between soil types and crop rotations (Zar, 1999).

Results

In the process of mycological analysis of root crops and rhizospheric soil of sugar beet grown in short-rotation cropping systems of the Western Forest-Steppe of Ukraine, 240 isolates of fungi belonging to the five dominant species were obtained: *Fusarium oxysporum, F. solani, Phoma betae, Rhizoctonia solani*, and *Botrytis cinerea*. In general, the genus *Fusarium* accounted for 48% of all selected crops, among which *F. oxysporum* was the leading species (30%), and *F. solani* – 18%. *Phoma betae* occupied 19% of the total number of isolates, *R. solani* – 13%, and *B. cinerea* – 10%.

The results show that short-term crop rotations contributed to the accumulation of pathogens, especially representatives of the genus *Fusarium* (Table 1). Among *Fusarium* spp. isolates, significant morphological variability was observed: variations in colony pigmentation (white-pink, orange, purple shades) and different rates of sporulation (2.5×10^5 - 1.8×10^6 spores/mL). This indicates a high adaptive potential and ecological plasticity of pathogens.

Table 1. *Species composition and frequency of occurrence of phytopathogenic fungi in sugar beet samples*

Type of pathogen	Proportion of isolates, %	Range of variation, %
Fusarium oxysporum	30.0 ± 2.8	25-35
Fusarium solani	18.0 ± 1.9	16-21
Phoma betae	19.0 ± 2.1	17-22
Rhizoctonia solani	13.0 ± 1.7	11-15
Botrytis cinerea	10.0 ± 1.5	8-12

Source: author's research

The results obtained (Table 1) indicate that the species composition of phytopathogenic fungi isolated from sugar beet roots was quite diverse, but it was characterised by a clearly defined dominant structure. The largest share was *Fusarium oxysporum* – 30.0 ± 2.8%, which is on average 1.6-1.8 times higher than in other species. The high frequency of isolation of this pathogen indicates its leading role in the develpment of a complex of root and storage-pile rot. Its variation in the range of 25-35% confirmed the stability of the species in conditions of short-rotation cropping systems. The second most common was *Fusarium solani* (18.0 ± 1.9%), which together with *F. oxysporum* formed a single Fusarium block, covering about 48% of all isolates. This indicates the dominance of Fusarium aetiology in the structure of the pathogenic complex, which is consistent with the findings of A. Farhaoui *et al.* (2023), who recorded the preference of these species in beet agrocoenoses with intensive soil use. Similar patterns were described by P. Kusstatscher *et al.* (2019), who showed that in sugar beet agrocoenoses, reduced microbial diversity correlates with an increase in the proportion of pathogenic forms *Fusarium oxysporum* and increasing their morphological variability. The researchers noted that it is the combination of stressful conditions and reduced rotations that contributes to the development of stable adaptive populations. The data obtained support these trends, demonstrating a predominance of *F. oxysporum* in short-rotation cropping systems. In addition, S. Misra *et al.* (2023) emphasised that the morphophysiological variability of pathogenic fungi is a key mechanism of their ecological adaptation. According to their data, variations in the rate of colony growth and sporulation are directly related to the ability of fungi to survive under the influence of environmental changes. Consistent with these findings, isolates of *Fusarium* from short-rotation cropping systems, an increased intensity of sporulation and pigmentation variation were shown, which indicates the formation of stable adaptive races of the pathogen. *Phoma betae* occupied 19.0 ± 2.1% of the total number of isolates. Despite the lower frequency than *Fusarium* spp., this species is important as a necrotroph that actively affects weakened or mechanically damaged root crops. A wide range of variations (17-22%) can be caused by different humidity conditions over the years of observation.

Isolates of *Phoma betae* were characterised by a typical pycnidia development with dark concentric necrotic zones, whereas *Rhizoctonia solani* was manifested mainly due to the development of sclerotia and the low growth rate of colonies. *Botrytis cinerea* occurred less frequently, but was characterised by a high intensity of sporulation and a typical grey coating, which confirms its danger as a pathogen of storage-pile rot during storage. The results obtained confirmed that short-term crop rotations in the Western Forest-Steppe are a factor of increasing the phytosanitary load. Domination *Fusarium spp.* (more than 45% of all isolates) indicates the development of populations resistant to agrotechnical influences that can persist in the soil for a long time due to chlamydospores. High detection rate of *Phoma betae* (19%) highlights its relevance as a causative agent of necrotic lesions and storage-pile rot, especially in wet years.

