Diagnosis of soil fungi that cause root rot

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Abstract. The relevance of studying the pathogens of soil fungi that cause root rot is due to their wide distribution, high plasticity in agrobiocenoses and harmfulness. The aggressive nature of root rot pathogens, their diverse species composition and the variability of the structure of pathogenic complexes in agrobiocenoses create enormous difficulties in resolving issues of plant protection from these diseases. The objective of this work is to diagnose the species composition of fungi that cause root rot of various genera of cultivated plants.

1 Introduction

Damage to plants by root and root rot can occur during the entire period of plant vegetation, causing the death of seedlings, lagging plants in growth, and the death of stems. Root rot is widespread in all regions of the Russian Federation in various cultures. The decline in plant productivity, the deterioration of product quality, environmental instability and economic damage are consequences of the disease [1-3].

The causative agents of the disease are fungi from the genera: Fusarium, Bipolaris, Alternaria, Pythium, Gaemnomnomyces, Cercospora, Typhula, Rhizoctonia, Aureobasidium living in the soil, on seeds and plant debris. In terms of frequency of occurrence and severity, priority belongs to fungi of the genus Fusarium, Bipolaris and Alternaria. Fungi that cause root rot have a wide range of different enzymes, with the help of which they destroy the tissues of the nourishing plant and cause its death. The root rot pathogens are most intensely affected by crops, grasses, and to a lesser extent trees and shrubs. The disease externally manifests itself in the form of browning of the roots, the underground internodes, the base of the stem and vagina of the lower leaves. When pathogens are affected by root rot, the underground internode and tillering nodes lose their strength, become loose, brittle and break off when pulling plants out of the soil. In agrobiocenoses, root rot pathogens represent a mixed infection, which is often localized in the rhizosphere of plant roots.

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2 The Methods

The work was performed in the Mycology and Immunity All-Russian research Institute of Phytopathology VNIIF with the aim of scientific justification and improvement systems for protecting crops from root rot in various regions of the Russian Federation based on data long-term study of the structure of agrobiocenoses and assessment of stability and tolerance. Develop an assessment of the most fast and objective methods for the diagnosis of root and root rot of plants, we analyzed previously obtained data on the identification and identification of the species composition of pathogens, seasonal and long-term dynamics of the development of the disease, the study of the biological characteristics of pathogens, patterns of formation of pathogenic fungus populations under the influence of biotic and abiotic factors.

3 The experimental results and discussion

The study of morphological and cultural properties of pathogens. The research material was identified cultures of root rot pathogens: *Fusarium*, *Bipolaris*, *Alternaria*, *Pythium*, *Gaemennomyces*, *Cercosporella*, *Typhula*, *Rhizoctonia*, *Aureobasidium*. Morphological and Cultural Types (MKT)fungal colonies were studied on potato glucose agar (KGA). To this end, cultures were sown in tenfold repetitions in Petri dishes (KGA) and grown for two weeks at 22-24 °C. Assessment of morphological and cultural signs (MCP) of fungal colonies was carried out according to the color and topography of air and substrate mycelia, sporulatingactivity on the 12-14th day of growth [4-6]. Study of pathogenic and toxic properties of strains of fungi, pathogens of root rot of grain crops. Clonal cultures of root rot pathogens of cereals from the genus Fusarium served as a material for research, Bipolaris, Alternaria shown in the picture 1-3. Pathogenic and phytotoxic properties of strains were studied using the method of bioassay on seeds. Experiences conducted in three replications. The pathogenicity of spore suspensions was determined as follows. Petri dishes with potato-glucose agar was seeded with fungal cultures with a piece of mycelium using a microbiological loop. Each strain was sieved into 5 Petri dishes. The cups were incubated in a thermostat at 25°C for 2 weeks. At the end after incubation, 10 ml of sterile tap water was added to each Petri dish, and the mycelium was washed off the surface agar with a scalpel, pipette with a cut nose was transferred to sterile flasks Erlenmeyer 250 ml and shaken for 5 min. Then the spore suspension was filtered from the remnants of the mycelium through two layers of cheesecloth and bring the volume to 50 ml. the Number of spores in 1 ml suspension were counted in the camera Goryaeva. In sterile Petri dishes on sterile filter paper was placed on 10 seeds and 6 ml of spore suspension was added. In control variant stap water was added. Petri dishes with seeds were incubated for 5 days at room temperature an din natural light, then counted the germination of seeds and measured the length of coleoptiles and roots of seedlings. Toxinogenics trains was determined by studies of phytotoxicity of culture liquid. For this purpose prepared liquid nutrient medium and poured into flasks of 250 ml of 100 ml of medium in each. As a nutrient medium liquid medium of the following composition was used: NaNO₃-3.0 g; K₂HPO₄-1.0 g; KCl-0.5 g; Mg SO₄ x 7H₂O-0.5 g;FeSO₄ x 7H₂O-0.01 g; ZnSO₄-0.01 g; CuSO₄-0.001 mg; Sucrose-30.0 g; distilled Water-up to 1 liter; (PH-6.0). The flasks were sterilized at 0.5 ATM for 30 minutes and fungal strains were seeded with a piece of mycelium using micro biological loop. Each strain was seeded into two flasks. Cultures were incubated on a rocker at 200 rpm and 25°Cfor 10 days. At the end of the
cultivation process, the contents of the flasks were filtered through paper filters for separation of the culture fluid from the mycelium, and then through bactericidal filters with a pore size of 0.22 microns. Filtrates fungal strains were tested for toxicity to barley and wheat seedlings in Petri dishes. In a Petri dish 10 seeds were placed on the filter paper and 6 ml of culture filtrate were added. In control variant tap water was added. Incubation of Petri dishes with seeds and accounting of experience was carried out in the same way as in determination of pathogenicity of strains. Statistical processing of the results was carried out using a modified a program developed in the Windows 98 environment based on Excel. The work was performed using the equipment of the Central Control Facility GKFM FGBNU VNIIF. Pathogenicity was determined by inhibition of spores by suspension pathogens of growth of roots and seedlings of wheat varieties.

