Improving methods for preventing diseases of pasture grasses and securing the quality of feedstuff

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Abstract. The species composition of phytopathogenic fungi isolated from seeds of lawn grasses (pasture ryegrass, shoot grass birch and meadow bluegrass) was revealed and the dominant genera and species were identified. The main components of the pathogenic mycobiota of seeds were fungi: Fusarium – 44 %, Alternaria sp. – 37 %, Penicillium sp. – 12 %, Mycelia sterilia – 5 %, Cladosporium sp. – 2 %. In laboratory conditions, the biological effectiveness of the preparations was evaluated: Strekar, VRK (d.v. phytobacteriomycin + carbendazim), Fitolavin, VRK (d.v. complex of streptotricin antibiotics + phytobacteriomyacin), TMTD-plus, KS (d. tiram + immunomodulators), Celest Top, KS (AI thiamethoxam + fludioxonil + diphenlonazole), of which Strekar, VRK, maximally increased the length of ryegrass seedlings, however, the length of the coleoptile was higher in the variant with TMTD-plus, KS, and the best effect on bluegrass seeds was provided by Celest Top, KS and TMTD-plus, KS. The contamination of seeds was reduced after treating with TMTD-plus.

1. Introduction
Quality of the grass stand of sports lawns contributes to the development of football and golf. Like any plants, lawn grasses are susceptible to a number of infectious and non-infectious diseases; both seed material and vegetative plants can be affected [1]. The stage of seed germination and rooting are the most vulnerable to various stress-related factors, which can be very harmful. Plants are less resistant to water scarcity, extreme temperatures and toxicity of pesticides [2]. Infectious diseases often have a focal nature of spread and are erroneously considered as adverse abiotic factors. The use of appropriate agricultural techniques, such as the water regime, the optimal cutting height and the fertilizer application mode, improve plant immunity and can reduce the harmfulness of diseases [3].

Root diseases are widespread and harmful. Due to a root damage, disturbances in vital processes reduce the viability and longevity of plants.

The highest frequency of Fusarium, Bipolaris, Pythium was observed [4]. Fusarium usually dominates endophytic pathogenic complexes of microorganisms specializing in roots of herbaceous plants [5]. Our studies and other authors [6] confirm that the roots of lawn grasses are a significant ecological niche for several species of Fusarium fungi. Phytosanitary disease monitoring on football fields in the North Caucasus (Sochi and Essentuki) and the Non-Chernozem zone (Moscow) identified fusarium fungi and other phytopathogens of root rot. If in the spring, in Moscow Fusarium fungi (including Microdochium nivale) were dominant, in Sochi Rhizoctonia prevailed (P = 58 %), and Fusarium had a lower frequency of occurrence (P = 45 %), slightly different in Cladosporium (P = 42 %).

In the summer, Rhizoctonia and Alternaria prevailed on football fields of the southern region, and
Alternaria sp. – on the football fields of Moscow. Although various growing conditions of grass stands, caused by the weather and climatic conditions of the regions affected the species composition of the dominant phytopathogens, Alternaria and Fusarium were present in the foci of affected lawn grasses [7].

According to Bacon and Yates [8], the endophytic species of Fusarium are hemibiotrophs, that is, they infect healthy tissues as biotrophs, but can infect host plants after a latent period. Under certain conditions, some isolates can infect plants without causing symptoms of the disease [9]. Asymptomatic root infections in plants caused by F. graminearum, F. nivale, F. culmorum [10] and F. croookswellense [11] have been reported.

Regular phytopathological examinations of grass stands and seeds of sports lawn plants are necessary in order to identify the species composition of pathogens, the degree of their negative impact on plants, as well as to determine the feasibility and effectiveness of the use of various preparations for seed treatment which can ensure optimal plant protection.

The purpose of our study is to specify the prevalence and species composition of phytopathogens of lawn grass seeds, as well as to identify the biological effectiveness of a number of fungicidal preparations against fusarium fungi and root rot pathogens.

2. Methods and materials
The main objects are 1) seeds (2017–018) and seedlings of lawn grasses: pasture ryegrass (Lolium perenne L.) produced by Jacklin Seed (USA), meadow bluegrass (Poa pratensis L.) produced by Seed research of Oregon (USA) and DLF (Denmark), shoot-forming moths (Agrostis stolonifera L.) produced by DLF (Denmark), which form the basis of the lawn grass stand of football fields and golf courses; 2) Fusarium oxysporum Schlecht. isolated from the rhizosphere of bluegrass plants; 3) a number of fungicides with different active substances and a wide spectrum of action recommended for seed treatment: Strekar, VRK (active phytobacteriomycin + carbendazim), Fitolavin, VRK (active complex of streptotricin antibiotics and phytobacteriomycin), TMTD-plus, KS (AI thiram, immunomodulators), Celest Top, KS (AI thiamethoxam + fludioxonil + diphenoxonazole). The biological effectiveness of the preparations was evaluated in laboratory conditions against an artificial infectious background of F. oxysporum. The turf of sports fields is grown on artificial soil containing sand and a small amount of organic matter. For the experiment, we used soil consisting of 85 % washed river sand corresponding to GOST 8736-2014 (particle size 0.002-1mm) and 15 % of low-lying separated peat (pH – 5.5–6.0). Creating an artificial infectious background, it is necessary to take into account that both insufficient and excessive amounts of conidia will not create conditions close to natural epiphytoties, and the results will not be reliable. At a magnification of x10, the optimal number of macroconidia in the field of view of the microscope should be 10–15 pcs. To produce the required concentration of F. xysporum spores in suspension, a Goryaev chamber was used (Fig. 1) at x10, x40, x100 magnifications, in tenfold repetition, calculating the number of conidia in a certain volume by formula:

