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THESIS OF Ph.D. DISSERTATION

FUNGAL DISEASES OF PROTECTED PLANT SPECIES AND OF WHITE MISTLETOE IN THE NATIONAL PARK OF ŐRSÉG

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1. Preliminaries and aims of the research work

Among plant population endangering factors the changes, disruption, and disappearance of living places, changing of climate or even illegal plant collection will be mentioned by nature conservation experts. Beside them however microscopic fungi infecting the saved plant populations has to be taken into consideration in this respect. Mapping of health status and fungal infection rate of a landscape or a nature park would be therefore very important.

In Hungary, research work on fungal diseases started about 1850 (BOGNÁR 1994). Still, up to now no sufficient data has been collected on species diversity, distribution, dominance of plant infecting microscopic fungi, as well as on their damaging effect. And no data occurs about their appearance time and importance. Practically, we can state, that we do not know the health situation and pathology of protected plants.

Former research works at national parks were focussed mainly on higher plant and animal world. Therefore, our aims, which are directed to investigate species distribution, hosts and effects of microscopic fungi present at the Őrség National Park, can be considered as a pioneer work.

Our mapping work on diseases of protected plant species has started in 2002 at the Őrség National Park. As a result a dissertation has been made titled: „Az Őrségi Nemzeti Park néhány védett növényfaja és kórokozói" (Some Protected Plant Species of the Őrség National Park and Their Diseases). In this work populations of four plant species and their pathogens were discussed. At that time raised the claim to extend the investigations to other protected plant species of the Park. Although this research do not induce directly economic benefits, still it has indisputable value because of the genetical value, rarity and aesthetic role of the protected plant species.

It is to be noted, that this subject includes more fields of the science. First a botanical work was necessary to identify the plant species of the Park, then to make studies on their ecological demands, on preferred living place, and the types of their living places. Within the plant pathology the knowledge of mycology was important.

The investigations on white mistletoe (*Viscum album* L.) was not strictly belonging to the subject of microscopic fungi, since it is not considered to be a protected species. FISCHL and cooworkers made studies on infection of mistletoe by microscopic fungi populations at Keszthely from 1978 on, and this made me initiate investigations on microscopic fungi on protected plant species of the Őrség National Park.

The main topics of investigations

Identification and description of microscoping fungi living on protected plant species of the Őrség NP at their natural circumstances, including the description of symptoms of the pathogen-host relations and determination of the damage by these diseases.

Determination of occurrence, distribution and spreading of fungal diseases, as well as the damages caused.

The influence of fungal species on the populations of host species.

Identification and description of protected plant species, determination of endangering factors.

Determination of infection rate of tree and bush species infected by the white mistletoe (*Viscum album* L.) with special respect to the traditional fruit species (apple, plum, pear, cherry) of the Őrség district. Description and identification of microscopic fungi parasitic on the mistletoe, determination of the damage by the fungal pathogens, description of the symptoms of diseases.

2. Materials and methods

2.1. Place of the investigations

We have had the possibility to make the investigations on the 44.000 ha Őrség National Park. In case of some protected plant species, Kőszeg Protected Landscape District, Sághegy District and a part of County Vas were also included.

For denomination of sampling places we used the place and number of the forest plot or its topographical number, and in many cases the local name. In case of meadow plots we used their topographical number or its local name.

The laboratory tests has been made in the labors of Institute of Plant Pathology of the Pannon University.

2.2 Field investigations

The field observations of the protected plant species has been made at their original places, in the Őrség NP. During the survey of places we have striven to evaluate most of the protected species, as well as to identify their different populations. These data were completed by observations and informations of workers of the National Park. The data collection was not easy, since during the first part of the investigations the NP did not have uniform database, except for botanical report cards. We have identified also provenances, for which until now no botanical data occurred.

Plant sample collection has been made based on the symptoms caused by the pathogens (discolouration or spots on the leaves, drying of leaves or sprouts, leaf and sprout deformations, leaf drop, part of died plants). The frequent sampling has been extended also to the resting time of the plants to find fungal pathogens, which appear eventually over the vegetation period at a given time. This has an importance mainly at the evergreen trees and bushes.
We have recorded the visible symptoms and the severity of the diseases. Also digital photos has been made. The exact geographical living places of the infected plants were determined by a GARMIN Etrex Legend type GPS. The east-west and north-south coordinata were determined by the GPS.

We have made a short description of the provenances and the populations we have found there. This was necessary, since the place, territory has an influence on the host-parasite relation, and because this gives a possibility to make a comparison later to see possible changes. 1000 photos has been made to record the symptoms, we have found on 74 protected plant species. Symptom showing leaves, stems, and fruits were collected also from the mistletoe plants.

