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Kateryna Davydenko, PhD [1,2]

G.M. Vysotsky Ukrainian Research Institute of Forestry & Forest Melioration[1.] Department Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden[2]

FUNGI ASSOCIATED WITH DECLINE OF SCOTS PINE SEEDLINGS IN FOREST NURSERIES

Afforestation and sustainable conversion of old farmland to woodland require healthy and high-quality seedlings. Container nurseries provide optimal conditions for the growth and development of forest seedlings (Szabla 2009). Controlled mycorrhization and biological and chemical protective treatments are applied, and pests and pathogens are actively eliminated. Unfortunately, container nurseries are not common practice in forestry in Ukraine and most Scots pine seedlings are grown in greenhouses.

Therefore, seedling blight and root rot, which develop several weeks after germination, are ubiquitous diseases in forest nurseries. They are caused by soil pathogens as well as pathogens without host specialization disseminated by seeds. Those pathogens contribute to rapid decomposition of plant tissue, and they prevent seed germination and seedling emergence (Menkis et al. 2006). Fungi which cause the greatest losses in nurseries include *Alternaria*, *Cylindrocarpon, Cylindrocladium*, *Fusarium, Trichothecium* and *Rhizoctonia*, most of which are identified in the conidial stage (Sutherland et al. 2002; Lilja et al. 2010; Meshkova et al., 2012, Davydenko and Meshkova, 2017, 2018). Those dangerous pathogens with a wide host range are transferred to forests with seedlings from infected nurseries, and they colonize both annual and perennial plants. Environmental factors play an important role in the spread of disease and the transmission of pathogens in conventional forest nurseries.

The aim of this study was to determine the fungal pathogens responsible for the pine seedling decline in the field and nurseries.

The experiment was carried out in six nurseries in State Forest Enterprises of Kharkov, Sumy and Poltava regions. Scots pine (Pinus sylvestris) seedlings were sampled in the greenhouse for the study one year after sowing. In the field nurseries, seedlings were grown in accordance with nursery standards, and pine seedlings were sampled for the experiment in the first year after planting on similar dates in all nurseries. Ten pine seedlings were randomly selected for the experiment. In a laboratory, seedlings were divided into roots and stems which were analyzed separately.

The study was carried out with the involvement of conventional culture methods as well as PCR with specific primers for Fusarium, Phytophthora root pathogens. By pure cultural method, small fragments were cut out from plant tissues selected for the study, they were rinsed in distilled water and disinfected in 96% ethyl alcohol solution for 30 seconds and in 1% sodium hypochlorite (NaOCl) for 30

seconds. The samples were rinsed three times in sterile water and dried, and 2 mm sections were transferred to Petri dishes (six sections per plate) containing malt extract agar (MEA). The plates were incubated at a temperature of 20°C in darkness for 7-10 days. The emerging filamentous fungi were transferred to new Petri dishes with 2% MEA and identified based on the available keys and published data.

Isolation of DNA, amplification and sequencing followed method described by Davydenko & Meshkova (2017). The thermal cycling was carried out using an Applied Biosystems GeneAmp PCR System 2700 thermal cycler (Foster City, CA, USA): initial denaturation step at 95°C for 5 min was followed by 35 amplification cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30s and final extension step at 72°C for 7 min. Raw sequence data were analyzed using the SeqMan Pro version 10,0 software from DNASTAR package (DNASTAR, Madison, WI, USA). The criteria used for identification were: sequence coverage > 80%; similarity to taxon level 98-100%, similarity to genus level 94-97%.

Species diversity was examined by principal component analysis (PCA) in PAST software (Hammer et al., 2001). The database of environmental factors was screened by PCA to evaluate the main trends in species diversity in the analyzed sites. The diversity of endophytic fungi was quantified based on the species richness index and the Shannon diversity index

A total of 228 fungal isolates were obtained from pine roots in both years of the study, including 124 in the first year and 104 in the second year.

