

MYCOFLORA OF STRAWBERRY PLANTS

Goal. To identify fungi associated with the tissues of strawberry plants and evaluate the frequency of their isolation at different stages of plant growth. **Methods.** The research was conducted in the Right Bank Forest Steppe of Ukraine (Cherkasy Region) in 2021–2023, cv. ‘Clery’. Plant samples were taken in the phases of rosette formation (April), budding-flowering (May), fruit ripening (June) and in the post-harvest period (July–August). The analysis was carried out in laboratory conditions using macroscopic and biological methods. **Results.** During the research period, fungi belonging to 27 genera were isolated from strawberry tissues. In general, they can be divided into three groups: pathogens of leaves, which is the most numerous, berries, roots and crown. Representatives of the genera *Alternaria*, which were isolated from 57–100% of samples, and *Fusarium* (40–88%) occurred most often. Less often, but also during the entire growing season, *Penicillium* spp. (36–69%), *Podosphaera aphanis* (11–80%), *Botrytis cinerea* (27–67%), *Paraphomopsis obscurans* (24–77%) were isolated. Among the leaf diseases powdery mildew was recorded during all growing season, with a maximum in the fruit ripening phase. White leaf spot was observed starting from the budding-flowering phase, and leaf scorch was found from the fruit ripening phase. Gray rot was the most common fruit disease. The root system was more often affected by *Fusarium* spp. **Conclusions.** The obtained results demonstrate that during the entire growing season fungi of the genera *Alternaria*, *Fusarium*, *Penicillium*, as well as *Podosphaera aphanis*, *Botrytis cinerea* and *Paraphomopsis obscurans* were most often isolated from strawberry tissues. *R. grevilleana* was isolated starting from the budding-flowering phase, and *D. earlianum* from the fruit ripening phase. Species from the genera *Podosphaera*, *Ramularia*, *Diplocarpon*, *Colletotrichum*, *Pestalotiopsis*, *Verticillium*, *Sclerotinia*, *Gnomoniopsis*, *Rhizoctonia*, *Coniella*, *Rhizopus*, *Septoria*, *Cylindrocarpon* require constant control of their spread. Under favorable conditions for their development, they can cause damage to plants and a significant

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lack of harvest. The seasonal monitoring of fungi on strawberry provides a means for establishing the optimal periods for their control and developing effective disease protection system.

strawberry; mycoflora; species composition; isolation frequency; monitoring; leaf diseases; berry diseases; root and crown rots

According to the State Statistics Service, the total area of strawberry plantations in Ukraine is 7.1 thousand hectares. At the same time, only 1.1 thousand hectares are in commercial production. The average crop yield reaches 7.48 t/ha [1]. Strawberry (*Fragaria* L.) is valuable for the high taste, nutritional, medicinal and dietary properties of the fruits. The strawberry is the first to open the season of fresh berries. They are especially rich in sugars, organic acids, pectin, vitamins (C, P, Bg, B2, E, K) and mineral ele-

ments (potassium, phosphorus, calcium, sodium, magnesium, iron, iodine). Berries are consumed fresh and processed. The yield and quality depend on the place where strawberries are grown, the weather conditions of the year, the cultivar, the special cultural practices.

