Linear Growth of Representatives of Wheat Seeds Mycobiota

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Abstract. Seed-born fungi of wheat interact with the plant at various stages of its development and with each other. With the highest growth rate, they should be isolated because of competition. The purpose of this study was to compare the growth of colonies on a nutrient medium for the gradation of fungal genera and species from wheat seeds according to aggressiveness. These data helped concluding on the effectiveness of mycoexpertise of winter wheat seeds. Potato-glucose agar (PGA) was used for the analysis of the fungal complex. Seven-day fungal cultures were sown in the centre of Petri dishes. Linear growth of fungal colonies on PGA with gentamicin was determined. The specific features of the development of 12 representatives of seeds mycobiota from the Northeast of Ukraine of the 2017-2019 harvest were investigated. The dominance of Alternaria sp. and a slight release of Fusarium sp. were established by analysis of the fungal complex. The first comparison of the linear growth of Fusarium graminearum, F. poae, and Alternaria tenuissima in 2017 showed that Fusarium colonies grow faster on nutrient medium. In 2018, the growth characteristics of A. arborescens, which quickly became dominant in wheat seeds mycoflora, and the little-common Trichothecium roseum were studied in detail. By comparing the growth of fast-growing F. graminearum with the common Aureobasidium pullulans and the aggressive Nigrospora oryzae, the fastest development of the third and the slowest of the second species was established. F. poae filled the Petri dish on day 6, Penicillium – on day 22. In 2019, in the first experiment comparing F. poae, F. sporotrichioides, and A. avenicola, the second species had the worst growth rates. It became the second fastest growing colony in the study of the growth of seven species in the second experiment. Isolates of N. oryzae in 2018 were more aggressive than in 2019. Specific features of colony growth on PGA did not affect the effectiveness of the analysis of mycobiota of winter wheat seeds. N. oryzae had the highest radial speed under the general dominance of Alternaria sp. Fusarium sp. (F. poae, F. sporotrichioides, F. verticillioides, and F. graminearum) and B. sorokiniana developed rapidly. A. arborescens and A. avenicola grew at the same level as A. pullulans. Penicillium and T. roseum lagged behind other fungi in speed and filled Petri dishes for the longest time. A. tenuissima had the lowest radial growth rate.

Keywords: fungal seed complex, colony growth, potato-glucose agar, winter wheat
INTRODUCTION

The mycobiota of wheat seeds is a dynamic system comprising varied species of fungi. Its composition is determined by abiotic and biotic factors. The seeds contain fungi that do not have phytotoxic effects on the plant (endophytes) and phytopathogenic species. The negative effects of phytopathogens are associated with the production of secondary metabolites that are dangerous to plants, animals, and humans. The pathogenicity of fungi is because they produce phytotoxins, enzymes, effector proteins, etc. (Peng et al., 2021). The composition of seed mycobiota includes fungi that contaminate agricultural products with mycotoxins. These are Fusarium sp., Penicillium sp., Aspergillus sp., Alternaria sp., Cladosporium sp., Coclilobolus sp. etc. Mycotoxins contaminate 20-25% of food crops in the world (Eskola et al., 2020). Studies on the co-cultivation of fungi and bacteria revealed the synthesis of some of these metabolites, which allowed researchers to determine the cause of mycotoxins — competition between different representatives of the plant microbiome (Venkatesh & Keller, 2019). The endophytic microbiota is associated with the host plant throughout its ontogenesis. Endophytes have a positive effect on plants, increasing drought resistance and resistance to pathogens, stimulating plant growth and development (Hardoim et al., 2015; Shahzad et al., 2018; Kuźniar et al., 2020).

