International Journal of Horticultural Science 2009, 15 (3): 31–36.
Agroinform Publishing House, Budapest, Printed in Hungary
ISSN 1585-0404

The microscopic fungi of orchid species in the Őrség National Park

Jandrasits, L.[1] & Fischl, G.[2]

[1]*Directorate of the Őrség National Park, H-9941 Őriszentpéter, Siska szer 26/1, Hungary*
[2]*Plant Protection Institute, Georgikon Faculty, Pannon University, H-8360 Keszthely, Deák F. u. 57, Hungary*

Summary: The wild orchids growing in Hungary are some of the most decorative and interesting members of the country's flora. The majority of species are rarely spotted, and some are only found in very few habitats, though others are quite common. All the species known in Hungary are protected, and 11 species are strictly protected. It is thus important to monitor the health status of these plants, to determine what diseases affect them, what pathogens are found on them and how severely they are infected, and to take the necessary precautionary measures. Eleven of the 14 orchid species occurring in the Őrség National Park were included in the study and the presence of microscopic fungi was detected on eight of these. Pathogenic species were found on lesser butterfly orchid (*Platanthera bifolia*), green-winged orchid (*Orchis morio*), burnt orchid (*Orchis ustulata*), sword-leaved helleborine (*Cephalanthera longifolia*), common twayblade (*Listera ovata*), autumn lady's-tresses (*Spiranthes spiralis*), western marsh orchid (*Dactylorhiza majalis*) and broad-leaved helleborine (*Epipactis helleborine*). A detailed account is given of the symptoms of major diseases and of the microscopic traits of the pathogens. An attempt was made to determine to what extent the pathogenic fungal species found on protected orchid species influence the lives of these plants. Current knowledge on this subject is extremely deficient, as practically no data are available from Hungary.

Key words: *Colletotrichum*, fungal diseases, orchid, Őrség National Park, protected plant species

Introduction

The Őrség National Park is the tenth and youngest of the national parks in Hungary. It is situated in the south-western corner of Vas County in the rolling hills of the Őrség region, which is characterised by forests, meadows, stream-side copses and deep valleys. The flora of the region is extremely diverse and is well-documented, the first data being recorded in 1583 by the famous botanist, Clusius. Comprehensive research on the flora was begun by Vince Borbás in 1887. The most valuable part of the park from the botanical point of view is the Vend region, which belongs to the Styrian flora group (Stiriacum), making up part of the Noricum flora of the Eastern Alps, while the rest of the park belongs to the varied, transitional flora of Vas County in the Praenoricum region (*Bonczóné*, 1983).

The number of protected plant species growing in the region is put at 111, and these include many that are found nowhere else in Hungary, such as *Alnus viridis, Alchemilla xanthochlora, Pyrola chloranta* (*Barbácsy & Tolnai*, 1996).

It is estimated that there are 20,000 orchid species in the world, making this family one of the biggest in the plant kingdom. They are an extremely interesting group from the botanical point of view. Europe counts as a marginal area, with only 215 wild species (*Buttler*, 2000). Until the early 1980s a total of 47 orchid species, 5 subspecies and approximately 100 hybrids were registered in Hungary (*Csapody*, 1982), but thirteen years later there was a report of 50 species and 11–13 hybrids (*Molnár et al.*, 1995). By the end of the century the number of known species had risen to 54 (*Farkas*, 1999) and since then further species have been identified, so the list now contains around 60 names.

The Red List of extinct and threatened vascular plants published in the Hungarian Red Book includes 53 endangered orchid species (*Németh*, 1989), while only 50 are considered to be endangered in the most recent Red List (*Király*, 2007).

Four of the 11 orchid species analysed in the present work (*Epipactis helleborine, Neottia nidus-avis, Orchis morio, Platanthera bifolia*) are not included in the current Red List, and are thus not considered to be endangered. Of these 11 species, the Red List published for the Burgenland region of Austria, which borders the national park, lists one in the severely endangered category, two as endangered, two as regionally endangered and five as regionally endangered in part of the region. *Neottia nidus-avis* is listed as not endangered (*Weber*, 2005).

No complete description is available of the fungal diseases attacking protected plants, but many mycological works (*Bánhegyi et al.*, 1985–1987, *Brandenburger*, 1985, *Farr et al.*, 1995, *Sutton*, 1980) identify fungal species collected from major protected plant species. A brief description of these fungal species is to be found in the publications listed above, but little information is given on the appearance of the diseases on given species, on the symptoms or spread of the disease, on the extent of damage, etc.