The most widespread strawberry diseases in the world are anthracnose (black spot) — the pathogens are *Colletotrichum* spp., phytophthora root rot — *Phytophthora fragariae* var. *fragariae* Hickman, fusarium wilt — *Fusarium oxysporum* f. sp. *fragariae* Winks et Williams, powdery mildew — *Podosphaera aphanis* (Wallr.) U. Braun & S. Takam (*Sphaerotheca macularis* (Wall. ex Fries) Jazz f. sp. *fragariae* (Peries), white spot — *Ramularia grevilleana* (Tul. & C. Tul. ex Oudem.) Jurrst. (*Ramularia tulasnei* Sacc., *Cylindrosporium grevilleanum* Tul. & C. Tul. ex Oudem, *Mycosphaerella fragariae* (Tul.) Lind.), leaf scorch — *Diplocarpon earlianum* (Ellis & Everh.) F.A. Wolf (*Marssonina potentillae* (Desm.) J.C. Fisch., *Ascochyta fragariae* Sacc., *Phyllosticta potentillae* Desm.), leaf blight — *Paraphomopsis obscurans* (Ellis & Everh.) Udayanga & Castl. (*Phomopsis obscurans* (Ellis & Everh.) B. Sutton) [2–5]. These diseases lead to a significant decrease in the productivity of plantations (from 15 to 92%), and their epiphytotic development can cause up to 100% yield losses [2, 6, 7]. In addition, due to diseases the general weakening of plants occurs, and as a result fruit-bearing plantations form lower yield next year and the yield of standard seedlings in nurseries decreases [6, 7, 8].

In Ukraine, root rot, leaf scorch, white spot, powdery mildew, verticillium wilt and gray rot are considered the main diseases of strawberries [6, 7, 9, 10, 11]. At the same time, when studying pathogens of strawberry diseases, the main atten-

tion was focused on their harmfulness, plant resistance, and the impact of protective measures. Little attention was paid to the processes of formation of mycoflora complexes on strawberry. A wider study of this issue will be important for the improvement of protective measures, may become the basis for their rational selection and increase the effectiveness of their application.

The goal of the research was to identify fungi associated with the tissues of strawberry plants and to estimate the frequency of their isolation in different periods of the growing season.

Methods of investigation. Surveys of fruit-bearing strawberry crops were carried out under conditions of the Right Bank Forest Steppe of Ukraine (Cherkasy Region) in 2021–2023 on the cv. ‘Clary’. Plant samples were taken in the phases of rosette formation (April), budding-flowering (May), fruit ripening (June) and in the post-harvest period (July-August). Samples were labeled and delivered in paper bags to the laboratory, where they were analyzed using macroscopic and biological methods. At the first stage, microscopic methods were used to detect the sporulation of fungi. In order to stimulate mycelial growth and improve sporulation, a moist chamber and nutrient medium were used. The tissue segments were washed with tap water, sterilized with 96% ethanol and washed twice with sterile water. Then they were placed in a humid chamber and in Petri dishes with potato-glucose agar (PDA) and kept at temperature 24°C. Pathogens were identified by cultural and morphological characteristics of colonies and morphometrics of sporulation [12].

The frequency of isolation (IF, %) was calculated according to the formula:

$$IF = (m/M) \times 100,$$

where m is the number of samples from which this genus or species was isolated; M is the total number of samples.

Results and discussion. During the research period, fungi belonging to 27 genera were isolated from strawberry tissues (Table 1).

The most common were *Alternaria* spp. (in average 82%) and *Fusarium* spp. (70%). *Botrytis cinerea* Pers. and *Paraphomopsis obscurans* (Ellis & Everh.) Udayanga & Castl. were isolated from 48% of samples, *Penicillium* spp. — from 46% of samples. These fungi were present at all stages of plant development. Representatives from the genera *Podosphaera Kunze*, *Ramularia Unger*, *Diplocarpon F.A. Wolf*, *Colletotrichum Corda*, *Pestalotiopsis Steyaert*, *Trichoderma Pers.* were found less frequently. Fungi from the genera *Acremonium Link*, *Epicoccum Link*, *Verticillium Nees*, *Mucor P. Micheli*, *Sclerotinia Fuckel*, *Gnomoniopsis Berl.*, *Trichothecium Link*, *Rhizoctonia DC*, *Aspergillus P. Micheli*, *Coniella Höhn.*, *Rhizopus Ehrenb.*, *Chaetomium Kunze*, *Septoria Sacc.*, *Cylindrocarpon Wollenw.*, *Doratomyces*

myces Corda occurred periodically in certain phases of plant development or in small quantities.