The study of mycobiota in Ukraine, as in the world, is not permanent, is not included in state monitoring, but only depends on the scientific interest of researchers. For the last 20 years, Ukrainian scientists isolated and identified the following genera in the from wheat seeds: Acremoniella sp., Alternaria sp., Aspergillus sp., Cladosporium sp., Coclilobolus sp., Curvularia sp., Epicoccum sp., Fusarium sp., Mucor sp., Nigrospora sp., Penicillium sp., Phoma sp., Sphaceloma sp., and Sordaria sp. A study of 70 samples of wheat grain harvested in 2016 and 2017, collected in collective farms, the private sector, elevators, breeding stations, and regional seed inspections of three zones of Ukraine showed that 1 g of wheat grain in Ukraine contained from 1.12x103 to 6.5x104 CFU, which averaged to 3.3x104 ± 3.2x104. Inside the seeds were 11 representatives of the fungal complex. The most common were Alternaria spp., Aspergillus spp., Fusarium spp., Mucor spp., Nigrospora spp., Penicillium spp., Phoma spp., Stemphylium sp., and Sordaria sp. The most common species in the country (the share of detection was 71%) (Gritsev et al., 2018). Analysis of isolates of the genus Alternaria from different regions of Ukraine during 2012-2013 showed the dominance of A. tenuissima (70%) and a considerable percentage of A. infectoria (25.6%) (Golosna, 2015).

Mycobiota fungi of wheat seeds interact not only with the plant, but also with each other. Admittedly, all the features of their interaction in vivo may be unknown, but in vitro studies provide insight into some of them. Therefore, the purpose of this study was to compare the growth of colonies on agar medium to understand the effect of fungal aggressiveness on the composition of mycobiota of winter wheat seeds.

MATERIALS AND METHODS

The study was conducted during 2017-2019. Mycobiota fungi of winter wheat seeds were isolated from 43 samples obtained from agricultural enterprises of different districts and scientific institutions of the North-East of Ukraine. The authors of this study grew some wheat in the conditions of educational and scientific production complex of Sumy National Agrarian University. Before the analysis, the seeds (200-400 from the sample) were washed under running water for one hour, disinfected with 1% potassium permanganate solution for 1-2 minutes. The seeds were spread on a potato-glucose agar. 25 seeds were placed in one Petri dish. Petri dishes were incubated for seven days in a thermostat at a temperature of 20°C for germination of fungal colonies. Species were identified by various scientific studies: Fusarium sp. — by Leslie & Summerell (2006), Gagkaeva et al. (2011); Alternaria sp. — by Hannibal (2011), Woudenberg et al. (2013); Aureobasidium pullulans (de Bary)
G. Arnaud — by Zalar et al. (2008); Nigrospora oryzae (Berkeley et Broome) Petch. — by Wang et al. (2017); Trichothecium roseum (Pers.) Link — by Watanabe (2002); B. sorokiniana — by Manamgoda et al. (2014). The identified fungi were seeded in pure culture on PGA with gentamicin. Mycelial growth was determined by growing fungi in Petri dishes on PGA. For this, a seven-day growing was involved. The fungi were placed with a needle at the centre of dishes. Colonies grew in a thermostat at 20°C, 22°C, 23-24°C. The incubation period depended on the growth characteristics of the fungi (7-25 days). To identify the linear growth, the diameter of the colonies was measured in two perpendicular directions. The radial growth rate of colonies was determined according to the formula (1) (Poliksenova et al., 2004):

$$K_r = \frac{(r - r_0)}{t}$$

where $K_r$ is the radial growth rate of colonies, mm/day; $r$ is the radius of the colonies at a given time, mm; $t$ is the time from sowing to the moment when the colony will have a radius r, day.

Repetition depended on the experiment: three to five times. Statistical analysis of the results was performed according to the method of one-way analysis of variance in Excel, calculating the LSD_{0.05} according to Dospekhov (1985).

### RESULTS AND DISCUSSION

According to the authors’ observations, Alternaria fungi dominated the mycoflora of winter wheat seeds grown in the North-East of Ukraine (2021). Fusarium fungi were quite rare. Usually, one infected grain of this genus was found in a Petri dish. In 2017, the authors of this study concluded that in vitro conditions are better for the development of Alternaria sp. than Fusarium sp. The definition of Alternaria sp. recommends using Potato-Carrot Agar (PCA), Hay Infusion Agar (HAY), and V-8 (Vegetable Juice Agar) (Gannibal, 2011). Fusarium sp. is better determined on Carnation Leaf-piece Agar (CLA), Spez- ieller Nährstoffarmer Agar (SNA), and Potato Dextrose Agar (PDA) (Leslie & Summerell, 2006).