The purpose of the present work was to map this information for the Őrség National Park. Some of the results have already been published in the form of proceedings (*Jandrasits & Fischl*, 2004, 2006a, 2007a) and papers (*Jandrasits & Fischl*, 2006b, 2007b, *Fischl & Jandrasits*, 2008).

Materials and methods

Leaves, stems, flowers, seeds or in some cases whole plants of orchid species exhibiting disease symptoms were collected during the vegetation periods of 2004–2008 in various habitats (forests, forest boundaries, dry hillside meadows, marsh meadows, roadside ditches) in the Őrség National Park. Visible symptoms were recorded, together with the extent of disease incidence. Digital photographs were taken of diseased plants in their habitats and the locations were identified using a GPS system. After transferring the samples to the laboratory

the diseased plant organs were placed in a moist chamber and examined under a microscope

every two days. In some cases pure cultures were established on BDA medium. The size of the fungal spore forming on the original plant material and on pure cultures was recorded. The conidia of some isolates were germinated in tap water.

In the case of *Orchis morio* a 5×5 m sampling area was set up on a grassland area which was no longer cut and where bushes and trees were beginning to grow. The number of plants on this area and the proportion exhibiting symptoms were recorded each year. A further 5×5 m quadrat was established approx. 100 m north of this on a regularly cut grassland area in April 2008.

This latter quadrat was divided into 1×1 m subquadrats and, starting from one corner of the area, the whole area of every other subquadrat was scored and the number of plants exhibiting symptoms was recorded. The numbering of the subquadrats followed this pattern. A total of 13 1×1 m sample areas and 12 control areas (where no plant survey was made) were thus designated within the 5×5 m quadrat.

Due to the small number of plants, the designation of sample areas was not justified for the other orchid species.

Plants suspected of being diseased were collected for the given species from the locations marked on the map (*Fig. 1*).

Sites of occurrence:

A fuller description of the sites where orchid species were found is given below.

Cephalanthera longifolia (Huds.) Fritsch – sword-leaved helleborine

Found in ditches and forest boundaries beside the Szentgotthárd-Farkasfa road.

Dactylorhiza majalis (Rchb.) – western marsh orchid

Found in Keserűszer, Őriszentpéter; on a rich fen next to the football pitch in Orfalu; in Pityerszer, Szalafő; on a rich fen in Kétvölgy-Ritkaháza; on a strictly protected rich fen beside the cemetery in Alsószölnök; on a rich fen in Hampó-völgy, Felsőszölnök.

Epipactis helleborine Cr. – broad-leaved helleborine

Found in a peasantwood in Rábatótfalu; in spruce woods (Cena, Majczán-házi, Szentgotthárd, Szentgotthárd-Farkasfa); in the Szentgotthárd-Máriaújfalu upper wood; in the wood next to a farm track in Jakabháza; in virgin woods in Szalafő.

Listera ovata (L.) R. Br. Ex Ait. – common twayblade

Found on the edge of a ditch near Ördög Lake in Szentgotthárd-Farkasfa

Platanthera bifolia (L.) Rchb. – lesser butterfly orchid

Found in the Áfi-házi wood in Kétvölgy; in the meadow near the cross in Orfalu; in spruce woods (Szentgotthárd-Farkasfa, Szentgotthárd-Rábatófalu, Majczán-házi); in the Götz-majori wood in Alsószölnök; in the wood behind the forester's hut in Szakonyfalu; in Hosszú-rét, Szakonyfalu; in the upper borderland in Alsószölnök; on the edge of forest section 105B in Kétvölgy; on the edges of forest sections 1E, F and ditches in Szentgotthárd-Farkasfa; in the wood under the electric lines in Apátistvánfalva; on a strictly protected rich fen in Gödörháza; on dry grassland on a hillside by the Szentgotthárd-Rábakethely church.

Orchis morio L. – green-winged orchid

Found on dry grassland in Gáj, Alsószölnök; in the meadow near the cross in Orfalu; in Keserűszer, Őriszentpéter; in Pityerszer, Szalafő; on dry grassland on a hillside by the Rábakethely church.

