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ORIGINAL ARTICLE

The potentional use of hyperparasitic *Tuberculina persicina* and *Fasarium sambucinus* for biocontrol of *Puccinia* sp.

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We have discovered and introduced in culture *in vitro* hyperparasitic fungi: *Tuberculina persicina* (Ditmar) Sacc. and *Fusarium sambucinus* Fuckel. In nature, these species caused a significant damage and antagonistic effect against the rust fungus *Puccinia* sp. on elderberry. The isolation and identification of regional hyperparasitic fungi are necessary to provide a pool of biological agents for biocontrol. The high activity of isolated isolates, as well as the possibility of obtaining spores on solid nutrient media, is promising for the biocontrol of rust fungi infecting wild and cultivated trees.
Key words: Tuberculina, Fusarium, biocontrol, plant diseases, hyperparasitism.

Due to the high load on biosystems with synthetic insecticides and fungicides, the use of biological agents is a promising direction. Most often, natural antagonists of insects, bacterial and fungal rots, and rust fungi are used as biological agents. Many anamorphic forms of fungi are hyperparasites of rust fungi, and due to the complex life cycle, new species are constantly found. Verticillium lectures and spherelopsis phylum are often used as promising biocontrol agents, as well as *Tuberculina photiniae, Zygosporium gibbum, Cladosporium uredinicola, Scytalidium uredinicola, Aphanocladium album, Cladosporium tenuissimum, Cladosporium cladosporioides, Cladosporium pseudocladosporioides, Ramularia* sp. and many others (Spencer & Atkey, 1981; Plachecka, 2005; Bartkowska, 2007; Manimohan & Mannethody, 2011; Zhao et al., 2017; Torres et al., 2017). *Tuberculina persicina* is an anamorph stage of *Helicobasidium purpureum* which is rust parasites (Lutz et al., 2004). In this regard, often discussed the possibility of using *Tuberculina* sp. for biological control of rust fungi. In our study was detected hyperparasitism species *T. persicina* and *F. sambucinum* in infected trees *Sambucus racemose* by rust fungus *Puccinia* sp., and were put in culture *in vitro*.

Methods

T. persicina and *F. sambucinus* was isolated from the infected with *Puccinia* sp. trees in the South-Siberian Botanical Garden. The fungi were transferred to potatodextrose agar (PDA) and incubated at 25 ºC, under diffuse light. The individual fungus colony was transferred to fresh media until a clear culture was isolated. Identification was carried out morphologically and by PCR analysis (BLAST algorithms). For isolate, a portion of a colony was scraped from the agar plates and transferred into a 1.5-ml Eppendorf tube. DNA was isolated by DiamondDNA kit (ABT LLc., Russia). The primers ITS1 5'- TCCGTAGGTGAACCTGCGG -3', ITS4 5'- TCCTCCGCTTATTGATATGC -3' were used for amplification (White et al., 1990). PCR(s) and sequencing were carried out as our previous studies (Skaptsov et al., 2017; Skaptsov et al., 2018).

Results and Discussion

Puccinia sp. infected the trees species *Sambucus racemosa*. At the same time on the spots of *Puccinia* sp. two types of hyperparasitic fungi were identified. Morphological and BLAST search data using ITS markers allowed us to identify hyperparasites. In most cases, *T. persicina* sclerotia were found. In some cases, its teleomorphic stage of *H. purpureum* was formed on sclerotia. Both stages were isolated and cultured in vitro. (fig. 1). To a lesser extent, *Puccinia* sp. was infected with *F. sambucinum*. A significant reduction in the growth of *Puccinia* spots has been identified. In most cases, after the appearance of *T. persicina* sclerotia, *Puccinia* sp. suspended and the spot dries up. Parts of the *T. persicina* sclerotia and mycelium of *F. sambucinum* were transferred to nutrient media. The isolates showed a good growth rate on the PDA agar. Mature conidia were formed in 7 days after inoculation. *T. persicina* sclerotia formed dense masses on nutrient media and did not form a teleomorphic stage in in vitro culture. Many species are bio control agents of plant pathogenic rust fungi. *Tuberculina* plays a major role in controlling of various plant diseases. A promising use of anamorphic forms may be to protect the forest due to the high risk of reproduction of teleomorphic forms in cultural species that are their parasites. In this regard, long-term field studies are necessary conditions for the transition stage in anamorphic teleomorphic to prevent the risk of fungal diseases of crop species.