Alternaria spp. are known as pathogens of strawberry leaf spot, which is considered an economically significant disease. It was found in many Asian countries and in Europe [13, 14]. In addition, fungi of this genus can occur as a secondary infection, i.e. they colonize tissues damaged by hail, pests, or have other physical or biological injuries [15]. They are also included in the complex of pathogens that cause mold and rotting of berries during the fruiting period of strawberries [13].

Fusarium spp. can cause significant damage to strawberry plants. The complex of fungi of this genus (*Fusarium oxysporum* Schlecht., *F. commune* K. Skovg., *F. equiseti*

The frequency of isolation of fungi during the growing season (Cherkasy region, cv. ‘Clary’, average for 2021–2023), %

Genera/species	Development phase			
	rosette formation	budding-flowering	fruit ripening	post-harvest period
<i>Alternaria</i> sp.	89.0 ± 11.5	83.3 ± 17.5	56.7 ± 10.1	100.0
<i>Fusarium</i> sp.	40.0 ± 5.8	76.7 ± 8.8	87.7 ± 3.3	76.7 ± 6.7
<i>Botrytis cinerea</i>	56.7 ± 5.8	43.3 ± 6.7	66.7 ± 10.6	26.7 ± 7.6
<i>Paraphomopsis obscurans</i>	76.7 ± 8.8	63.3 ± 6.6	24.3 ± 6.8	26.7 ± 3.3
<i>Penicillium</i> spp.	35.7 ± 8.8	43.3 ± 4.7	36.7 ± 5.0	69.0 ± 4.4
<i>Podosphaera aphanis</i>	11.0 ± 3.3	23.3 ± 4.4	80.0 ± 8.8	46.7 ± 5.8
<i>Ramularia grevilleana</i>	0.0	26.7 ± 6.6	83.3 ± 10.1	93.3 ± 12.0
<i>Diplocarpon earlianum</i>	0.0	0.0	52.3 ± 7.6	76.7 ± 8.6
<i>Cladosporium</i> sp.	60.0 ± 15.3	33.3 ± 7.6	33.3 ± 7.6	26.7 ± 6.6
<i>Colletotrichum</i> sp.	40.0 ± 8.7	26.7 ± 6.7	16.7 ± 5.8	26.7 ± 6.7
<i>Pestalotiopsis</i> sp.	16.7 ± 3.3	11.0 ± 3.3	34.3 ± 5.8	46.7 ± 4.4
<i>Trichoderma</i> sp.	20.0 ± 8.0	16.7 ± 4.4	16.7 ± 4.4	26.7 ± 5.8
<i>Acremonium</i> sp.	20.0 ± 8.8	10.0 ± 3.3	16.7 ± 4.4	6.7 ± 3.4
<i>Epicoccum nigrum</i>	36.7 ± 10.9	10.0 ± 5.8	0.0	0.0
<i>Verticillium</i> sp.	0.0	0.0	16.7 ± 3.3	23.3 ± 9.3
<i>Mucor</i> sp.	0.0	10.0 ± 4.4	16.7 ± 5.8	0.0
<i>Sclerotinia sclerotiorum</i>	0.0	0.0	16.7 ± 5.8	6.7 ± 3.3
<i>Gnomoniopsis comari</i>	20.0 ± 11.5	0.0	0.0	0.0
<i>Trichothecium roseum</i>	0.0	0.0	0.0	17.7 ± 13.2
<i>Rhizoctonia</i> sp.	0.0	0.0	0.0	16.7 ± 6.7
<i>Aspergillus</i> sp.	0.0	0.0	16.7 ± 6.7	0.0
<i>Coniella fragariae</i>	13.3 ± 4.8	0.0	0.0	0.0
<i>Rhizopus</i> sp.	0.0	0.0	6.7 ± 3.3	0.0
<i>Chaetomium</i> sp.	6.7 ± 3.4	0.0	0.0	0.0
<i>Septoria fragariae</i>	0.0	0.0	3.3 ± 3.0	0.0
<i>Cylindrocarpon</i> sp.	0.0	0.0	3.3 ± 3.0	0.0
<i>Doratomyces</i> sp.	0.0	0.0	3.3 ± 3.0	0.0

(Corda) Sacc., *F. solani* (Mart.) Sacc., *F. tricinctum* (Corda) Sacc., *F. sambucinum* Fuckel) are causative agents of root and collar rots. *Fusarium oxysporum* also cause fusarium wilt [15, 16].