\[ O(n \times V \times 1000) / 0.9 \]

where, \( n \) is the average number of conidia on the grid of the Goryaev chamber; \( V \) – volume of the suspension – 150 ml.

Before sowing, bluegrass and ryegrass seeds were sprayed with a suspension of F. oxysporum. The drugs were recommended by manufacturers for treating cereal seeds in terms of 100 ml of water:

- **Option 1** – control, the seeds were wetted.
- **Option 2** – Celest Top, KS – 0.14 ml per 100 ml of water.
- **Option 3** – TMTD-Plus, KS – 0.28 ml per 100 ml of water.
- **Option 4** – Strekar, VRK – 0.6ml per 100ml of water.
- **Option 5** – Fitolavin, BPK – 0.2 ml per 100 ml of water.

The soil surface was sprayed with inoculum of 10 zilch in each pot. The seeds (25 pieces per vessel) were placed on the soil substrate, ryegrass was sprinkled with 1.5 cm of soil, bluegrass – with 0.5 cm of soil. After that, the substrate was moistened, covered with a cling film and placed in a bright
place. The experiment was carried out in three replicates for each option. The state of the plants was assessed on the 10th and 20th days respectively. The plants were carefully removed from the soil and washed in running water. The ruler measured the length (mm) of the root system, coleoptile and aerial parts of the seedling of the test culture with photofixation. The following symptoms were taken into account: root length, coleoptile length, shoot length, and damage to the ground and underground parts of the plant, where

- 0 – healthy plant;
- 1 – single signs of disease (strokes, ulcers, chlorosis, wilting, etc.);
- 2 – single signs of disease (slight browning / darkening of the coleoptel, root or single strokes at the base of the stem)
- 3 – severe browning or darkening, rot
- 4 – non-viable plant.

![Figure 1. Macroconidia of Fusarium in the Goryaev chamber at x40](image)

According to the results, the following indicators were calculated: prevalence of the disease (P, %) and biological effectiveness of the drug (BE, %).

\[
P = \frac{n}{N} \times 100,
\]

where \( n \) is the number of affected and dead seeds in the sample; \( N \) is the total number of seeds analyzed in the sample.

\[
BE = 100 \times \frac{(P_k - P_o)}{P_k},
\]

where \( R_k \) and \( P_o \) are the prevalence of the disease in the control and experimental versions.

Surface infection with micromycetes was evaluated on the filter paper in Petri dishes.

The identification of pathogen fungi was carried out using traditional phytopathological methods, including wet chambers, MIKMED-5, and microbiological methods – isolating micromycetes on the artificial nutrient medium (potato-glucose agar (KGA) and using reference books.

3. Results
An analysis of seed contamination was carried out in 2018–2019.

Using the traditional phytopathological methods, the following pathogens were revealed: Fusarium sp., Alternaria sp, Penicillium sp., Cladosporium sp., Mycelia sterilia (Fig. 2).

An analysis of the phytosanitary state of seeds showed that the dominant fungi were Fusarium fungi (P-44 %). Therefore, the main model object was one of the isolates (F. oxysporum) isolated from the roots of the affected plants. During the growth of seedlings in the vegetation vessels, the change in root, shoot and coleoptile lengths was evaluated, which helped assess the degree and nature of the drug effect on the plant. Data on the drug effect on pasture ryegrass are presented in Table 1.
Figure 2. Diagrams of seed micromycetes prevalence in 2018

Table 1. Influence of drugs on biometric indicators of raygrass pastural (Lolium perenne L.)

| Option            | Coleoptile, cm | Length, cm | Root, cm | First Leaf, cm |
|-------------------|----------------|------------|----------|----------------|
| Control (water)   | 1.9±0.12       | 3.9±0.21   | 9.3±0.58 |                |
| Phytolavine, VRK | 1.8±0.12       | 4.2±0.31   | 9.0±0.52 |                |
| Selest Top, KS    | 1.7±0.11       | 3.4±0.37   | 8.7±0.55 |                |
| TMTD-plus, KS     | 2.3±0.18       | 4.4±0.25   | 10.5±0.53|                |
| Strekar, VRK      | 2.0±0.14       | 4.9±0.3    | 11.2±0.66|                |

All drugs improved the biometric parameters of the pasture ryegrass seedlings. Strekar, VRK, maximally contributed to an increase in the length of the root and the first leaf, however, Length coleoptile was more efficient in Option with the TMTD-plus drug, KS, which positively influenced the development of seedlings. The lowest values of the lengths were observed in Option, where the treatment was carried out using Selest Top, KS, which slowed down the growth and development of ryegrass seedlings.

