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THE FIRST REPORT ON THE MYCOPARASITE *TRICHOHECIUM ROSEUM* (PERS. 1809) ON *VENTURIA INAEQUALIS* (COOKE) G. WINTER IN RUSSIA

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*A hyperparasite was observed on *Venturia inaequalis* during a survey in Krasnodar region, South Russia. Morphological characterization using light microscopy and molecular characterization by sequencing ITS region of nuclear ribosomal DNA and phylogenetic analysis revealed the identity of the pathogen as *Trichothecium roseum*. This is the first record of hyperparasite *Trichothecium roseum* on *Venturia inaequalis* from Russia.*

Keywords: hyperparasite; *Trichothecium roseum*; *Venturia inaequalis*

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ПЕРВОЕ СООБЩЕНИЕ О МИКОПАРАЗИТЕ *TRICHOHECIUM ROSEUM* (PERS. 1809) НА *VENTURIA INAEQUALIS* (COOKE) G. WINTER В РОССИИ

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В результате обследований садов Краснодарского края, Юг России, на пяти-нах парши яблони был найден белый мицелиальный налёт. После оценки мор-

фологических и генетических характеристик чистой культуры гриба, с использованием метода секвенирования, он был идентифицирован как *Trichothecium roseum*. Факт паразитирования подтверждён с соблюдением постулатов Коха.

Ключевые слова: гиперпаразит; *Trichothecium roseum*; *Venturia inaequalis*
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Venturia inaequalis (Cooke) Winter is a haploid fungus from the Ascomycota subdivision that is responsible for an economically-important apple disease reported in all areas of temperate climate – apple scab [6]. *V. inaequalis*, anamorph – *Fusicladium dendriticum* (Wallr.) Fuckel, affects only this culture and its wild relatives and is characterized by high intrapopulation variability and adaptability due to the annual sexual process leading to recombination of traits [7; 18]. After infection of the host plant, the pathogen develops in the subcuticular space of the leaf or some other terrestrial organs of the apple tree [1;16]. Krasnodar Region is characterized by favorable weather and climatic conditions for the epiphytotic development of apple scab. There has been an increase in the aggressiveness of the *V. inaequalis* population and an increase in its parasitic activity in the region since 2004 [4]. The epiphytotic development of the disease often results in the reduction of fruit quality or almost complete devastation of an apple crop [14]. In the absence of disease control, over 80 % of the fruits of susceptible cultivars can be damaged. Depending on the degree of development of the disease, 10 to 15 or even more fungicidal applications are usually needed for efficient control. An alternative to fungicides is the use of apple scab-resistant varieties and biological control of the disease [15].

During a regular survey of orchards in the South of Russia in August 2021, a significant number of signs of white bloom were found on apple scab spots, both on fruits and on leaves.

The purpose of this study: to determine the species identity of the hyperparasite found on *Venturia inaequalis* using classical and genetic methods. The research tasks included confirmation of hyperparasitism in the detected *Trichothecium roseum* by the Koch method, deposition in GenBank and in the departmental collection of beneficial microorganisms. The scientific novelty lies in the first detection of the hyperparasite *T. roseum* on *Venturia inaequalis* in Russia.

As a result of microbiological analysis of these symptoms, spores of the hyperparasitic fungus *T. roseum* were found on scab-affected leaves of the Jonagold

apple tree (Krasnodar Region) (Fig. 1A). The mycelium of the hyperparasite was transferred with a sterile needle from a scab spot without surface sterilization of the leaf to potato glucose agar (PGA) in 3 replicates. The growth of the colony lasted 7 days at a temperature of 25°C. The isolated strain received the code L21/22. Using microscopy, the fungus was identified as *T. roseum*. Colonies are moderately fast growing, flat, suede-like to powdery, initially white but becoming rosy, pink or orange with age (Fig. 1C). The conidiophores are indistinguishable from the vegetative hyphae until the first conidium is produced. They are erect, unbranched, often septate near the base, more or less rough-walled, bearing basipetal zig-zag (alternating) chains of conidia at the apex. Conidia are two-celled ellipsoidal to pyriform, with an obliquely truncate basal scar, hyaline, smooth to delicately roughened and thick-walled [11, 24]. (Fig. 1B).