Botrytis cinerea (Fig. 1) causes gray mold — a commercially harmful disease of strawberries. This pathogen infects fruits in the field, during storage, and transportation [16]. The fungus can develop as a parasite and as a saprophyte [7].

Fruit rot can also be caused by *Rhizopus* spp. and *Mucor* spp. In the field, symptoms appear on mature or nearly mature fruits. At the same time, damage to the fruit surface is necessary for infection. The process develops very intensively, the mycelium of the fungus can completely cover the fruit in almost a day. Pathogens can continue to grow on harvested infected but still asymptomatic berries, as well as on healthy fruits that have been exposed to spores, causing fruit losses during processing and storage [7, 15, 17].

Penicillium fruit rot (mostly *Penicillium expansum* Link and some other species of this genus), can also cause significant post-harvest losses [17]. However, endophytic *Penicillium* spp. have a wide spectrum of antimicrobial activity. Investigations of Zargar et al [18] showed that *P. hordei* Stolk and *P. polonicum* K.W. Zaleski isolated from the leaves and shoots of strawberries have antagonistic properties against the causative agent of anthracnose.

Powdery mildew (*Podosphaera aphanis*) was the first to appear among strawberry leaf diseases. Its

symptoms were recorded starting from the phase of rosette formation with an average isolation frequency of 11% over the years of research. Over time, the damage by the disease increased and reached a maximum during the ripening period of the fruits.

The pathogens of the most common strawberry leaf spots — white spot (*R. grevilleana*) and leaf scorch (*D. earlianum*) (Fig. 2) were found mainly in the second half of the growing season. The frequency of their isolation reached 93.3 and 76.7%, respectively.

In general, among the selected pathogenic species, the causative agents of leaf diseases are: *Pestalotiopsis* sp., *Diplocarpon earlianum*, *Septoria fragariae* Desm., *Paraphomopsis obscurans*, *Ramularia grevilleana*, *Colletotrichum* sp., *Podosphaera aphanis*, *Gnomoniopsis comari* (P. Karst.) Sogonov, *Alternaria* spp. The following species caused berry diseases — *Botrytis cinerea*, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Colletotrichum* sp., *Rhizopus* sp. Some of the isolated fungi were associated with collar and root diseases — *Fusarium* spp., *Gnomoniopsis comari*, *Rhizoctonia* sp., *Cylindrocarpon* sp., *Coniella fragariae* (Oudem.) B. Sutton.

In addition, the following fungi were isolated: *Aspergillus* sp., *Penicillium* spp., *Cladosporium* sp., *Acremonium* sp., *Trichotecium roseum* (Pers.) Link, *Chaetomium* sp., *Mucor* sp., *Epicoccum nigrum* Link, *Trichoderma* sp., *Doratomyces* sp.

It is necessary to pay attention to the rather high frequency of detection of the fungi *Paraphomopsis ob-*

scurans and *Pestalotiopsis* sp. Fungi of the genus *Pestalotiopsis* (Fig. 3) can cause necrotic spots on leaves and flowers, as well as affect fruits [19, 20]. There are also reports that they are pathogens of root and collar rot [21, 22].

