Greenhouse Test for the Resistance to Root and Stem Rot of *Hypericum perforatum* L. Accessions

A. KAČERGIUS and D. RADAITIENĖ*

Institute of Botany, Laboratory of Phytopathogenic Microorganisms, Vilnius LT-2021, Lithuania

**E-mail: audrius@botanika.lt*

Abstract

Root and stem rot caused by soil-borne agent *Fusarium avenaceum* is a major disease of wild *Hypericum perforatum* accessions in the field collection of Medicinal and Aromatic Plants (MAP) of the Institute of Botany in Lithuania. These wild accessions of *H. perforatum* are growing as an initial material for breeding. In 1998–2001 the monitoring of epidemiological situation of field collection of *H. perforatum* showed differences among accessions considering the resistance to root rot. High intensity of root rot was observed in the third–fourth years of cultivation. The most damaged plants (> 50%) were among the accessions 219, 379, 381, and cv. Zolotodolinskaja. Fungi of the *Aspergillus, Cladosporium, Penicillium, Rhizoctonia,* and *Verticillium* genera were associated with *H. perforatum* roots together with the rot agent *Fusarium avenaceum*. Seven accessions from Lithuania and cv. Zolotodolinskaja of *H. perforatum* were tested for the resistance to root rot under greenhouse conditions. Two accessions (219, 381) were highly susceptible to the disease, another two (218, 383) were less susceptible, others were free of the symptoms of root rot. Accessions and single plants, survived after artificial infection, have been selected for further investigations.

Keywords: *Hypericum perforatum;* greenhouse test; *Fusarium avenaceum;* resistance

INTRODUCTION

Hypericum perforatum L. is one of many medicinal plants with constantly rising requirements in modern phytotherapy. Recently collecting from wild satisfied most needs for raw materials in pharmacy industries. Now in many countries medicinal and aromatic plants are grown commercially in huge areas. In order to provide plant material with high productivity and resistance, the breeding work has been started in many countries, including Lithuania. According to previous data, *H. perforatum* is characterised by a wide range of its morphological and productivity features. It gives an opportunity for wild accessions of this herb to become an important source of genetic variation for new cultivated material (RADUŠIENĖ 2000). This variation is favourable for the selection of resistance. Root and stem rot, caused by a soil-borne fungus *Fusarium*, may attack plants in any stage of growth. The disease symptoms may appear as yellowing. Other symptoms of the above-ground part are difficult to notice. Plants infected with this disease may be often killed by the pathogen. The monitoring of epidemiological situation in the field collection of Medicinal and Aromatic Plants (MAP) of the Institute of Botany showed differences among accessions of *H. perforatum* considering the damage caused by root rot (RADAITIENĖ *et al.* 2001).

The present research is the second part of a broader framework for the examination and selection of *H. perforatum* in Lithuania. The aim of this study was to screen *H. perforatum* accessions for resistant to root rot caused by *Fusarium* in greenhouse and compare with the data from field.

MATERIALS AND METHODS

Plant material. Seven accessions from Lithuania and one cultivar Zolotodolinskaja originating from Novosibirsk (Russia) were used in the experiment. The plants were cultivated in seedling trays containing peat under non-controlled greenhouse conditions.

Pathogen material. The isolate of *Fusarium avenaceum*, used in the experiment, was isolated from

surface-sterilised diseased roots of *H. perforatum* into malt extract agar (MEA) containing of 0.075 g/l streptomycin. For inoculation, the fungus was cultivated for 20 days on MEA (pH 6.5) in Petri dishes. Sporulating mycelium was replaced from the medium, homogenised in distilled water, and inoculum suspension prepared.

Inoculation. Thirty days after sowing, the seedlings were inoculated by pathogen inoculum suspension. Twenty-five plants per accession were used for inoculation. The suspension was spilled on soil 500 ml per tray.

Visual evaluation of disease parameters. The disease symptoms were measured using the disease index I_i (in %) according to JENKINSON and PARRY (1994) as well as WALER *et al.* (1998):

$$I_i = \frac{\sum (V_i a_i)}{\sum a} \times 100$$

where: V_i – the class indices that represent the disease value of a particular plant

a_i – the numbers of plant in each disease category

The values awarded to these indices are summarised below:

Class	V_0	V_2	V_4	V_6	V_8	V_{10}
Value	0	0.2	0.4	0.6	0.8	1
Disease severity*	0	< 20	40	60	80	100

*% of yellowed and wilting leaves

In order to verify that disease symptoms were a direct result of artificial inoculation, the pathogen was re-isolated from the infected plants by placing surface-sterilised segments of stem-base on MEA. After the incubation of 10–14 days, the isolates were identified.

RESULTS AND DISCUSSION

The disease resistance is the second important character after the productivity of cultivars and species of cultivated plants. The results indicate that the greenhouse test for the selection of wild accessions considering their resistance to root rot was successful. First symptoms of root rot were observed in plants of accession 219 on the 5th day after inoculation. Above-ground symptoms first appeared on older leaves as their yellowing and then progressed upward into younger leaves. Defoliation was revealed on severely infected plants. Under field conditions during wet season sporulating mycelium on the stem-base of diseased plants may appear (Figure 1). Accession 219 suffered extremely hard infection – all plants were killed during 10 days (Figure 3). Accession 381 was highly susceptible to *Fusarium* infection, too. Accessions 218 and 383 were more resistant (Figure 2), and other three ones together with cv. Zolotodolinskaja were free of symptoms. Figure 2 demonstrates the quantitative disease parameter – amount of diseased plants and Figure 3 qualitative parameter – infection severity. In order to confirm that the observed disease

