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Wintering ability of *Calophoma complanata* under the conditions of Saint Petersburg area

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Abstract. Giant hogweed is one of the most widespread invasive alien species of the Baltic region. The pycnidial fungus, *Calophoma complanata* is being evaluated as a potential bioherbicide for control of *Heracleum sosnowskyi*. The aim of this work is to evaluate an ability of the potential mycoherbicide *C. complanata* MF-32.121 to overwinter in the conditions of Saint Petersburg area. Plants inoculated by *C. complanata* MF-32.121 successfully survives wintering in the conditions of Saint Petersburg area. Pearl barley covered by *C. complanata* MF-32.121 mycelium successfully survives wintering in the same conditions as well. When favorable conditions have arisen, the *C. complanata* MF-32.121 mycelium is capable to infecting young giant hogweed. Moreover, it was revealed that a change in the biochemical composition, namely, an increase in the level of trehalose, contributes to the manifestation of a greater tolerance of *C. complanata* MF-32.121 mycelium to temperature-humidity conditions.

1 Introduction

Giant hogweed is one of the most widespread invasive alien species of the Baltic region [1]. The bioherbicides are used as part of complex methods for managing troublesome weeds when the classical methods are insufficient to control their distribution [2]. The isolate of phoma-like fungus *Calophoma complanata* MF-32.121 is being evaluated as a potential bioherbicide for giant hogweed control [3]. This is a highly specialized plant pathogen that causes the canker of roots, stems and leaves of *Apiaceae* plant species. It is shown that in Canada *C. complanata* overwinters in the soil and seed of *Pastinaca sativa* [4]. The aim of this work is to evaluate an ability of the potential mycoherbicide *C. complanata* MF 32.121 to overwinter in the conditions of Saint Petersburg area.

2 Materials and methods

The strain of *C. complanata* MF-32.121 was obtained from the collection of pure cultures of the Mycology and Phytopathology Laboratory of the All-Russian Institute of Plant Protection.

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The *C. complanata* MF-32.121 mycelium was grown on a pearl barley (15 g / 10 ml of water) until concentration 0.7×10^8 colony-forming units (CFU) per gram. Then, the pearl barley colonized by *C. complanata* MF-32.121 mycelium was placed on the soil surface, depth 20 cm and at the height of 1.5 m above the ground for wintering in November 2018 at the coordinates of 59.735287°, 30.428694° (Pushkinsky district, Saint Petersburg). Alternatively, it was placed in 4° C refrigerator. After 4 months *C. complanata* MF-32.121 mycelium was evaluated for viability and pathogenicity.

Plants of *Heracleum sosnowskyi* at the rosette stage were inoculated with mycelium of *C. complanata* MF-32.121 ($5*10^4$CFU/ml) and incubated for 24 h at 100% relative humidity. By the 14-th day post inoculation, the plants of *H. sosnowskyi* were placed on the surface of the soil for wintering in November 2018 at the coordinates of 59.735287°, 30.428694° (Pushkinsky district, Saint Petersburg).

Survival of overwintering *C. complanata* MF-32.121 mycelium was determined by quantity of CFU on potato sucrose agar media. The pathogenicity of reisolates was assessed by the area of *H. sosnowskyi* leaf necrosis on the 4th day post inoculation (dpi). The *H. sosnowskyi* plants in the rosette phase was inoculated with an infectious dose of 25 mg / ml (2 ml / plant) at a 24-h period of increased moisture.

Microphotographs of pycnidia and conidia of isolate *C. complanata* MF-32.121 was obtained using a Leica M165 Stereo Microscope equipped with a digital camera.

Overwintered *C. complanata* MF-32.121 mycelium was analyzed by the multilocus analysis included amplification and sequencing of a large subunit ribosomal DNA (LSU) and partial beta-tubulin gene were sequenced using primers listed in table 1.

The sugar content in the *C. complanata* MF-32.121 mycelium was determined using the automated HPTLC system (CAMAG, Muttenz, Switzerland). The TLC plate was developed in 6% boric acid-acetic acid-ethanol-acetone-ethyl acetate (10: 15: 20: 60: 60). Staining was carried out with a resorcinol / sulfuric acid reagent in methanol under heating.

Statistical processing was performed by analysis of variance and when appropriate means comparisons among were tested using an LSD test at p=0.05.

3 Results and discussion

The strain *C. complanata* MF-32.121 successfully wintered in the Pushkinsky District of Saint Petersburg between November 2018 and February 2019. Similarity original *C. complanata* MF-32.121 and isolates obtained from the overwintering infectious materials was confirmed by molecular phylogenetic analysis as well by morphological features. Obtained sequences of LSU and beta-tubulin loci of wintered reisolates were compared with those of original *C. complanata* MF-32.121 (accession numbers of sequences are MH634681 and MH665689.1 for LSU and beta-tubulin gene respectively).

Also, the morphological features of pycnidia, that were formed on the surface of wintered plant material and pearl barley, of the reisolate corresponded to the description of the native strain *C. complanata* MF-32.121 pycnidia (fig. 1).

The greatest survival of *C. complanata* MF-32.121 mycelium was observed during wintering on the soil surface (fig. 2). Reisolates from inoculated plants, that were laid on the soil, formed more CFU, than isolates, obtained from overwintered both over and in the soil plants. Also, reisolates from plants, that were wintered on the soil surface, were the most aggressive.