The size of the populatiosns were determined by counting. The estimation of bigger population size has been made according to the directives of the National Biodiversity System, Vol. IV (TÖRÖK 1997). In case of bigger population spots the number of plants were counted in circles of 5 m diameter, and the coordinata of the plot determined by GPS EOV. In other cases 5 x 5 m sampling quadrates were marked out, in which the plant number were counted. In case of small, dense plant species (e.g. *Vaccinium vitis-idaea* L., *Leucojum vernum* L., *Orchis morio* L.) these 5 x 5 m quadrates were divided into 1 x 1 m subquadrates and in every second quadrate the plant (sprout) number were counted. An exception were made in case of a Erythronium dens-canis L., where the size of quadrat was 3 x 3 m , to ensure the necessary sprout number for 50 plants. In case of trees (birch) and protected bushes (alder, willow) no sampling quadrats were created, since they grew sporadically. Their observation were made individually by their counting.

The severity of pathogen occurrence were determined by estimation. A scale of 0-5 were used to characterize the infection rates caused by rusts, and the MacKinney index were created by the formula as follows:

$$F_i \% = \{\Sigma (a_i \times f_i) / (n \times k)\} \times 100 , \quad \text{where}$$

a_i = scale of infection
f_i = frequency of the scale
n = number of plants investigated
k = highest scale value

The rate of rust infection on *Erythronium dens-canis* L. were measured in 3 years at 3 locations and the data were taken on a diagram also in function of time.

2.3. Laboratory investigations

Classical micological tests with wet chamber has been made on 400 disease suspicious samples collected from 74 protected plant species. Beside these, 24 similar samples of non-protected 17 plant species were tested. White mistletoe infection occurred on 18 tree and bush species at the territory of Őrség NP. Their 30 samples were also tested.

The samples were incubated in wet chambers at 22-23 °C for 1-7 days. Every two days microscopic observations were made with stereomicroscope and normal light microscopes. Size determination of spores were made by using ocular and object micrometers. Also microscopic photos were made and recorded on a PC.

In some cases pathogen cultures were produced to determine their macro- and micro-morphological traits. For these PDA (potato-dextrose-agar) medium were used. The morphological traits were measured on the 3rd, 6th and 9th day following the inoculation. The size of fungal bodies were measured on the plant samples and also on the cultures. Conidia from the cultures were left to grow in tap water. Reproduction organs (picnidia, acervulus, conidia) of the fungi were photographed by a Flex-cam video and recorded on a PC. Similar way were treated the samples collected from mistletoe.

To help the identification of the fungi, data from Hungarian books, (BÁNHEGYI et al. 1985-87, UBRIZSI and VÖRÖS 1968, PINTÉR 1997) and other books (FARR et al. 1995, VÁNKY 1985, BRANDENBURGER 1985, HANLIN 1992, SUTTON 1980 etc.) were studied and used. These latters have more data regarding the protected species, while the Hungarian books mention fewer protected species and are not so well-detailed.

No pot trials and artificial re-infection with the protected species and the fungi has been made, partly because of special regulations (see ANDRÉSI 2002), partly because the reproduction of the protected species would need much more years, and partly because such a work is basicly a hard nature-conservation question!.

3. Results

The main results of the research work made between 2004-2009 are as follows:

In case of *Erythronium dens-canis* L. we have proven, that the infection rate by the rust-causing fungus *Uromyces erythronii* (de Candole) Passerini has one peak in the year, which had a maximum value at the three sampling area of 72 %, 54 %, and 75,6 % respectively, always in the last days of April.

In case of an *Astrantia major* L. at Kétvölgy location on a 5 x 5 m sampling plot the infection rate by the fungus *Colletotrichum dematium* (Persoon) Fries and by *Septoria* species changed between 36,88 % and 80,95 % during 2006-2009. The highest value was measured in 2009, the lowest in 2006.

The infection rate of ***Vaccinium vitis-idaea*** L. were measured at two 5 x 5 m sampling plot, divided into 1 x 1 m quadrats. One of the 5 x 5 m quadrate, plot 71D, is located at village Szakony, in a forest. At this location the rate of infection changed between 10,50 % to 36,72 % during 2006-2009. The pathogens we have found were: *Phyllosticta vaccinii* Earle, *Thekopsora vacciniorum* Karsten, *Podosphaera myrtillina* (Schubert ex Fries) Kunze et Schmidt, *Exobasidium vaccinii* (Fuckel)

Woronin , *Truncatella angustata* (Pers.) S. Hughes, and *Discosia strobilina* Lib. The highest infection rate was found in 2006, the lowest in 2007.
The other sampling plot is located at Kétvölgy, in a forest, plot number 92B. The rate of infection changed here between 15,49 % and 18,76 % during 2007-2009. The highest value was measured in 2008, the lowest in 2009.