Black spot can appear on leaves, stems, shoots, fruits and collar of strawberries. Its causative agents are fungi of the genus *Colletotrichum*. *C. acutatum* J.H. Simmonds, *C. gloeosporioides* Penz.) Penz. & Sacc., *C. fragariae* A.N. Brooks are most often mentioned as pathogens of black spot of strawberry [23]. Frequency of isolation of *Colletotrichum* sp. varied during the growing season from 16.7 to 40%. Although the pathogen was more often released during the beginning of vegetation period, no symptoms were observed on leaves at this time. It is known that *C. acutatum* can be present as a latent infection, causing no symptoms on leaves, but being an important source of infection [24, 25].

Identification of *Colletotrichum* species is quite difficult, their morphometric features are very similar, therefore most modern studies are aimed at using the PCR method of diagnosis for accurate identification. Based on the results of research by Pei-Che Chung et al. [26] 5 species of this genus were isolated from strawberry plants and identified by the PCR diagnostic method: *C. miaoliense* P.C. Chung & H.Y. Wu, *C. boninense* Moriwaki, Toy. Sato & Tsukib., *C. karsti* You L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, *C. fructicola* Prihast., L. Cai & K.D. Hyde ra *C. siamense* Pri-

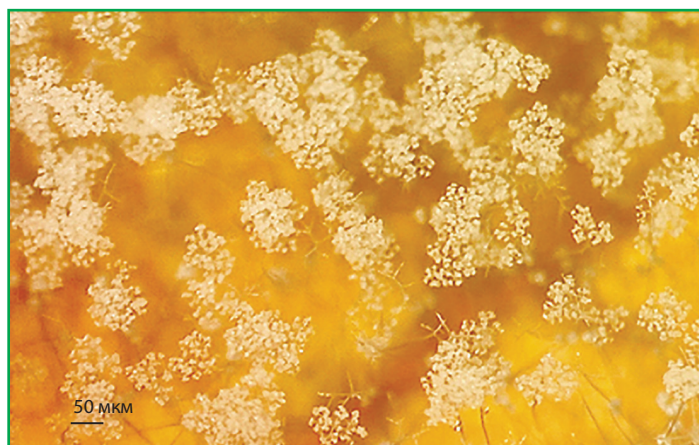


Fig. 1. Conidiophores with conidia of *Botrytis cinerea*

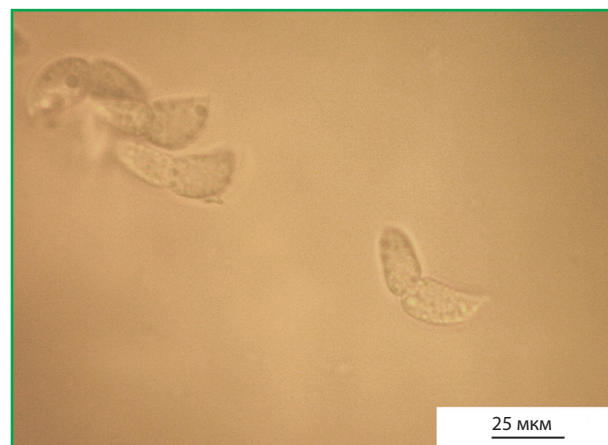


Fig. 2. Conidia of *Diplocarpon earlianum*

hast., L. Cai & K.D. Hyde. Ying Ji et al [27] isolated and identified 23 species on strawberry. The most spread were *C. nymphaea* (Pass.) Aa, *C. acutatum*, *C. fructicola*, *C. siamense*, *C. theobromicola* Delacr. and *C. simmondsii* R.G. Shivas & Y.P. Tan [27].

Gnomoniopsis comari (Fig. 4) transfers with planting material and appears mainly at the beginning of the season. It can exist as a systemic infection in shoots that does not become apparent until favorable conditions occur and the fungus begins to infect leaves and calyxes [28].

Rhizoctonia sp. (Fig. 5) was isolated from strawberry roots in the post-harvest period. This fungus is considered as one of the main pathogens of strawberry root rot and is often found in combination with other pathogens, in particular *Fusarium* spp. [15, 28].



Fig. 3. Conidia of *Pestalotiopsis* sp.