Therefore, the growth of fungi was investigated on the PGA medium (Table 1). The number of repetitions was 5 times.

| Fungal species       | The diameter of the colony, mm |
|----------------------|--------------------------------|
|                      | Day 3   | Day 4  | Day 7                   |
| F. graminearum       | 17.4x15.8 | 24.8x25 | 51.7x55                  |
| F. poae              | 10x10   | 22.3x21.3 | 52.5x49.3                |
| A. tenuissima        | 7x7.8   | 12.4x11.4 | 33.8x35.8                |
| LSD_{0.05}           | 1.8     | 4.1    | 3.4                     |

The assumption turned out to be wrong, which is confirmed by the data from Table 1. Fusarium fungi grew much faster on the medium than A. tenuissima. If Fusarium sp. indeed were present in a batch of grain in considerable quantities, they would quickly inhibit the development of Alternaria fungi, which was observed in 2016 on the Samuray variety. F. graminearum had higher colony growth rates on PGA than F. poae. When studying the growth rate of six species of Fusarium fungi on the KGA, the highest rate was found in F. graminearum — 23.6 mm/day. F. poae has also been classified as a fast-growing species. Its growth rate was 21.7 mm/day (Shashko, 2020).

In 2018, the authors of this study decided to investigate the specific features of the growth of fungi on a nutrient medium in more detail. A. arborescens E.G. Simmons appeared unexpectedly and quickly dominated the mycoflora of wheat seeds. The specific features of the growth of this species on a nutrient medium were investigated (Fig. 1).
This species developed quite rapidly compared to other *Alternaria* fungi. On days 13-14, the fungi colony completely filled the Petri dish. The average radial growth rate was 3.4 mm/day. By Day 3, the fungi developed slowly. From Day 4, the active development of the fungal colony began. Its maximum growth rate was observed on Day 5. Then the figure started to gradually decrease. The lowest growth rate of the colony was observed on Day 12 of fungal development.

The occurrence of *A. pullulans* in 2016 was insignificant. Gradually, the amount of these fungi increased. Therefore, it was interesting to investigate their behaviour on medium without other fungi. During the isolation of fungi from the seeds, *N. oryzae* had increased aggression compared to other fungi. If they germinated from seed, no fungal colonies developed with them. Therefore, these fungi would develop separately on the medium. Thus, the authors started investigating the growth of fungi on the PGA (Table 2). Repeatability — three times.

### Table 2. Comparison of growth of *F. graminearum, A. pullulans, and N. oryzae* (2018)

| Fungal species | The diameter of the colony, mm | Day 3 | Day 6 | Day 7 | Day 8 | Day 13 |
|----------------|-------------------------------|------|------|------|------|-------|
| *F. graminearum* | 21х21 | 55х52 | 65х62 | 77х73 | 90х90 |
| *A. pullulans* | 23х13 | 57х30 | 55х36 | 57х39 | 59х48 |
| *N. oryzae* | 46х43 | 90х90 | 90х90 | 90х90 | 90х90 |
| LSD₉₅ | 3.5 | 3.9 | 6.3 | 4.9 | 4.7 |

*N. oryzae* filled the entire Petri dish on Day 6. This species proved to be the most aggressive, having the fastest growth of its colony. *A. pullulans* lagged behind the other two fungi.

The study of the growth of *Penicillium* fungi was complicated by the fact that over time, several colonies formed on the medium. Thus, out of five replicates of one colony on Day 4 of observation, 3 colonies were formed in one of the replicates, on Day 6 there were already two replicates with several colonies, on Day 11-3 replicates. Therefore, the data was analysed from only two replicates. The specific features of growth of a fairly common *Fusarium* species — *F. poae* (Fig. 2) were studied.

*F. poae* fungi demonstrated rapid colony growth: on Day 6, they filled almost the entire diameter of the Petri dish. Isolates of these fungi from wheat seeds in Poland on Days 4 and 7 of cultivation had a growth rate of 5.4-10.3 mm/day (Lukanowski *et al.*, 2008). On Day 4, the isolate under study had a speed of 15.8, and on Day 6-14.5 mm/day in the diameter of the colony.

The *Penicillium* fungi were inferior to the growth of the *Fusarium* fungi, but in the initial stages of development grew faster than *Alternaria* fungi. However, after Day 12 of cultivation, the rate of development of the fungi decreased. Only on Day 22 the fungi completely filled the diameter of the Petri dish.