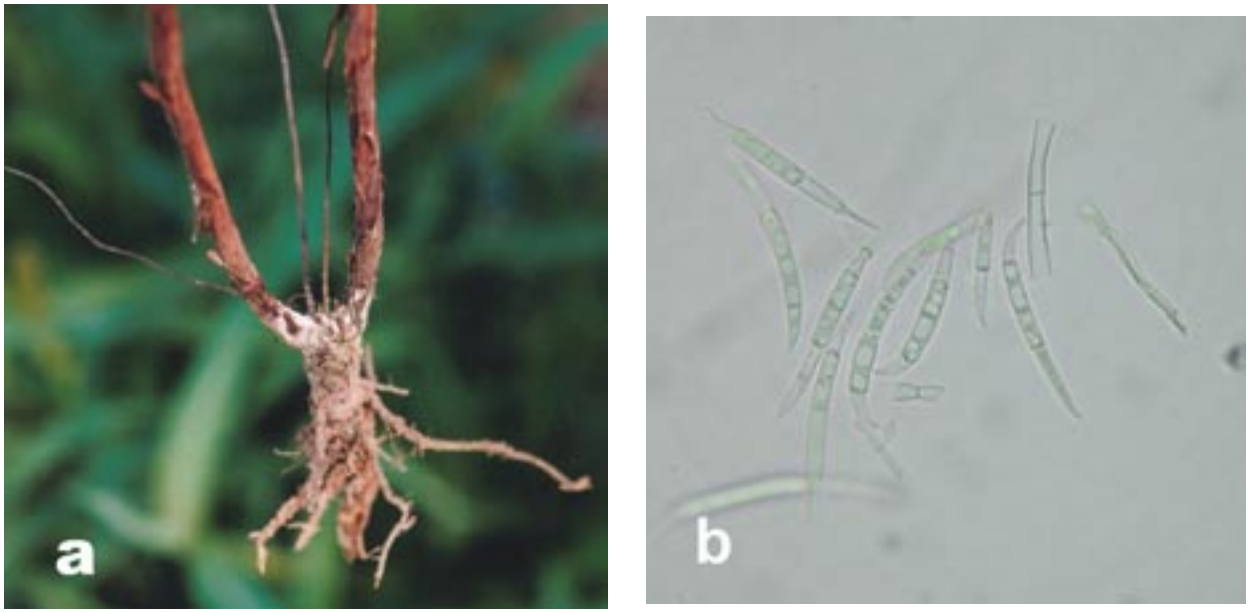

Figure 1. *Fusarium* root rot symptoms (a) and spores of *Fusarium avenaceum* (b)

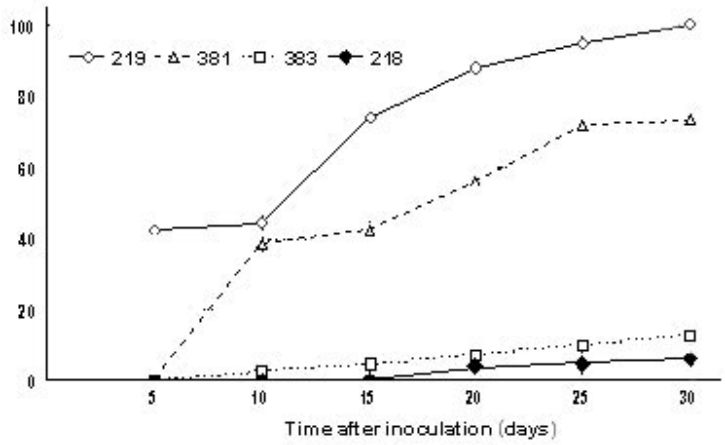

Figure 2. The severity of root rot of *H. perforatum* accessions after artificial inoculation

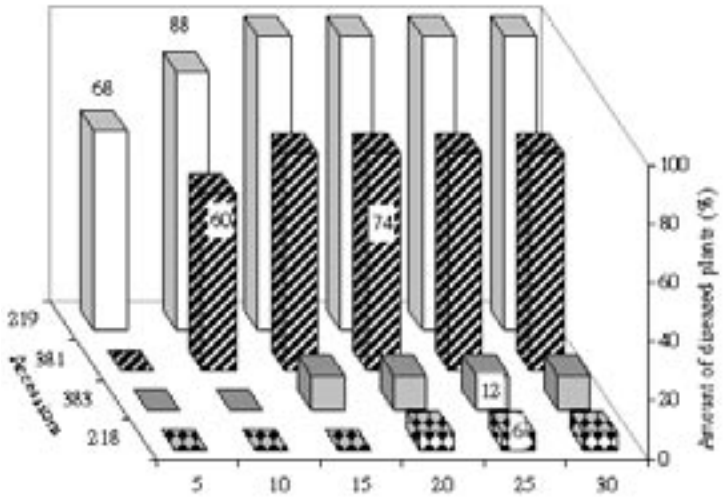

Figure 3. Amount of diseased plants of *H. perforatum* accessions after artificial inoculation

symptoms were really caused by artificial inoculation, the pathogen was re-isolated from the infected plant roots. These results were positive including some cases with saprotrophic bacterial disturbance. According to the field observation, the most diseased plants (> 50%) were identified in accessions 219, 379, 381, and cv. Zolotodolinskaja. High intensity of root rot was observed in the third–fourth years of cultivation. Fungi of the *Aspergillus, Cladosporium, Penicillium, Rhizoctonia*, and *Verticillium* genera were associated with *H. perforatum* roots together with the rot agent *Fusarium avenaceum*.

Accessions and single plants survived after artificial infection have been selected for further investigations.

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