Carbohydrate analysis of overwintered mycelium showed that the highest level of trehalose was observed in the case of the highest viability of pathogen and aggressivity to giant hogweeds (fig. 3). The remaining samples lost viability and pathogenicity by 40-50%, while the content of trehalose was also higher than in the original mycelium ($p \leq 0.05$). Trehalose is known to be a major factor in the resistance of microorganisms to drying [2]. However, during wintering, environmental factors also influence the biosynthesis of this disaccharide. Interesting, that the significantly accumulation of trehalose in the Arctic and Antarctic strains of micromycetes growing at low temperatures was shown [8].

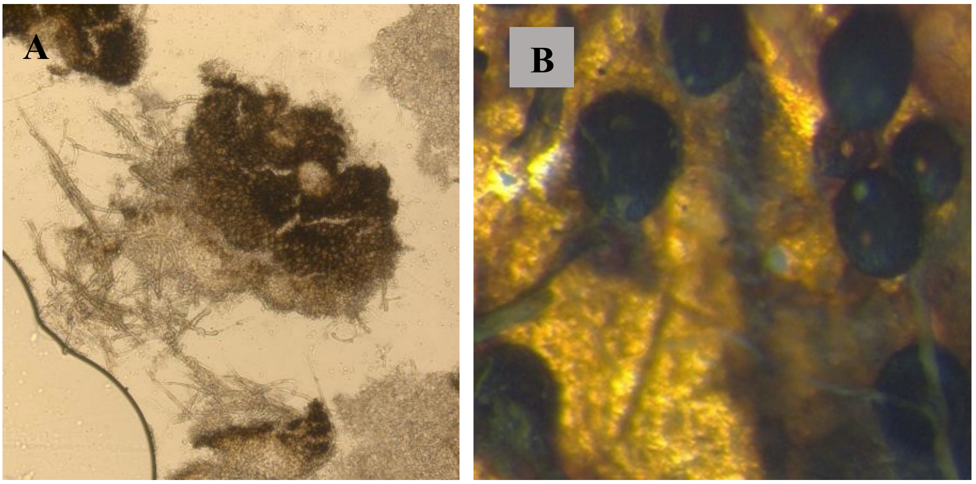

Fig. 1. A - the native strain *C. complanata* MF-32.121 pycnidia on the potato sucrose agar; B – the pycnidia observed on the overwintered plants

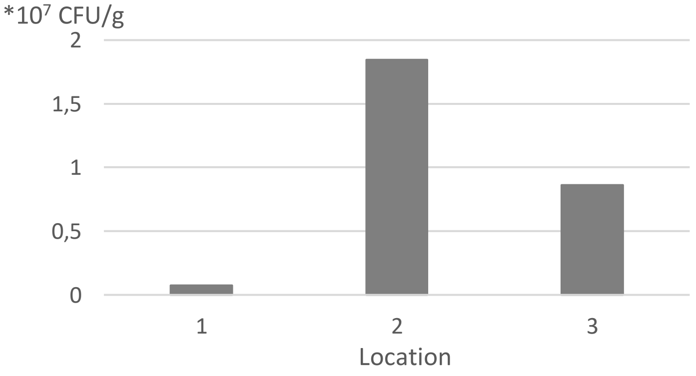

Fig 2. Survival of overwintering *C. complanata* MF-32.121 mycelium $LSD_{0.05}=0.5*10^7$. Probe location: 1 - 1.5 m above the ground; 2 - on the soil surface; 3 – 20 cm soil depths.

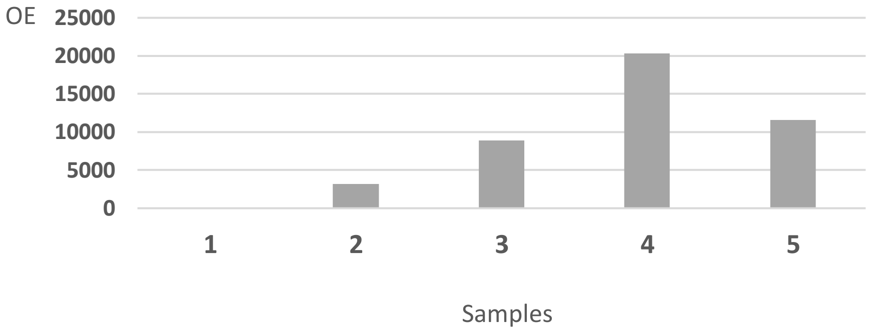

Fig 3. The level of trehalose. 1 - pearl barley; 2 - infected by *C. complanata* MF-32.121 barley before wintering; 3 – infected by *C. complanata* MF-32.121 barley after wintering at height of 1.5 m above the ground; 4 – infected by *C. complanata* MF-32.121 barley after wintering on the soil surface; 5 – infected by *C. complanata* MF-32.121 barley after wintering in 20 cm soil depths. $LSD_{0.05}=3*10^3$

Table 1. Primers used for PCR

Primers	Sequences 5'–3'	Annealing temperature, °C	References
LR0R	ACCCGCTGAACTTAAGC	58	5
LR5	TCCTGAGGGAAACTTCG		6
T1	AACATGCGTGAGATTGTAAGT	57	7
T2	TAGTGACCCTTGGCCCAGTTG		

Thus, plants inoculated by *C. complanata* MF-32.121 successfully survives wintering in the conditions of Saint Petersburg area. Pearl barley covered by *C. complanata* MF-32.121 mycelium successfully survives wintering in the same conditions as well. When favorable conditions have arisen, the *C. complanata* MF-32.121 mycelium is capable to infecting young giant hogweed. Moreover, it was revealed that a change in the biochemical composition, namely, an increase in the level of trehalose, contributes to the manifestation of a greater tolerance of *C. complanata* MF-32.121 mycelium to temperature-humidity conditions. In the future, this biochemical indicator can be used as an additional criterion for assessing the quality of mycoherbicides.

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