Pathogenicity was determined by the formula: \( N = 100\% - \left( \frac{L_{on}}{L_k} \times 100 \right) \), where:

- \( P \) – pathogenicity of the strain;
- \( L_{on} \) - length of roots (seedlings) in the experiment;
- \( L_k \) - length of roots (seedlings) in the control

Pathogenicity and toxicity of pathogen strains were differentiated into four groups:

- Non-pathogenic/non-toxic-inhibition of plant growth 0 -30%,
- Low pathogenic/low toxic-inhibition of plant growth 31 -50%,
- Moderately pathogenic/moderately toxic-inhibition of plant growth 51 -70%,
- Pathogenic/toxic-inhibition of plant growth over 70%.

The pathogenicity and toxicity of fungus isolates were judged by the degree of inhibition of seed germination, the length of coleoptiles and root. The most informative indicator of pathogenicity and toxicity of isolates is root length. Root length in the control variant corresponds to 100%.

The main parameters of root rot manifestation and the geography of fungi distribution are determined. The spread of root rot can be uneven. Fusarium root rot is caused by the fungi Fusarium culmorum, F. oxysporum, F. avenaceum and others. These are highly specialized pathogens that affect many cultures. In different zones is found in various complex species of Fusarium fungi. They affect shoots and adult plants. In wet weather, a pink or yellowish patina of pathogen sporulation forms on the affected tissue. In soil fungi of the genus Fusarium inhabit plant residues, live in the rhizosphere and on the surface of the roots, actively multiply in dead roots, withstanding the competition of the substrate settlement by other fungi, bacteria and actinomycetes. These pathogens are able to exist in the soil in
saprotrophic form and accumulate on plant residues with the transition under certain conditions to a parasitic form of existence, which makes the fight against this disease very difficult.

Helminthosporium root rot \((Bipolarissorokiniana = Helminthosporiumsativum)\). In the germination phase, the disease manifests on the coleoptile and at the base of the seedling in the form of dark necrosis. In the phase of exit into the tube, the underground internode, the base of the stems and the vagina of the basal leaves turn brown, the roots rot and die. When Helminthosporium infection on the infected tissue develops a dark olive or almost black conidia plaque. *Epioblasma* root rot \((Ophiobolus graminis (Sacc.))\). When ophiobolus is the death of productive stems during the whole vegetation period. Characteristic symptoms include blackening of the roots, then sheaths of basal leaves in the lower part of the stem, and then their gradual extinction. Plants are stunted, easily pulled out of the soil. Manifested in the form of distinct foci. *Cercosporella* the causative agent of basal rot, or eye / spot, *Pseudocercosporella herpotrichoides* on aerial parts of plants are formed of bright elliptical spots in the eye to the border dark color, sometimes banding the stem. The tissue in such places loses its strength, the stem breaks, which leads to lodging of crops, hollow ears. Inside the straw is a gray coating of fungus sporulation.

In recent years, in addition to the above pathogens of root rot, increased harmfulness of fungi genera *Alternaria, Pethium and Rhizoctonia*, which in some years can cause significant damage to cultivated plants.

The main sources of infection of all types of root and basal rot are soil, stubble residues, seeds. Factors that enhance the development of rot are violation of agricultural technology, non-compliance with crop rotations and the degree of saturation of one crop. The results allow us to develop new methods less complex taking into account (qualification composition of manpower), energy costs, time resources.

4 Conclusions

Root rot is a disease of plants weakened by adverse environmental factors, for example, sudden changes in temperature. The complex of pathogens is constantly changing depending on the cultivation zone, the degree of saturation of one crop, agricultural technology and varieties. Especially harmful are the manifestations of root rot at an early stage of plant development. Healthy planting material is the key to successful production. Diagnosis of root rot pathogens on seeds and during the growing season for plant damage will allow timely adoption of appropriate preventive measures and reduce the infectious load on agrobioecoses and crops.

The results are in good agreement with the results of studies by other scientists [6-18].

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