Fig. 4. Perithecia of *Gnomoniopsis comari* on PDA



Fig. 5. T-shape mycelia of *Rhizoctonia* sp.

CONCLUSIONS

The obtained results demonstrate that during the entire growing season fungi of the genera *Alternaria*, *Fusarium*, *Penicillium*, as well as *Podosphaera aphanis*, *Botrytis cinerea* and *Paraphomopsis obscurans* were most often isolated from strawberry tissues. *R.grevilleana* was isolated starting from the budding-flowering phase, and *D.earlianum* — from the fruit ripening phase.

Species from the genera *Podosphaera*, *Ramularia*, *Diplocarpon*, *Colletotrichum*, *Pestalotiopsis*, *Verticillium*, *Sclerotinia*, *Gnomoniopsis*, *Rhizoctonia*, *Coniella*, *Rhizopus*, *Septoria*, *Cylindrocarpon* require constant control of their spread. Under favorable conditions, they can cause damage to plants and a significant lack of harvest.

Monitoring of strawberry mycoflora during the growing season provides an opportunity to establish optimal periods for disease control and the development of effective protection systems.

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**Мікофлора рослин
суниці садової**

Мета. Ідентифікація грибів, пов'язаних з тканинами рослин суниці, та оцінка частоти їх ізоляції в різні фази розвитку рослин. **Методи.** Польові й лабораторні. Дослідження проводили в Правобережному Лісостепу України (Черкаська обл.) у 2021—2023 рр. на сорті Клері. Зразки рослин відбира-

ли у фази формування розетки (квітень), бутонізації-цвітіння (травень), достигання плодів (червень) та у післязбиральний період (липень — серпень). Аналізували результати в лабораторних умовах із застосуванням макроскопічного та біологічного методів. **Результати.** Протягом періоду досліджень з тканин суниці було виділено гриби, що належать до 27-ми родів. Загалом їх можна поділити на три групи збудників хвороб: листя (найбільш чисельна), ягід, коренів і кореневої шийки. Найчастіше траплялися представники родів *Alternaria*, яких виділяли з 57—100% зразків, та *Fusarium* (40—88%). Рідше, але також протягом всього періоду вегетації, ізолювали *Penicillium* spp. (36—69%), *Podosphaera aphanis* (11—80%), *Botrytis cinerea* (27—67%), *Paraphomopsis obscurans* (24—77%). З хвороб листя впродовж періоду вегетації фіксували борошністу росу з максимумом у фазі достигання плодів. Починаючи з фази бутонізації-цвітіння спостерігали ураження білою плямистістю, а з фази достигання плодів — бурою плямистістю. З хвороб плодів найбільше була поширена сіра гниль. Коренева система частіше уражувалась фузаріозом. **Висновки.** Впродовж всього вегетаційного періоду найчастіше з тканин суниці виділяли гриби родів *Alternaria*, *Fusarium*, *Penicillium*, *Podosphaera aphanis*, *Botrytis cinerea* і *Paraphomopsis obscurans*. Починаючи з фази бутонізації-цвітіння виділявся *R. grevilleana*, а з фази дозрівання плодів — *D. earlianum*. Види з родів *Podosphaera*, *Ramularia*, *Diplocarpon*, *Colletotrichum*, *Pestalotiopsis*, *Verticillium*, *Sclerotinia*, *Gnomoniopsis*, *Rhizoctonia*, *Coniella*, *Rhizopus*, *Septoria*, *Cylindrocarpum* вимагають постійного контролю поширення. За сприятливих умов для їхнього розвитку гриби можуть спричинити ураження рослин та значний недобір урожаю. Моніторинг мікофлори суниці протягом вегетаційного періоду дає можливість встановлювати оптимальні періоди для контролю хвороб та розробки ефективних систем захисту.

суниця; видовий склад грибів; частота ізоляції; моніторинг; хвороби листя; хвороби плодів; гнилі коренів та кореневої шийки

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