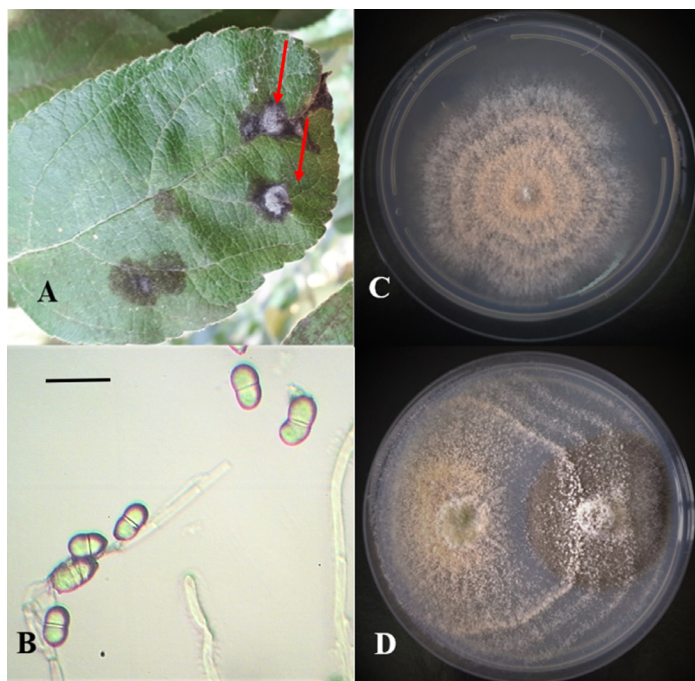


Fig. 1. **A** – white mycelial plaque on apple scab spots on the leaf (indicated by arrows); **B** *Trichotheceum roseum* conidia (Bar=20 µm); **C** – pure culture of *Trichotheceum roseum* (PGA); **D** – growth of the antagonist *Trichotheceum roseum* (on the left) on the colony of the pathogen *Venturia inaequalis* (on the right) under *in vitro* conditions (PGA)

Total DNA was isolated by the sorbent method on magnetic particles («Sintol», Moscow). Amplification of the internal transcribed spacer (ITS) was performed according to White et al. [25]. A 25 μ L reagent mixture for one reaction contained 5 μ L of 5X PCR MasterMix MagMix («Dialat Ltd», Moscow), 10 pM of each primer and 20 ng of target DNA. PCR products intended for sequencing were purified using the GeneJET PCR kit (Fermentas). Sanger sequencing was performed in ABI PRISM 3500 apparatus (Applied Biosystems). The sequencing reaction was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Alignment of sequences using the program «BioEdit». The nucleotide sequence of the ITS region was analyzed for belonging to the specific species using the NCBI BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). Then obtained sequence was deposited in GenBank (GenBank number: OM462526).

Phylogenetic tree was constructed in MEGA X program using additional sequences uploaded from GenBank: NR_165998, NR_111321, NR_160148, AB298698, AJ621773, LC514693, MF161110, MH470248 and outgroup: AJ230675. A phylogenetic species hypothesis showed the causal agent was conspecific with *T. roseum* (Fig. 2).

The pathogenicity of the fungus against *V. inaequalis* was determined *in vivo* and *in vitro*. Apple leaves with fresh scab symptoms were inoculated with a suspension of *T. roseum* spores. The spore suspension was obtained by washing off the surface of a pure culture and filtered through four layers of gauze to remove mycelium fragments. The spore concentration was determined using a hemocytometer and adjusted to 10^6 conidia/ml. The treated leaves were placed in Petri dishes with a dampened filter paper disk to create a humid chamber. At the same time, no more than two leaves were placed in each cup. The experiment was repeated on ten leaves. After 2 weeks of incubation, a similar white coating was observed on scab spots. The hyperparasite was re-isolated on PGA medium. Hyperparasitism of *T. roseum* in laboratory conditions was determined by the method of counter (double) cultures with joint splicing with a 30-day monospore culture of *V. inaequalis* on PGA medium. Sowing of double cultures was performed by injection, in three repetitions. After 7 days of incubation at a temperature of 25°C, an increase in the antagonist on the pathogen colony (hyperparasitic zone) was noted (Fig. 1D).

