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## FUNGAL INFECTIONS OF *ADONIS VERNALIS* L. FRUITS\*

**ABSTRACT:** Yellow pheasant's Eye is a herbaceous plant from dry ressy areas. Owing to habitat destruction and over- collection for ornamental and medical purposes, *A. vernalis* L. has become scarce in central and south Europe. The reasons for *A. vernalis* threatened are manifold. The low seeds germination rate is significant. According to our investigation the main cause of fruit destruction is fungal infection. From the surface of the fruits, collected in Deliblato Sands, the following micromycetes has been isolated and determined: *Fusarium solani* (Mart.) Sacc., *Fusarium sporotrichioides* Sherb., *Alternaria* sp. and *Drechslera* sp. Histological analysis showed the presence of conidiomata and conidia *Phoma* sp. in the seeds.

**KEY WORDS:** *Adonis vernalis*, fruits, micromycetes, *Phoma* sp., seeds

## INTRODUCTION

*Adonis vernalis* L. (Yellow pheasant's Eye) is a herbaceous perennial and a typical steppe plant. In the middle and southwest Europe the area is disjunct with some isolated growth places in a mainly azonal habitats scattered from south-east Sweden to south-east Spain (J a l a s & S u o m i n e n, 1989). Its grown places in central and south Europe are restricted to isolated grown places, but in more easterly Europe populations are increasing. Owing to habitat destruction and over-collection for ornamental and medical purposes, *A. vernalis* has become scarce in central and south Europe.

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Within the manyfold reasons for *A. vernalis* threatened the low seed germination is very significant. *A. vernalis* is an element of dense grassy places in which the seeds often have difficulties to reach open soil surface. In spite of this, regeneration by seeds does not take place each year. The seeds lose their viability very quickly, and no seed reserve is built up in the soil. Many seedlings die off due to soil desiccation in summer and frost in winter. An individual plant does not flower before its third or fourth year. Fertile reproduction only take place if seeds are abundant and the weather is rainy and cool in July to August, enabling the seeds to germinate immediately after maturing. Thus, vegetative growth is far more important than generative reproduction and happens by rhizomes producing new shoots each year (Melnik, 1998).

## MATERIAL AND METHODS

The specimens for mycological and histological analysis (fruits of *Adonis vernalis*) were collected from Deliblato Sand, protected area.

### *Mycological analysis*

The seeds were surface disinfected with 4% NaOCl and another seed samples left untreated and then placed, in moistening chambers and on malt agar (MA) (Booth, 1971a). The morphologically different micromycetes were reisolated on selective mycological media. After the period of incubation, the fungal structures were placed on microscopic slides and stained with Lactophenol cotton blue. Reproductive structures were measured and photographed on Reichert microscope with Canon Power Shot S40. The micromycetes from seed surface were determined using Booth (1971b) and Ellis (1997) identification keys.

### *Histological analysis*

Specimens were fixed in FAA (formalin-glacial acetic acid-ethanol, 10:5:85) at 4°C, 3 days. Fixed material was dehydrated in graded ethanol series and embedded in paraffin at 57°C. Sections (8–10 mm thick) were stained with haematoxylin and a second contrasting stain safranin (0.8%). All sections were photographed on Reichert microscope with Canon Power Shot S40.

## RESULTS AND DISCUSSION

A high degree of black and destroyed fruits probably caused by fungi was observed. From the surface of *A. vernalis* fruits, the following micromycetes have been isolated: *Alternaria* sp., *Drechslera* sp., *Fusarium solani* (Mart.) Sacc and *Fusarium sporotrichioides* Sherb. (Hyphomycetes, Deuteromycotina). After the seed disinfection from the seed surface it was isolated

only *Drechslera* sp. Species from genus *Alternaria* and *Drechslera* are transmitted through seeds. Some species are known as seed-borne pathogens. Their conidia colonize the seed coat during the seed development stage and when the seed germinates, they become active (W a t a n a b e, 2002). *Fusarium solani* is one of the most ubiquitous soil fungus and a destructive plant pathogen of hundreds of hosts, causing root and fruit rots (S h a m i a m et al., 2003).