The infection rate of *Leucojum vernum* L. was measured at Alsószölnök, in the valley of a creek. Originally two 5 x 5 m sampling plot were marked out but one of them were destroyed because of a deforestation. On the other sampling plot the rate of infection changed between 9,47 % and 19,23 %, the highest value found in 2007, the lowest in 2008. The following microscopic fungi were found: *Botrytis cinerea* Persoon, *Botrytis narcissicola* Klebahn, *Colletotrichum dematium* (Persoon) Fries, *Ramularia septata* (Bonord.) Bubák, *Septoria malisorica* Bubák, *Puccinia schmidtiana* Dietel (Syn.: *Puccinia sessilis* Schroet.)

The infection rate of *Orchis mario* L. were measured on two 5 x 5 m sampling plots, divided into 1 x 1 m subplots. Both location was at Orfalu, on a meadow. One of them was cultured, that is used for hay making, the other was not under culture. We have observed 57,60 % to 63,80 % infection caused by *Colletotrichum dematium* (Persoon) Fries, *Colletotrichum gloeosporioides* Penzig, *Cladosporium herbarum* (Persoon) Link, and by *Penicillium* species during 2008-2009. The highest value was measured in 2008. This was 6,2 % lower in the next year. On the other plot the infection rates changed between 23,65 % and 100 %, the highest value was in 2009, the lowest in 2006.
We have collected data of the microscopic fungi of Őrség district, since up to now no data were collected here. During the period 2004-2009 we have identified a considerable number of biotrophic, necrotrophic and saprophitic fungal species found on 74 plant species.
As regard **biotrophic** fungi, rust species are to be mentioned. Rust causing species were detected on 9 protected plant species as follows (table 1.):

1. táblázat Rust causing species detected on protected plant species of the Őrség NP

Hostplant species Primary host plant	Alternate host plant	**Rust species**	**Progress of development symbol**
1. *Vaccinium vitis-idea* L.	*Tsuga canadensis (*L.*)* Carr.	*Thekopsora vacciniorum* Karsten	0 I/II III IV
2. *Phalaris arundinacea* L.	*Leucojum vernum* L.	*Puccinia schmidtiana* Dietel	0 I/II III IV
3. *Erythronium dens-canis* L.		*Uromyces erythronii* (de Candolle) Passerini	0 I – III IV
4. *Fritillaria meleagris* L.		*Uromyces lilii* (Link)	0 I – III IV
5. *Carlina acaulis* L.		*Puccinia carlinae* Jacky	0 – II III IV
6. *Salix fragilis* L.	*Galanthus nivalis* L.	*Melampsora galanthi-fragili* Klebahn	0 I/II III IV
7. *Iris sibirica* L.	*Urtica* sp., *Valeriana* sp.	*Puccinia iridis* (de Candolle) Wallroth	0 I/II III IV
8. *Inula helenium* L.		*Coleosporium tussilaginis* (Persoon) Léveillé	0 I II III IV
9. *Salix aurita* L.	*Allium* sp.	*Melampsora salicina* Lév.	0 I/II III IV

We have sent leaf samples of *Erythronium dens-canis* L.infected by rust to the Museum of Natural Sciences and they were deposited under code number: Uromyces erythronii 2007.04.03. BP99423 (an ecidium form) and Uromyces erythronii 2007.04.17 BP99424 (a teleuto form). By this the number number of *Uromyces erythronii* samples in the museum increased to 5.

Among rusts we have identified only two caused high infection rate, where the dog's tooth violet and leaves of a eared willow species died. We suppose however, that despite high infection rate these populations will not be highly damaged. As regard the other protected plant species, we did not find correlations between host number and rust frequency or infection rate.

Also the smut species are biotrophic. We could identify smut on protected plant in case of a *Carex fritschii* Waisb., which was infected by *Schizonella melanogramma* de Candole. This rare smut species was earlier unknown at the Őrség NP. A *Carex fritschii* population was highly infected at Sorokpolány in May 2009. We have found *Anthracoidea subinclosa* Körnicke on seeds of the non-protected *Carex acutiformis* Ehrh. plants. Also this smut species was unknown at the Őrség NP.