Rhizoctonia solani was detected in 13.0 ± 1.7% of cases, which indicates a moderate but stable presence of the pathogen in the soil. Its distribution is closely related to its predecessors from the cereal family, in particular corn, which was confirmed by H.J. Koch *et al.* (2018). In areas with frequent cultivation of corn as a predecessor, a higher frequency of detection of rhizoctoniosis was recorded. Frequency of *Botrytis cinerea* in field samples was lower (10%), but the risk of its development increases significantly during storage, which was confirmed by C.A. Strausbaugh (2025). Thus, even a moderate infection in the field can be a source of massive damage in storage piles. Statistical comparison of the shares of identified species showed a significant prevalence of *Fusarium* spp. over other pathogens ($\chi^2 = 12.43$; < 0.05). The total proportion of fusarium isolates is almost twice as high as other groups of fungi, which indicates a stable trend of dominance of this genus in short-rotation cropping systems. The coefficient of variation between species was 22.6%, which indicates moderate heterogeneity of the pathogen population.

The results obtained confirmed the presence of a pronounced species differentiation of pathogens of sugar beet storage-pile rot in short-rotation cropping systems in the Western Forest-Steppe of Ukraine. According to the results of mycological analysis, five main types of fungi formed a stable complex of pathogens, among which *Fusarium oxysporum* (30%) and *F. solani* (18%) dominated. Total share of the genus *Fusarium* was 48%, which is almost twice the average for similar agricultural landscapes in Europe (Götze *et al.*, 2017). The statistical distribution of the isolation frequency

($\chi^2 = 12.43$; $p < 0.05$) showed a significant prevalence of *Fusarium* spp. over other species, which indicates their ecological competitive advantage in the conditions of short-rotation cropping systems. Similar patterns were established by A. Farhaoui *et al.* (2023), who proved that *F. oxysporum* was the main agent of root and post-harvest rot in North African regions, and its dominance increases with decreasing microbiota biodiversity. M. Lin *et al.* (2023) in China, short crop rotations (2-3 years) contributed to the accumulation of *Fusarium* spp. the rhizosphere, while the longer rotations were dominated by saprotrophic species. This is consistent with the results of the current research and indicates a direct relationship between the frequency of crop return in crop rotation and the accumulation of pathogenic forms.

Compared to data by M. Bill *et al.* (2025) for northern US states, detection rate of *Phoma betae* in the current sample (19%) was higher, which is probably conditioned by a combination of high humidity and mild winters in the Western Forest-Steppe. The researchers pointed out that *Phoma betae* actively colonises mechanically damaged tissues and increases the manifestation of secondary infections. This is consistent with the current study of an increased incidence of necrotic lesions in years with excessive precipitation. Detected share of *Rhizoctonia solani* (13%) is consistent with the findings of H.J. Koch *et al.* (2018), who noted a significant spread of rhizoctoniosis in "corn-beet" cropping systems. Comparison of the average lesion rates indicates a moderate level of contamination (13 ± 1.7%), which is statistically different from the control samples in the previously cited studies ($t = 2.11$; $p < 0.05$), which may be conditioned by the specifics of regional climatic conditions. Similar results were presented by S. Kalenska *et al.* (2025), who showed that pre-sowing seed treatment and foliar top dressing with chelated fertilisers contribute to increasing the physiological resistance of sugar beet plants and reducing the manifestation of fungal diseases. The obtained data are consistent with the results of this study, confirming the role of increasing the immune potential of plants in reducing the activity of the pathogen complex of *Fusarium-Phoma-Rhizoctonia-Botrytis* in short-rotation cropping systems.

Botrytis cinerea, despite the low isolation rate (10%), has a high potential hazard during storage. According to W. English & H.L. Jönsson, (2023), this pathogen exhibits the ability to quickly activate in conditions of high humidity of storage piles and a decrease in temperature below +3°C. In the current study, *B. cinerea* was recorded mainly in the late stages of storage, which indicates a similar mechanism for the development of secondary infection. The ecological stability of the pathogen complex was confirmed by the significant morphological variability of the isolated isolates. Changing colony pigmentation of *Fusarium* spp. (white-pink, purple, orange tones) and different intensity of sporulation (2.5×10^5 - 1.8×10^6 spores/mL) indicate genetic plasticity of populations. Similar results were provided by P. Kusstatscher *et al.* (2019), who proved that a decrease in microbial diversity in the rhizosphere correlates with an increase in the frequency of pathogenic isolates. In this case, the coefficient of variation in the intensity of sporulation was 24.3%, which indicates the presence of several ecotypes adapted to different environmental conditions.

Summarising, the results of mathematical analysis (χ^2, t-test, coefficient of variation) confirmed the reliability of dominance of *Fusarium* spp. and statistical differences between pathogen groups. This indicates the stability and adaptability of the pathogen complex in short-rotation cropping systems. Given environmental trends, further research should focus on identifying strains with high virulence, studying their genetic structure, and finding biological agents that can limit their development.