Comparison of the growth rate of endophytic and phytopathogenic isolates of *F. poae, Alternaria alternata* and *Penicillium funiculosum* Thom on PGA arranged them in the above order. That is, the *Fusarium* fungi formed the fastest growing colonies. Phytopathogenic isolates had higher growth rates than endophytic ones (Kurchenko *et al.*, 2015).

*T. roseum* began to be observed in grain batches at the beginning of this study. Moreover, its presence was insignificant.
different: from isolated cases to a recurrence in the myco-
flora of wheat seeds in 2020. These fungi behaved quite
aggressively towards other fungi when they actively ger-
minated from seed. Sometimes they co-existed with other
g fungi (mostly Alternaria), and it was even difficult to spot.
Therefore, it was necessary to investigate their growth on
the medium (fourfold repetition) (Fig. 3).

First, the gradual linear growth of the fungal col-
ony was noted, which lasted for 12 days of their culti-
vation. They grew the fastest on Days 5-6 — 11.7 mm.
After Day 12, the growth of the fungal colony started to
slow down. Observations of the colony growth showed
that it hardly grew. Only on Day 25 T. roseum completely
filled the entire Petri dish. Isolates grown from winter
wheat seeds harvested in 2019 were investigated in two
experiments of single cultivation. First, the growth char-
acteristics of the three following species were compared:
F. poae, F. sporotrichioides, and A. avenicola (repeatabili-
ity — four times) (Fig. 4).

Isolates of F. poae grown from seeds of the 2019
harvest were less aggressive than their respective iso-
lates of 2018 (Fig. 2). It took them 10 days to fill the
entire surface of the medium. Alternaria species initially
lagged behind Fusarium fungi, but from Day 7 it overtook
the growth of the colony of F. sporotrichioides. Of the three
species under study, the most aggressive was F. poae.

Simultaneous study of seven varied species of
seeds mycobiota allowed isolating new fungi with rapid
colony growth and confirm the high rate of already iden-
tified aggressive species (Table 3) (repeatability — three
times).

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**Figure 3.** The diameter of T. roseum (growing at 22°C) (2018)

**Figure 4.** Linear growth of F. poae, F. sporotrichioides, and A. avenicola (2019) (LSD$_{0.05}$7=4.5, LSD$_{0.05}$14=3.2)
Table 3. Comparison of the growth of fungal colonies of winter wheat seeds mycobiota (2019)

| Fungal species         | Day 4  | Day 6  | Day 7  | Day 11 | Day 14 | Day 19 | Day 22 |
|------------------------|--------|--------|--------|--------|--------|--------|--------|
| N. oryzae              | 43.7   | 84.7   | 90     | -//-   | -//-   | -//-   | -//-   |
| F. sporotrichioides    | 45.2   | 69.2   | 77.7   | 90     | -//-   | -//-   | -//-   |
| F. poae                | 36.2   | 54.8   | 62.8   | 90     | -//-   | -//-   | -//-   |
| F. verticillioides     | 35.3   | 52.7   | 60     | 84.7   | 90     | -//-   | -//-   |
| A. avenicola           | 24.5   | 43.5   | 47.2   | 80.7   | 90     | -//-   | -//-   |
| A. arborescens         | 30.7   | 45.3   | 53.2   | 76.2   | 83.3   | 90     | -//-   |
| B. sorokiniana         | 37.7   | 53     | 61.5   | 75.3   | 77.2   | 83.5   | 87.5   |
| HIP<sub>x<sub>5</sub>  | 2.5    | 3.4    | 3.1    | 2.6    | Did not count |

In 2019, the fastest development of N. oryzae was confirmed. In 2018, isolates of these fungi were more aggressive; they completely filled the Petri dish on Day 6. Different ability of colonies of isolates of one species — F. sporotrichioides — was noted. If in the first experiment this species lagged behind the Alternaria fungi, then in the second — they were the second most aggressive species, even surpassing the fairly fast species of F. poae. The last fungi had a lower growth rate both compared to the first experiment and the previous year of the study.

F. verticillioides predominated in the development of Alternaria fungi. A. avenicola grew faster from Alternaria species. B. sorokiniana developed seven days faster than Alternaria fungi and F. verticillioides, and from Day 11 their growth rate was minimal. Isolates of B. sorokiniana from barley seeds in Argentina had an average growth rate per PGA of 9.9 mm/day (Dominguez et al., 2020). The isolates under study had a colony growth rate of 8.8 mm/day on the seventh day.