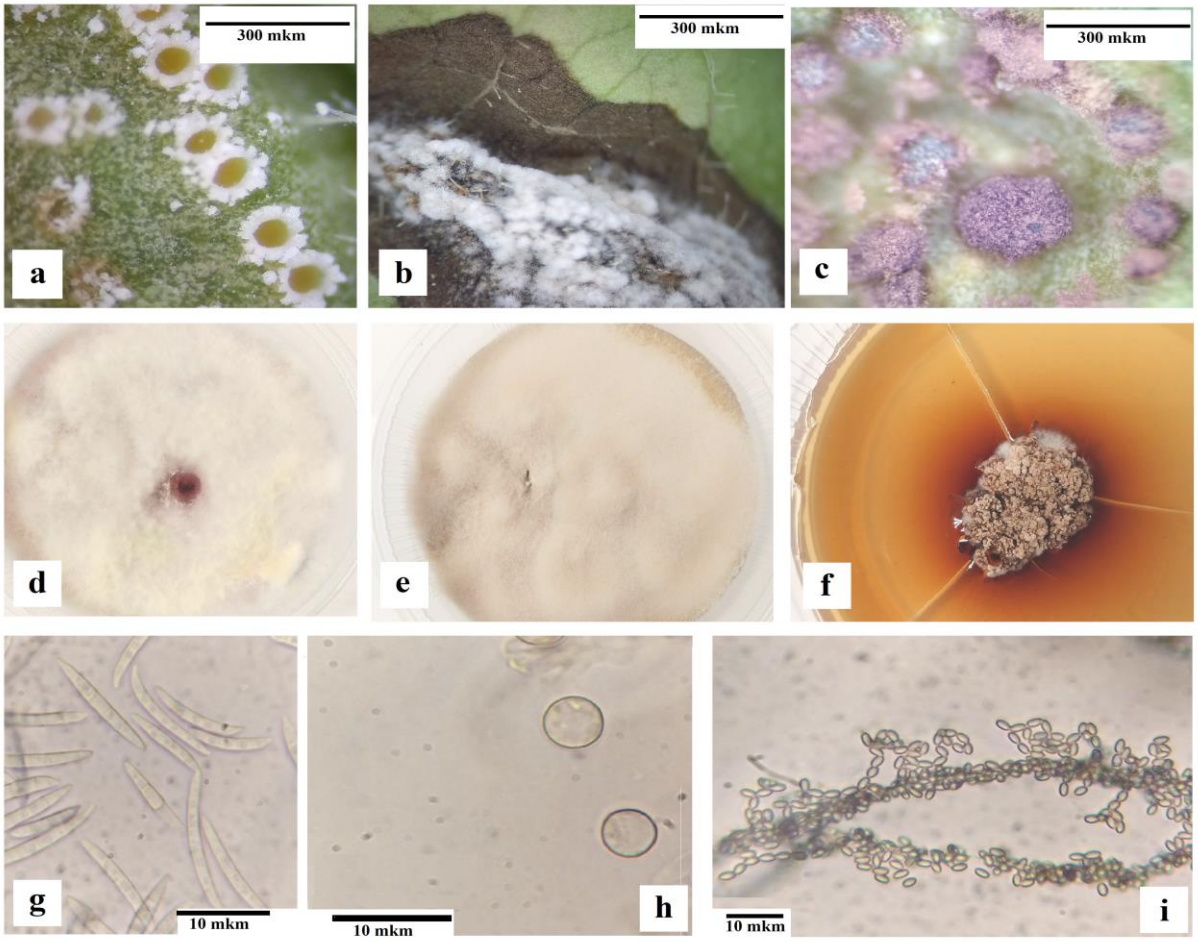

Figure 1. Morphology of *T. persicina* and *F. sambucinum* on nature and *in vitro* culture. a. Puccinia sp. on *Sambucus racemosa* leaves; b. *F. sambucinum* on Puccinia sp. spot; c. *T. persicina* sclerotia on Puccinia sp. spot; d – f. *F. sambucinum, T. persicina* teleomorph and anamorph on PDA plate; g – i. *F. sambucinum, T. persicina* anamorph and teleomorph spores

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