Table 2. Influence of drugs on the contamination of raygrass seeds (LOLIUM PERENNE L.)

| Option                  | Contamination, % | BE, % |
|-------------------------|------------------|-------|
| Control (water)         | 49.3             | –     |
| Phytolavine, BPK        | 42.0             | 42.4  |
| Selest Top, KS          | 53.3             | 51.5  |
| TMTD-plus, KS           | 42.6             | 69.6  |
| Strekar, VRK            | 58.0             | 18.8  |

* biological intensity (R, %)

Similar results (Table 2) showed TMTD-plus and Phytolavine, VRK – 42.6 and 42.0 %, respectively, but the biological effectiveness of TMTD-plus was higher by 27.2 %. The highest contamination was observed in Option with Strekar, its biological effectiveness was minimal – 18.8 %.

Data on the effect of the drugs on the biometric indices of meadow bluegrass seedlings are presented in Table 3.

The development of bluegrass seedlings had a similar positive effect on selest Top, KS and TMTD-plus, KS (Table 3), all biometric indicators increased. However, for ryegrass, Selest Top had the opposite effect, slowing the growth and development of seedlings. Phytolavine, VRC inhibited the growth and development of bluegrass seedlings.
Table 3. Influence of drugs on biometric indicators of meadow bluegrass (*Poa pratensis* L.)

| Option                  | Coleoptile | Length, cm | First Leaf |
|-------------------------|------------|------------|------------|
| Control (water)         | 0.9±0.09   | 0.8±0.13   | 3.8±0.27   |
| Phytolavine, VRK        | 0.6±0.09   | 1.3±0.12   | 2.5±0.28   |
| Selest Top, KS          | 1.2±0.16   | 1.1±0.15   | 4.7±0.29   |
| TMTD-plus, KS           | 1.1±0.08   | 1.1±0.15   | 4.7±0.29   |
| Strekar, VRK            | 1.1±0.08   | 1.3±0.12   | 4.4±0.27   |

Table 4. Influence of drugs on the contamination of meadow bluegrass seeds (*POA PRATENSIS* L.)

| Option                  | Contamination, % | BE, % |
|-------------------------|------------------|------|
| Control (water)         | 98.6             | -    |
| Phytolavine, VRK        | 96.0             | 11.4 |
| Selest Top, KS          | 82.6             | 22.8 |
| TMTD-plus, KS           | 81.3             | 52.8 |
| Strekar, VRK            | 85.3             | 51.4 |

* biological intensity (R, %)

Meadowgrass seeds had the highest initial contamination (Table 4), and they were more susceptible to the artificial infectious background of Fusarium and fungal diseases. It is necessary to monitor the composition of phytopathogens and implement protective and agrotechnical measures to prevent the development of epiphytoties.

4. Conclusion

It was found that the main pathogens of seeds of pasture ryegrass, meadow bluegrass, and shoot-bent grass were Fusarium sp., With a frequency of occurrence of 44 %, Alternaria sp. – 37 %, Penicillium sp. – 12 %, Mycelia sterilia – 5 %, Cladosporium sp. – 2 %.

When growing seeds in vegetation vessels with an artificial infectious background *Fusarium*, after treating ryegrass seeds with Strekar, VBC, the length of the root and the first leaf increased by 25.6 and 20.4 %, compared to the control option, however, the length of coleoptile was higher in the option with TMTD-plus, KS, which had a positive effect on seedling development, increasing the average root length by 12.8 %, coleoptile by 21 % and the first leaf by 13 %. After treating the ryegrass seeds with the Selest Top, KS, the sprouting length was minimal. At the same time, the length of the coleoptile decreased by 10.5 %, the length of the first leaf decreased by 6.5 % and the length of the root – by 12.8 %.

TMTD-plus, KS was the most effective reducing the contamination by 6.7 %. Its biological efficiency was 69.6 %. Strekar, VRK, minimized the seed contamination.

As for bluegrass, the development of its seedlings was similarly affected by Selest Top, KS and TMTD-plus, KS which increased the root length by 37.5 %, and the length of the first leaf by 23.6 %; the length of the coleoptel increased by 33.3 and 22.2 %.

The maximum contamination of bluegrass seeds was reduced by TMTD-plus by 17.3 %, with a biological efficiency of 52.8 %. Phytolavine, VRK was ineffective, reducing the contamination by 2.6 %.

In general, the contamination of meadow bluegrass seeds on an artificial infectious background was significantly higher than that of pasture ryegrass.

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