We analyzed immature fruits of *Adonis vernalis* (Fig. 1). The structure of fruits was disrupted. The longitudinally-sectioned fruits showed an absence of normal pericarp layers: egzocarp, mesocarp and endocarp. Testa (seed coat), embryo and endosperm was destroyed. We did not notice normal structure of seed coat. The sections show only some parts of testa without visible cell layers. In addition, we observed an absence of embryo and endosperm (Fig. 1.1). The whole immature fruit with seed was full of mycelium and reproduc-

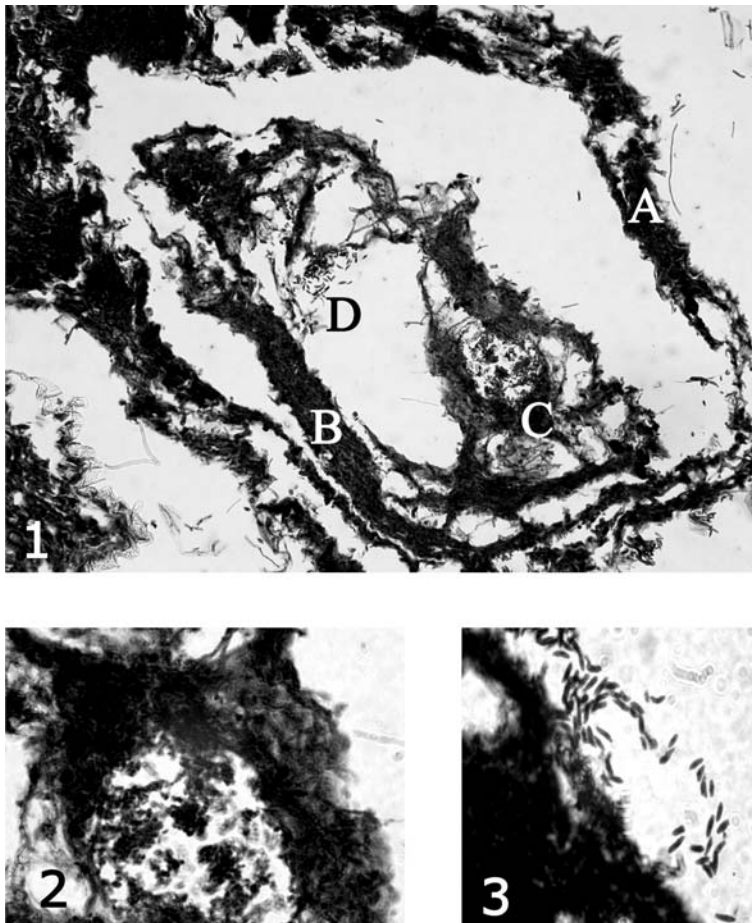


Fig. 1. The longitudinal section of infected *Adonis vernalis* fruits  
 Fig. 1.1. A. Destroyed pericarp; B. Destroyed testa; C. Reproductive structures of *Phoma* sp. Fig. 1.2. Conidiomata of *Phoma* sp. Fig. 1.3. Conidia of *Phoma* sp.

tive structures of fungi which was first determined as Coelomycetes. According to mycological experts from Kew Garden, England, who confirmed the identification, conidiomata and conidia belong to genus *Phoma* (Fig. 1.2 and Fig. 1.3). The investigations of development of reproductive structures of *Phoma macdonaldi* Boerema on sunflower seeds showed a complete disintegration of the cotyledon and the picnidia arranged in rows formed in the outer layers of the parenchyma (Stajić et al., 2001).

Long-term examination of *Dianthus superbus* ssp. *superbus* on habitat Brezi in Protected Landscape area (PLA) Litovelske Pomoravi (Czech Republic) showed similar results. Three pathogenic fungi, *Alternaria dianthi*, *Fusarium oxysporum* and *Verticillium albo-atrum* were isolated from seeds and capsules and their negative influence on the germination and development of young plants was proved (Mikulik et al., 2001—2002).

This results arise new questions, ideas and solutions in the concept of the threats of plant species.

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## ГЉИВИЧНЕ ИНФЕКЦИЈЕ ПЛОДОВА *ADONIS VERNALIS* L.

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### Резиме

Гороцвет је зељаста биљка сушних предела. У централној и јужној Европи, као типично степска биљка, има ограничено распрострањење, док је у источној Европи бројност популација у опадању. У централној и јужној Европи ова биљка постаје све угроженија због претеране експлоатације од стране човека, у медицинске сврхе, као и због нарушавања њеног станишта. *A. vernalis* је као угрожена биљка укључена у црвене књиге. Један од разлога угрожености *A. vernalis* је ниска способност клијавости семена. Према нашим истраживањима главни узрок деструкције плодова је инфекција гљивама. Са површине плодова *A. vernalis*, сакупљених у Делиблатској пешчари, изоловане су и детерминисане следеће микрочицете: *Fusarium solani*, *Fusarium sporotrichioides*, *Alternaria* sp., *Drechslera* sp. Хистолошки пресеци инфицираних плодова показују значајне промене: перикарп и семена су разорени мицелијом и плодноним телима гљиве из рода *Phoma*, према мишљењу миколошких експерата (Kew Garden, Енглеска) који су потврдили идентификацију. Ови резултати доносе нова питања, идеје и решења о концепту угрожености биљних врста.