The **necrotrophic** fungal species are partly weakness-parasites, causing leaf spots of different colors, shapes and sizes. Among them the *Colletotrichum* species are most important. We have identified *Colletotrichum* infections on six european orchid species (*Platanthera bifolia* L., *Orchis morio* L., *Orchis ustulata* L., ,*Gymnadenia conopsea* L.*, Listera ovata (*L) R. Br.*., annd a Cephalanthera longifolia* (L.) Fritsch) and on other seven protected species. In case of *Astrantia major* L. acervula emerged also on the leaf-stick. Also the stem of *Platanthera bifolia* showed symptoms of anthracnose. Most severe infection was observable on the *Platanthera bifolia* and other three orchid species. Among the *Colletotrichums C. dematium* occurred more frequent than *C. gloeosporioides* on different plant species. It seems however, that the *Colletotrichum* species do not endanger directly the plant populations investigated.
In some cases other necrothrophic species were identified on protected plant species, like *Phoma*, (*Phoma leptidea, Phoma salicicola*), *Phomopsis*, *Phyllosticta* (*Phyllosticta pirolae*, *Phyllosticta vaccinii*), *Discosia* (*Discosia strobilina*), *Septoria* (*Septoria cyclaminis*, *Septoria polygonorum*), which however did not cause severe infections.
Also some **saprotrophic** fungi were identified on protected species (e.g. *Penicillium* sp., *Botrytis cinerea*, *Alternaria alternata*, *Epicoccum nigrum*, *Cladosporium herbarum*, etc.).
As mentioned earlier, our aim was also to investigate, whether the identified microscopic fungi can decrease the number of protected plant individuals, or their populations. Up to now it seems, that directly they do not have such influence, which means, that no preceding measures for saving the protected plant species are necessary.

We have made investigations on **white mistletoe** (*Viscum album* L.), partly to see, which microscopic fungi infect its individuals, and partly to see, which protected and non-protected tree species were infected by the mistletoe.
We have identified the following microscopic fungi on mistletoe: *Phaeobotryosphaeria visci* (Kalchbr.) A.J.L. Philips et Crous, *Plectophomella visci* (Sacc.) Moesz, *Septoria visci* Bres., *Colletotrichum gloeosporioides* (Penzig) Penzig et Sacc., *Diplodia visci* (de Candolle) Fries, and *Fusarium* sp. Among them *Phaeobotryosphaeria visci* occurred most ferquently, causing leaf drop, and sometimes dying of mistletoe.
We have observed large mistletoe populations on 18 tree and bush species at the extensive meadowes at Őrség district, where traditional fruit trees are also usual. Apple trees were most infected by mistletoe, followed by plum, and pear varieties. No cherry trees were infected.
Infected are the trees on roadsides, and trees of old parks: mainly poplars, lindens, mapples, birches and american ashes. Infected are also the trees of the Rába-valley: willows, poplars and alders.

4. New scientific results

Based on our investigations and data of scientific literature may we propose to accept the following statements as new scientific results:

1. We have made the first report on microscopic fungi of protected plant species of the Őrség National Park.
2. This is the first time in Hungary, that microscopic fungi were identified on 74 protected plant species living at their natural circumstances. Symptoms, characteristics, and the damage caused by this pathogens were determined in details. Biotrophic fungi (rusts) were first identified on 9 protected plant species (detailed above). Mostly necrotrophic fungal species were identified and some saprophytes.Near to 100 fungal species were identified on the protected plant species.
3. This is the first report in Hungary on territory, occurrence, distribution and infection rate of microscopic fungi found on protected plant species.
4. First we have documented in Hungary, that the seasonal epidemic graph of rust infection caused by *Uromyces erythronii* (de Candolle) Passerini on the *Erythronium dens-canis* L. has one peak with its maximum at the last days of April.
5. We have made the first report in Hungary on occurrence of *Seimatosporium hypericinum* (Ces.) Hutton fungal pathogen on the protected plant *Hypericum barbatum* Jacky.
6. We have tried to determine first that the fungal pathogens we have identified what like influence have on the number of individuals of protected plant populations. Our investigation shows that these microscopic fungi do not endanger directly the populations of the investigated species. It means, that it is not necessary to make preceding measures for saving the protected species.
7. We were the first to give data on spreading of white mistletoe (Viscum album L.) on the traditional fruit trees (apple, plum. pear, cherry) and on protected species of the Őrség National Park, as well as about the rate of infection and frequency. We have observed infection on 18 tree and bush species. We have identified the parasitic fungi of mistletoe (6 species) detailed the symptoms and the rate of damage caused.