Pathogenic fungi colonizing pine seedlings belonged to the genera *Alternaria, Botrytis, Cylindrocarpon, Fusarium, Pestalotia, Phoma, Pythium* and *Rhizoctonia*. Pathogenic isolates accounted for 57.5% of fungi colonizing roots and 30.5% of fungi colonizing stems of pine seedlings. Pathogenic species accounted for 59% of total isolates in greenhouses and 41% in field nurseries. A comparison of the species diversity of fungi isolated from pine roots and stems revealed that pine roots in greenhouses were colonized by 19 taxa and non-sporulating fungi. Pine roots in field nurseries were colonized by 11 taxa and non-sporulating fungi. The presence of the pathogenic species *Cylindrocarpon destructans* and species belonging to the genera *Fusarium* and *Alternaria* on the roots and stems of pine seedlings was also determined.

Pathogenic fungi were more abundant on pine seedlings from the greenhouses, whereas a comparison of the species richness index with the Shannon diversity index revealed greater species diversity of fungal communities in samples from field nurseries than in seedlings from greenhouses.

The dominant fungal species colonizing pine stems was *F. avenaceum* and *F. oxysporum* in greenhouses. In field nurseries, a significant percentage of *Fusarium* sp., *Pythium* and the antagonistic species *Trichoderma harzianum* was noted, and a high proportion of *Cylindrocarpon destructans* was observed.

The presence of pathogenic fungi of the genera *Cylindrocarpon* in plant tissues was determined by PCR, but they were not identified in conventional mycological analyses, which indicates that PCR is a much more reliable diagnostic method.

Fusarium species were identified in most samples from greenhouses, whereas *Cylindrocarpo*n species were noted only in seedlings from field nurseries.

Therefore, fungal pathogens responsible for seedling decline and root rot, representing the genera Alternaria, Botrytis, Cylindrocarpon, Fusarium, Pestalotia, Phoma, Phytophthora, Pythium and Rhizoctonia, were identified in greenhouses and field nurseries. Pathogenic isolates accounted for 57.5% of fungi colonizing roots and 30.5% of fungi colonizing stems of pine seedlings. Moreover, the molecular method with PCR markers was a highly effective tool for identifying fungal pathogens on pine seedlings.

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В. М. Деменко, О. М. Ємець
Сумський національний аграрний університет

ЗАХОДИ ЗАХИСТУ ЯБЛУНІ ВІД ШКІДНИКІВ

У 2018 р. площа плодово-ягідних культур становила 228 тис. га, з них плодоносних – 200 тис. га. Валове виробництво плодово-ягідної продукції нараховувало2571 тис. т, у т. ч. в сільськогосподарських підприємствах – 556 тис. т, господарствах населення – 2015 тис. т. Серед плодово-ягідних культур основною культурою є яблуня. У 2014 р. вона вирощувалася на площі 115,2 тис. га, валове виробництво яблук становило 1090 тис. т. У 2015 р. площа насаджень яблуні дорівнювала 111,7 тис. га, виробництво яблук – нараховувало 1200 тис. т. У 2016 р. спостерігається зменшення площі яблуні до 106,1 тис. га, виробництво яблук – до 1100 тис. т. У 2017 р. площа насаджень яблуні була 103,1 тис. га, валове виробництво яблук – 1070 тис. т. У 2018 р. площа насаджень становила 101,6 тис. га, а виробництво яблук склало 1500 тис. т. Отже, в Україні є достатньо резервів для забезпечення населення яблуками за рахунок закладання нових інтенсивних насаджень, поліпшення технології вирощування, зберігання вирощеної продукції.

Ураховуючи, що плодові дерева вирощують на одному місці протягом багатьох років, у садових насадженнях утворюються певною мірою стабільні екологічні умови, які формують відносно постійний склад шкідливої та корисної фауни. В Україні відомо близько 400 видів комах, які пошкоджують плодові насадження. Враховуючи різну насиченість яблунею в різних природних зонах, видовий склад шкідників також неоднаковий. Для підвищення стійкості плодових культур проти шкідників, одержання високого врожайності необхідно проводити захисні заходи протягом усього року. Система захисних заходів для промислових насаджень яблуні включає до 10–12 обробок проти комплексу шкідників.

Дослідження проводили в умовах навчально-наукового виробничого комплексу Сумського національного аграрного університету у 2016–2018 рр. на сорті яблуні Флорина. Методика проведення досліджень загальноприйнята. Сад Сумського НАУ закладено у 2008 р. Ураховуючи те, що насадження яблуні