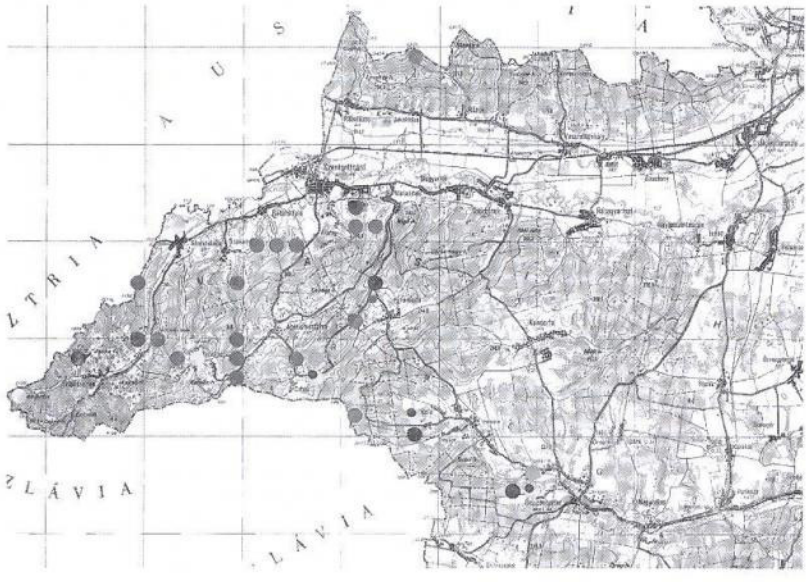

Figure 1. Occurrence of orchid species within the Őrség National Park

Orchis ustulata L. – burnt orchid

Found on dry grassland in Gáj, Alsószölnök; on the hill Alsó János-hegy in Felsőszölnök; in Pityerszer, Szalafő; in Keserűszer, Őriszentpéter.

Spiranthes spiralis (L.) – autumn lady's-tresses

Found in Keserűszer, Őriszentpéter; in the dry grassland known as "Nyíres" in Orfalu.

Despite their exhibiting symptoms suggesting disease, no pathogens were detected on the following orchid species: *Platanthera chlorantha, Neottia nidus-avis, Epipactis palustris*. The species *Gymnadenia conopsea* was only found on one occasion during the 2004–2008 period (a single strong plant), and no disease-like symptoms were observed on the plant.

Results

The number of symptom-exhibiting samples for each orchid species were as follows:

Cephalanthera longifolia (Huds.) Fritsch – sword-leaved helleborine 4
Dactylorhiza majalis (Rchb.) – western marsh orchid 13
Epipactis helleborine Cr. – broad-leaved helleborine 13
Epipactis palustris (Mill.) Cr. – marsh helleborine 1
Listera ovata (L.) R. Br. Ex Ait. – common twayblade 1
Neottia nidus-avis (L.) Rich – bird's nest orchid 1
Orchis morio L. – green-winged orchid 19
Orchis ustulata L. – burnt orchid 8
Platanthera bifolia (L.) Rchb. – lesser butterfly orchid 35
Platanthera chlorantha (Custer) Rchb. ex Mössler – greater butterfly orchid 1
Spiranthes spiralis (L.) – autumn lady's-tresses 4

The pathogenic and saprotrophic fungal species identified on the samples are listed in *Table 1*.

The most frequently occurring pathogenic fungal species was *Colletotrichum dematium*, which was identified from a total of 23 samples from three orchid species. The typical anthracnose symptom observed on the leaves of burnt orchid is illustrated in Figure 2.