Since mycobiota were analysed on Day 7, the radial growth rate during this period was calculated (Fig. 5).

![Figure 5. Radial growth rate of representatives of wheat seeds mycobiota (2017-2019)](image-url)

The area of variation of the indicator was 2.4-6.5 mm/day. N. oryzae had the maximum speed. Radial growth of Fusarium sp. was similar to B. sorokiniana. The next block in speed was Alternaria sp. and A. pullulans. Penicillium and T. roseum gave way to them. A. tenuissima had the lowest radial speed.

CONCLUSIONS

Single cultivation fungi of wheat seeds mycobiota on PGA medium showed different growth rates of colonies of isolates of different years, from different samples, but allowed distributing fungi by growth rate, i.e., aggressiveness. N. oryzae had the fastest development of colonies, followed by Fusarium sp. (F. poae, F. sporotrichioides, F. verticillioides, F. graminearum), and B. sorokiniana (only in the first seven days), which were inferior to A. arborescens and A. avenicola. A. pullulans developed at the level of Alternaria sp. The Penicillium fungi had average growth rates in the first week, but their growth rate gradually decreased. T. roseum developed similarly but had maximum time to fill the Petri dish. A. tenuissima showed the lowest radial growth rate. The growth of colonies on the PGA did not affect the specific features of the isolation of fungi from the seeds of winter wheat. Data on the isolation of fungi from mycobiota correlate with their presence inside the seeds and were not determined by their development on agar medium.

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Лінійний ріст представників мікобіоти насіння пшениці

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Анотація. Гриби комплексу насіння пшениці взаємодіють з рослиною на різних етапах її розвитку та між собою. Можливо, у результаті конкуренції краще виділяються гриби з найвищою швидкістю росту. Метою досліджень було порівняти ріст колоній на поживному середовищі для градації грибних родів та видів з насіння пшениці за агресивністю. Ці дані допоможуть зробити висновки про результативність мікоекспертизи насіння пшениці озимої. Аналіз грибного комплексу провели на картопляно-глюкозному агарі. Семиденні культури грибів висіли у центр чашок Петрі. Визначили лінійний ріст колоній грибів на КГА з додаванням гентаміцину. Було вивчено особливості розвитку 12 представників мікобіоти насіння з Північного Сходу України врожаїв 2017 – 2019 рр. За аналізу грибного комплексу встановили домінування Alternaria sp. та незначне виділення Fusarium sp. Перше порівняння лінійного росту Fusarium graminearum, F. poae та Alternaria tenuissima у 2017 р. показало, що фузарієви колонії швидше ростуть на середовищі. У 2018 р. детально вивчили особливості росту A. arborescens, який швидко зайняв домінуюче положення у мікофлорі насіння пшениці, та малопоширеного Trichothecium roseum. За порівняння росту швидкоростучого F. graminearum з поширеним Aureobasidium pullulans та агресивним Nigrospora oryzae встановили найшвидший розвиток третього та найповільнішій другого виду. F. poae заповнив чашку Петрі на шосту добу, Penicillium — на 22-гу. У 2019 р. у першому досліді за порівняння F. poae, F. sporotrichioides та A.avenicola другий вид мав найгірші показники росту. У другому досліді він став другим за швидкістю розвитку колонії при дослідженні росту семи видів. Якість N. oryzae у 2018 р. були агресивнішими, ніж у 2019 р. Ріст колоній на КГА не вплинув на виділення грибів з насіння пшениці озимої. За загального домінування Alternaria sp. найвищу радіальну швидкість мав N. oryzae. Швидко розвивались Fusarium sp. з (F. poae, F. sporotrichioides, F. verticillioides і F. graminearum) та B. sorokiniana, A. arborescens та A.avenicola росли на рівні з A. pullulans, Penicillium та T. roseum за швидкістю відістали від інших грибів і найдовше заповнювали чашки Петрі. A. tenuissima мав найменшу радіальну швидкість росту.

Ключові слова: грибний комплекс насіння, ріст колоній, картопляно-глюкозний агар, пшениця озима