PUBLICATIONS AND LECTURES CONNECTED WITH THE DISSERTATIONS

Scrutinized publications in Hungarian language

Jandrasits, L. és Fischl, G. (2006): Védtelen védett növények. A vörös áfonya nyavalyái. Élet és Tudomány. LXI (50): 1584-1587.

Jandrasits, L. és Fischl, G. (2007): A havasi éger /*Alnus viridis* (CHAIX) DC./ gombabetegségei és állományfelmérése az Őrségi Nemzeti Park néhány erdőrészletében. Erdészeti Lapok. CXLII (6): 205-209.

Fischl, G., **Jandrasits, L.,** Király, G. és Mesterházy, A. (2007): A szakállas orbáncfű (*Hypericum barbatum* JACQ. új gombabetegsége Magyarországon. Növényvédelem 43 (8): 364-367.

Fischl, G. és **Jandrasits, L.** (2008): A kakasmandikó (*Erythronium dens-canis* L.) rozsdabetegsége az Őrségi Nemzeti Parkban. Növényvédelem 44 (1): 19-25.

Jandrasits, L. és Fischl, G. (2008): Védett növényfajok rozsdagombái az Őrségben. Növényvédelem 44 (7): 360-364.

Jandrasits, L. és Fischl, G. (2008): Védett növényfajokon előforduló *Colletotrichum* fajok az Őrségben. Mikológiai Közlemények Clusiana 47 (1): 5-13.

Fischl, G., **Jandrasits, L.,** Varga, I. és Pásztor, Sz. (2009): A fehér fagyöngy (*Viscum Album* L.) parazita gombái. Növényvédelem 45 (4): 178-183.

Jandrasits, L. és Fischl, G. (2009): Tőzikevédelem mikroszkóppal. TermészetBÚVÁR 64(6):38-39.

Lectures in Hungarian language

Jandrasits, L. és Fischl, G. (2004): Védett növényfajok gombabetegségei. Növényvédelmi Tudományos Napok, Budapest. 2004.02.24.

Jandrasits, L. és Fischl, G. (2006): Védett növényfajok gombabetegségei az Őrségi Nemzeti Parkban. Természetvédelmi Konferencia. Őrségi Nemzeti Park Igazgatóság, Őriszentpéter 2006.02.16.

Jandrasits, L. és Fischl, G. (2006): *Colletotrichum* fajok előfordulása védett növényfajokon. Növényvédelmi Tudományos Napok, Budapest. 2006.02.23.

Jandrasits, L. és Fischl, G. (2007): Védett növényfajok rozsdagombái az Őrségben. Körkép a hazai rozsdagomba kutatásról. MTA Növénytermesztési Kutató Intézete, Martonvásár. 2007.11.06.

Fischl, G., **Jandrasits, L.,** Varga, I. és Pásztor, Sz. (2009): Újabb adatok a fehér fagyöngy parazita gombáiról. Növényvédelmi Tudományos Napok, Budapest. 2009.02.24.

Publications in foreign languages

Jandrasits, L. and Fischl, G. (2008): Fungal diseases of mountain cranberry (*Vaccinium vitis-idaea* L.) in the Őrség National Park. Acta Agronomica Óváriensis 50 (2): 27-34.

Jandrasits, L. and Fischl, G. (2009): The microscopic fungi of orchid species in the Őrség National Park. International Journal of Horticultural Science 15 (3): 31-36.

Publications on others topics

Jandrasits, L. (2003): Az Őrségi Nemzeti Park néhány védett növényfaja és kórokozói. Diplomadolgozat. Veszprémi Egyetem Georgikon Mezőgazdaságtudományi Kar, Keszthely.
Jandrasits, L. és Fischl G. (2004): Védett növényfajok gombabetegségei. Növényvédelmi Tudományos Napok. Budapest. Előadások összefoglalói, p. 84.

Jandrasits, L. és Fischl G. (2006): *Colletotrichum* spp. előfordulása védett növényfajokon. Növényvédelmi Tudományos Napok Budapest. Előadások összefoglalói, p. 46.

Fischl, G., Taller, J., Csöndes, I., Varga, Zs. és **Jandrasits, L.** (2008): Adatok a fehér fagyöngy elterjedéséhez (*Viscum album* L.) és a *Botryosphaerostroma visci* (DC.) PETRAK gazda-parazita kapcsolathoz.. XVIII. Keszthelyi Növényvédelmi Fórum Előadás Összefoglaló 3. old.

Jandrasits, L. (2008): Vörös áfonya (*Vaccinium vitis-idaea* L.*)* gombabetegségei az Őrségi Nemzeti Parkban. E-Tudomány Évkönyv I. 145-154.