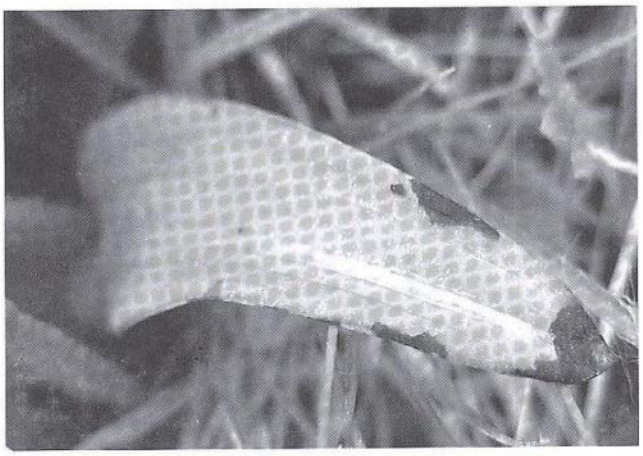

Figure 2. Typical anthracnose symptom on a burnt orchid leaf

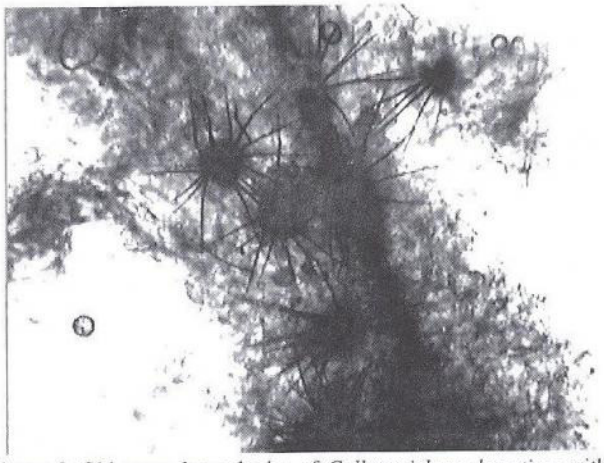

Figure 3. Old acervulus colonies of *Colletotrichum dematium* with setae, formed on the leaves of green-winged orchid

Table 1. Number of pathogenic and saprotrophic fungal species detected on each orchid species

Plant species	*Colletotrichum dematium*	*Colletotrichum gloeosporioides*	*Botrytis cinerea*	*Cladosporium herbarum*	*Penicillium* sp.	*Alternaria alternata*	*Periconia* sp.	*Phyllosticta epipactis*	*Phomopsis* sp.	*Epicoccum nigrum*	*Phoma* sp.	*Fusarium semitectum*	*Septoria* sp.	*Discosia strobilina*	*Trichoderma* sp.	*Heterosporium* sp.	*Drechslera* sp.	*Cercospora* sp.	*Rhizoctonia solani*	*Mucor* sp.
Platanthera bifolia	14	7	3	5	4	1	1	1	1	1	1	2	1	1						
Spiranthes spiralis			1	1	1															
Cephalanthera longifolia		1			1	1														
Listera ovata		1																		
Orchis morio	6	1		1	1	6									1		1			
Orchis ustulata	3															1				
Dactylorhiza majalis				1	2	2		2				1							1	1
Epipactis helleborine				1	1	5		2	1	1								1		1
Total	23	10	4	9	10	15	1	5	2	2	1	3	1	1	1	1	1	1	1	2

The species *Colletotrichum gloeosporiodes*, which also causes anthracnose, was detected on a total of 10 samples from four orchid species.

Old acervulus colonies of *Colletotrichum dematium* with setae, formed on the leaves of green-winged orchid, can be seen in Figure 3, and the single-celled, bent conidia of *Colletotrichum dematium*, which narrow at each end and have an oil drop in the middle, are shown in *Figure 4*.

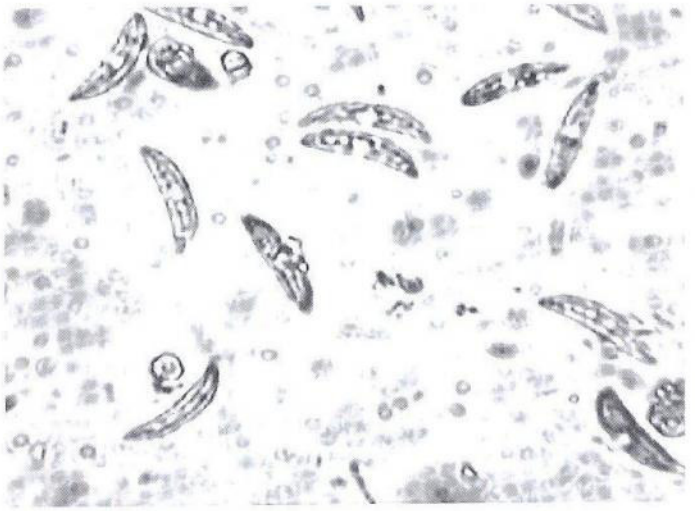

Figure 4. Single-celled, bent conidia of *Colletotrichum dematium*, narrowing at each end, with an oil drop in the middle

The flat, round, halved pycnidia of *Discosia strobilina* in leaf tissue can be seen in *Figure 5*, while *Figure 6* illustrates the elongated, sausage-shaped conidia of *Discosia strobilina*, which have three cross-walls and rounded ends, with filamentous appendages on the outer cells.