A pure culture of the fungus was deposited in the departmental collection of useful microorganisms for agricultural purposes of the Federal State Budgetary Scientific Institution «All-Russian Research Institute of Agricultural Microbiology», St. Petersburg, Russia under the number RCAM05940.

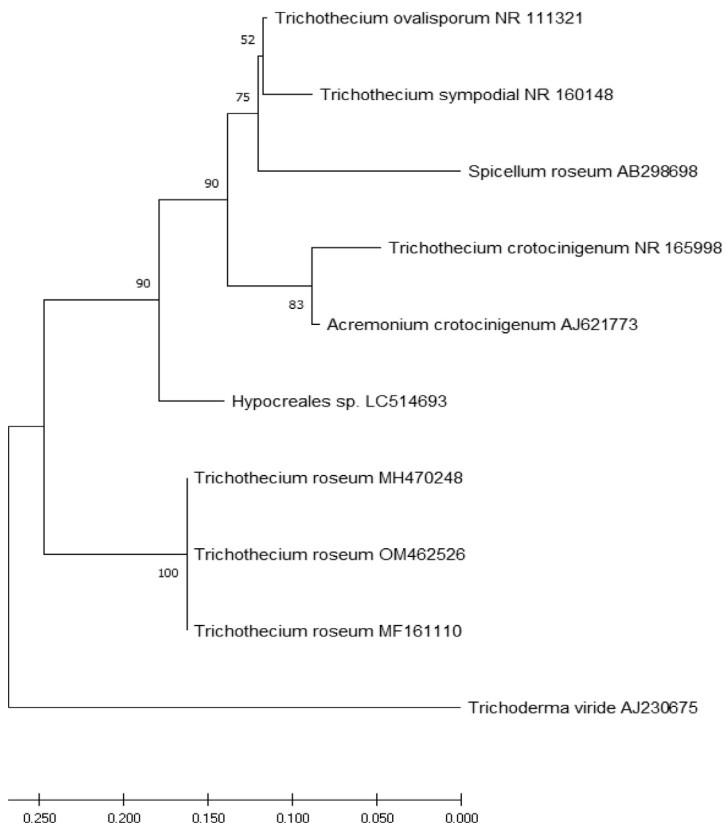


Fig. 2. Maximum likelihood tree calculated from the ITS region of studied sample together with 8 another isolates of *Trichothecium* sp. Isolates are indicated in the tree by accession number of collection. Numbers at each node indicate bootstraps based on 1000 replications.

T. roseum has a worldwide distribution [10]. It has been found in soils in various countries including Poland, Denmark, France, Russia, Turkey, Israel, Egypt, the Sahara, Chad, Zaïre, central Africa, Australia, Polynesia, India, China, and Panama. Known habitats of *T. roseum* include uncultivated soils, forest nurseries, forest soils under beech trees, teak, cultivated soils with legumes, citrus plantations, heathland, dunes, salt-marshes, and garden compost [10]. Commonly, this fungus can be isolated from the tree leaf litter of various trees [2], including birch, pine, fir, cotton, and palm [10]. It has also been isolated

from several food sources such as apples, grapes, hazelnuts, pecans, pistachios, peanuts, and coffee [19]. *T. roseum* is known to produce pink rot on apples [9]. *T. roseum* also causes apple core rot which is a serious problem in China [13]. This micromycete was not found in the apple core rotting pathocomplex in the Krasnodar Region [3].

It also is a destructive mycoparasite [8]. It is known that *Trichothecium roseum* exhibits parasitic properties against many species of macro- and micro-mycetes [17, 20], such as *Botrytis cinerea* [23], *Sclerotinia sclerotiorum* [12], *Bipolaris sorokiniana*, *Verticillium dahliae*, *Fusarium oxysporum*, *Phytophthora infestans*, *Puccinia graminis* as well as a number of others that cause grape mildew, root rot, tracheomycosis and potato cancer, clover anthracnose, tree species cytosporosis, maize blister smut, stone fruit and pome fruit moniliosis, sugar beet clamp rot, etc. [5, 21]. A hyperparasite on *Venturia inaequalis* was previously reported in India [22].

To our knowledge this is the first record of *Trichothecium roseum* on *Venturia inaequalis* in Russia. *Trichothecium roseum* has a potential to be used for managing this apple scab as a sustainable and ecofriendly alternative.

The authors declare that they have no conflict of interest.

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