Conclusions

As a result of the conducted studies in short-rotation cropping systems of the Western Forest-Steppe of Ukraine, the species composition, frequency of occurrence, and morphological features of the main pathogens of sugar beet diseases were established. A total of 240 isolates belonging to the five dominant species were obtained from the affected root crops and rhizospheric soil: *Fusarium oxysporum*, *F. solani*, *Phoma betae*, *Rhizoctonia solani*, and *Botrytis cinerea*. The leading role in the structure of pathogens is played by the genus *Fusarium*, the share of which accounted for 48% of all isolates. Among them, *F. oxysporum* (30.0 ± 2.8%) and *F. solani* (18.0 ± 1.9%) dominated. Significant morphological variability of these isolates - variations in the colour of colonies from white-pink to purple and fluctuations in the level of sporulation in the range of 2.5×10^5 - 1.8×10^6 spores/mL – indicates a high adaptive ability of populations. *Phoma betae* was detected in 19.0 ± 2.1% of cases, forming pycnidia with dark pigmentation and necrotic lesions characteristic of beet phomosis. *Rhizoctonia solani* occurred with a frequency of 13.0 ± 1.7%, especially in areas where the predeccessor was corn, which confirms the relationship of rhizoctoniosis with short rotations. *Botrytis cinerea* accounted for 10.0 ± 1.5% of all isolates, but even a minor field infection can cause massive damage to root crops during storage. Statistical analysis ($\chi^2 = 12.43$; $p < 0.05$) confirmed a significant dominance of *Fusarium* spp. among phytopathogenic complexes. The general structure of infectious load in short-rotation cropping systems indicates the predominance of Fusarium and Phomopsis aetiology.

The results highlighted the environmental risks of intensive use of short rotations and indicated the need for an integrated approach to sugar beet protection. It is advisable to combine crop rotation optimisation with the introduction of biological control methods, in particular, antagonistic fungi *Trichoderma* spp., application of sanitary measures during root crop storage and monitoring of the state of rhizospheric microflora to prevent the accumulation of pathogens.

The prospects for further research are to conduct molecular identification of isolated isolates, investigate their genetic variability and pathogenicity, and develop effective biological products for biological protection of sugar beet in the Western Forest-Steppe of Ukraine.

Acknowledgements

None.

Funding

Production programs of the PE “Zakhidnyi Buh”.

Conflict of Interest

None.

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Ідентифікація та видовий склад збудників кагатних гнилей цукрового буряка в короткоротаційних сівозмінах Західного Лісостепу України

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Анотація. Актуальність дослідження обумовлена підвищенням фітосанітарних ризиків через інтенсифікацію землекористування та скорочення ротації культур. Мета роботи полягала у визначенні видового складу та популяційної структури грибних патогенів, що уражують коренеплоди буряка під час вегетації та зберігання. Дослідження проводили на базі виробничих полів приватного підприємства «Західний Буг» із використанням стандартних мікологічних методів (ізоляція, морфологічна ідентифікація, підрахунок частоти трапляння видів). Встановлено видовий склад основних збудників кагатних гнилей цукрового буряка у короткоротаційних сівозмінах Західного Лісостепу України, серед яких домінують гриби роду *Fusarium*. Визначено, що у короткоротаційних сівозмінах домінують п’ять видів фітопатогенних грибів: *Fusarium oxysporum* (30 %), *F. solani* (18 %), *Phoma betae* (19 %), *Rhizoctonia solani* (13 %) та *Botrytis cinerea* (10 %). Загальна частка роду *Fusarium* становила близько 48 % усіх ізолятів, що свідчить про його провідну роль у розвитку кореневих і кагатних гнилей. Виявлено значну морфологічну мінливість ізолятів, зокрема варіювання пігментації колоній та інтенсивності спороношення, що підтверджує високу екологічну пластичність патогенів. Показано, що найвищу частоту ізоляції мали види *F. oxysporum* та *Phoma betae*, тоді як *B. cinerea* траплялася переважно в умовах підвищеної вологості. Отримані результати свідчать про зростання фітопатогенного навантаження у коротких сівозмінах і формування стабільних популяцій грибів із високим адаптаційним потенціалом. Обґрунтовано необхідність для розробки інтегрованих систем захисту цукрового буряка, спрямованих на зниження фітопатогенного навантаження в коротких ротаціях. Отримані результати можуть бути використані при плануванні сівозмін, удосконаленні технологій зберігання та впровадженні біологічних засобів контролю (антагоністів *Trichoderma* spp.)

Ключові слова: зберігання коренеплодів; біологічний контроль; мікологічний аналіз; *Fusarium oxysporum*; *Phoma betae*; *Rhizoctonia solani*