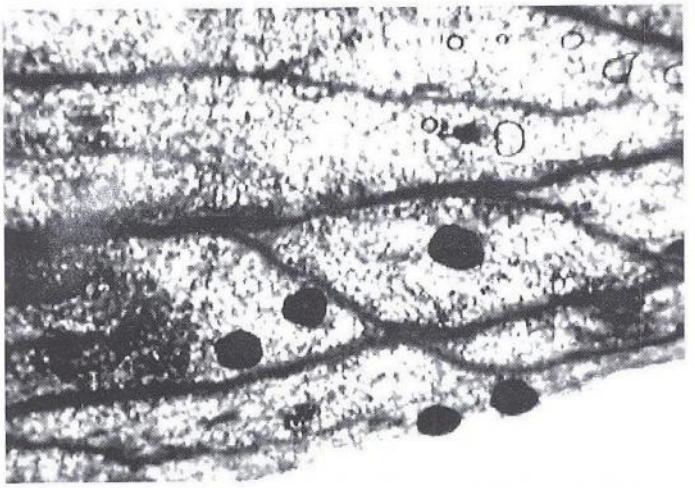

Figure 5. Fruiting bodies of *Discosia strobilina* in leaf tissue from lesser butterfly orchid

Surveys on the two sample quadrats established for green-winged orchid on a meadow near the cross in Orfalu, as described in the Materials and Methods section, showed a total of 444 plants in the 5×5 m quadrat in April 2006. Symptoms suggestive of fungal diseases were observed on 105 of these plants, representing a level of infection of 23.65%. The exact location of the site was identified using a hand-held GPS

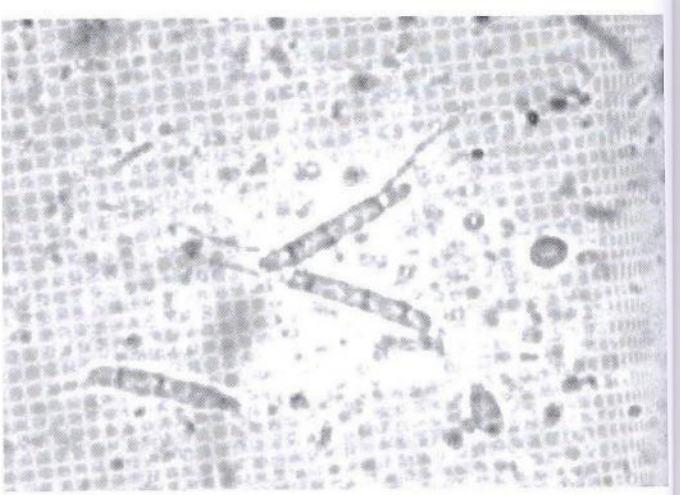

Figure 6. Elongated conidia of *Discosia strobilina*, with three cross-walls, rounded ends and a filamentous appendage at each end

instrument, which indicated EOV coordinates of E:438007, N:175186, with a precision of 4 m. In April 2008 only 83 plants were found in the same quadrat, 53 of which exhibited disease symptoms, a level of infection of 63.85%.

The other 5×5 m sample area, which was divided into 1×1 m subquadrats, was established on a regularly cut grassland on 30 April 2008 (EOV coordinates: E:437995, N:175215, with a precision of 4 m). When the whole area was surveyed, a total of 268 green-winged orchids were found, 171 of which exhibited disease symptoms on the rosette and true leaves, equivalent to an infection level of 63.80%. Detailed plant surveys were carried out on 13 of the subquadrats (*Table 2*).

The mean infection level calculated from the data was 82.34%, but this high infection level did not mean that the plants were severely damaged. In many cases, however, the infected leaves withered up, which could affect the later survival of the plant.

Phyllosticta epipactis, which causes small, round leaf spot symptoms, was detected on five samples from three orchid species.

Botrytis cinerea causes flower wilting, withering and browning. Dark-edged spots were detected on the tepals and spurs of the flowers in four samples from two orchid species.

Septoria sp. was only found in one sample of one species. The symptoms were small spots on the rosette leaves, rust-brown to start with and later becoming lighter in the centre. *Fusarium semitectum* was identified on three samples of two orchid species, causing rents with dark brown edges on the rosette leaves and the brown discoloration of leaf tips and edges.

Phoma sp. was also detected on only one sample of one species. The symptoms were oval-shaped, dark brown spots at the base of the seedstalk. *Phomopsis* sp. was identified from two samples representing two orchid species, causing light brown spots on the leaves, the brown discoloration of the leaf edges and tips, the withering of shoot tips and brown spots or stripes on the stem.

Rhizoctonia sp. was only detected on one sample of one species. The symptoms were brown discoloration on the rosette leaves, and light brown spots or rents with dark edges on the true leaves Brownish black stripes appeared at the base of the stem.

Table 2. Total number of green-winged orchids, number of diseased plants and% infection for the subquadrats

Parameters	Subquadrat code												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Total NO. Of plants	8	12	2	22	16	30	5	13	4	13	7	1	4
NO. of diseased plants	4	10	2	12	14	22	4	12	4	12	4	1	4
% Infection	50	83,33	100	54,54	87,50	73,33	80	92,30	100	92,3	57,14	100	100

Drechslera sp. was identified on one sample of one species, causing the yellowing or browning of the leaf tips, the yellowing of the rosette leaves and the appearance of round, dark brown spots on the true leaves.

Representatives of non-pathogenic, saprotrophic fungal species were also identified from the samples. Due to their way of life, these appeared on older leaves or on those injured by insect feeding, hail, trampling, etc. The most frequently detected was *Alternaria alternata*, found on 15 samples from five orchid species. The symptoms were splits or tiny black spots on the leaves, the browning of the tepals, brownish-black patches at the tips of rosette and true leaves, and brown discoloration of the leaf edges. Elongated brown patches were also formed along the veins.

Secondary infection with *Penicillium* sp. was observed on 10 samples from six orchid species. The symptoms were the yellow or brown discoloration of the rosette leaves, or the appearance of dark-brown patches with yellow edges. Leaf deformation was also observed in some cases. The tepals were seen to wilt and brown and the flower primordia turned brown before they opened. Brown streaks were formed on the seed stalk.

Cladosporium herbarum was detected on a total of nine samples from five plant species. The symptoms were almost identical to those of *Penicillium*. The general wilting of the flowers was preceded by the appearance of brown spots on the tepals and labella.

Epicoccum nigrum, which occurred on only two samples from two different orchid species, is also a saprotrophic fungus causing the yellowing or browning of the rosette leaves in the form of dark brown patches with lighter centres.

Heterosporium sp. was only detected on one sample of one species. The symptoms were brown discoloration or black spots on the tips of rosette and true leaves and brown patches on the true leaves.

Damage caused by insects (chewing, sucking, boring) was frequently observed on the samples, and nematodes were also found in the plant tissues. The highest incidence of insect damage (11 cases of chewing and one of boring) was recorded for *Platanthera bifolia*, probably due to the large number of samples (35). As the present work focussed on the identification of pathogenic microscopic fungal species, the insect species causing this damage were not investigated.

Discussion

The factors endangering orchid populations as the result of human activity can be summarised as follows: extensive tree felling in orchid habitats, trampling and compaction, the growth of weeds, bushes and trees when grassland is not regularly cut, the ploughing up of grassland, changes in land use and the plantation of Christmas trees on grassland; in the case of species growing next to roads, the salting or widening of the roads and the building of new roads; the drainage of marshes and fens, the regulation of stream beds, and flower collection (for private use).

Mention should also be made of drying processes caused by climate change and the uneven distribution of rainfall, and of the slow but continuous transformation of habitats.

In many cases, nature protection experts with no knowledge of microscopic fungi attribute negative changes in the populations of protected plant species to the above reasons, completely ignoring the fact that the presence of pathogenic fungi on these species could also induce or promote these changes.

The occurrence of pathogenic fungi may have a particularly serious effect on small, isolated plant populations.

It appears from the results achieved so far that the microscopic fungus species detected on orchids do not endanger these populations directly. Nevertheless, studies should be continued in the future. In order to determine the exact extent of the damage and the risk, it would be advisable to carry out artificial back-infections (for which a special permit will be required).

Anthracnose infection caused by *Colletotrichum* species was frequently observed on the orchid species included in the study. The most severe infections were detected on lesser butterfly orchid, green-winged orchid and burnt orchid, but damage was also seen on common twayblade and sword-leaved helleborine. Of the two *Colletotrichum* species, *C. dematium* occurred more frequently than *C. gloeosporioides*, causing typical symptoms on various orchid species.

There appears to be every justification for the regular investigation of microscopic pathogenic fungus species on other protected plant species.

References

Bánhegyi J., Tóth S., Ubrizsy G. & Vörös J. (1985–1987): Magyarország mikroszkopikus gombáinak határozókönyve, Vol. 1–3. (Identification Handbook of Microscopic Fungi in Hungary.) Akadémiai Kiadó, Budapest.

Barbácsy, Z. & Tolnai, K. (1996): Őrségi Tájvédelmi Körzet. (The Őrség Nature Protection District.) Fertő-Hanság Nemzeti Park Igazgatóság, Sarród.

Bonczóné, K. (1983): Őrség (Tájvédelmi Körzet). (Őrség. Nature Protection District.) Tájak Korok Múzeumok Kiskönyvtára series, Veszprém.

Brandenburger, W. (1985): Parasitische Pilze an Gefääpflanzen in Europa. Gustav Fischer Verlag, Stuttgart, New York

Buttler, K. P. (2000): Orchideák. (Orchids.) Gunter Steinbach series. Magyar Könyvklub, Budapest.

Csapody, I. (1982): Védett növényeink. (Protected plant species of Hungary.) Gondolat, Budapest.

Farkas, S. (1999): Magyarország védett növényei. (Protected plant species of Hungary) Mezőgazda Kiadó, Budapest.

Farr D.F., Bills G.F., Chamuris G.P. & Rossman A.Y. (1995): Fungi on Plants and Plant Products in the United States. Amer. Phytopath. Soc. Press, St. Paul. Minnesota

Fischl, G. & Jandrasits, L. (2008): A kakasmandikó (*Erythronium dens-canis* L.) rozsdabetegsége az Őrségi Nemzeti Parkban. (Rust disease of Dog's-Tooth Violet in Őrség National Park) Növényvédelem. 44 (1):19–25.

Jandrasits, L. & Fischl, G. (2004): Védett növényfajok gombabetegségei. (Fungal diseases of protected plant species.) Növényvédelmi Tudományos Napok. Budapest. Előadások összefoglalói, p. 84.

Jandrasits, L. & Fischl, G. (2006a): *Colletotrichum* spp. előfordulása védett növényfajokon. (Occurrence of *Colletotrichum* spp. on protected plant species.) Növényvédelmi Tudományos Napok. Budapest. Előadások összefoglalói, p. 46.

Jandrasits, L. & Fischl G. (2006b): Védtelen védett növények. A vörös áfonya nyavalyái. (Defenceless protected plants. Diseases attacking red cranberry.) Élet és Tudomány.LXI (50): 1584–1587.

Jandrasits, L. & Fischl, G. (2007a): Védett növényfajok rozsdagombái az Őrségben. (Rust fungi on protected plant species in the Őrség region.) MTA Mezőgazdasági Kutatóintézete, Martonvásár.

Jandrasits, L. & Fischl, G. (2007b): A havasi éger [*Alnus viridis* (CHAIX) DC.] gombabetegségei és állományfelmérése az Őrségi Nemzeti Park néhány erdőrészletében. Fungal diseases and forest stand survey on green alder [*Alnus viridis* (CHAIX) DC.] in the Őrség National Park.) Erdészeti Lapok CXLII (6):205–209.

Király, G. (2007): Vörös Lista. A magyarországi edényes flóra veszélyeztetett fajai. (Red list.of the vascular flora of Hungary.) Saját kiadás, Sopron.

Molnár, A., Sulyok, J. & Vidéki, R. (1995): Vadon élő orchideák. A hazai növényvilág kincsei. (Hungarian orchids growing int he wild.) Kossuth Könyvkiadó, Debrecen.

Németh, F. (1989): Száras növények. (Vascular plants.) In: Rakonczay Z. (ed.): Vörös Könyv. A Magyarországon kipusztult és veszélyeztetett állat-és növényfajok. (Red Data Book. Extinct and threatened animal and plant species of Hungary.) Akadémiai Kiadó, Budapest, pp. 265–325.

Sutton, B.C. (1980): The Coelomycetes. Fungi imperfecti with pycnidia and acervuli. CMI Kew, Surrey, England.

Weber, E. (2005): Liste der Farn- und Blütenpflanzen des Burgerlandes. Veröffentlichungen der Internationalen Clusius Forschungsgesellschaft Güssing, Heft 9.