ACTA MYCOLOGICA
Vol. 45 (1): 97–114
2010

Dedicated to Professor Barbara Gumińska on the occasion of her eighty-fifth birthday

The occurrence of cellulolytic fungi and *Fusarium* in nests of *Circus pygargus*

TERESA KORNIŁŁOWICZ-KOWALSKA[1], IGNACY KITOWSKI[2] and HELENA IGLIK[1]

Department of Agricultural Microbiology, Mycological Laboratory
University of Life Sciences in Lublin, Leszczyńskiego 7
PL- 20-069 Lublin, teresa.kornilowicz@up.lublin.pl
[2] Department of Nature Conservation, Institute of Biology
University of Maria Curie-Skłodowska, Akademicka 19
PL- 20-033 Lublin, ignacyk@autograf.pl

Kornilłowicz-Kowalska T., Kitowski I., Iglik H.: *The occurrence of cellulolytic fungi and Fusarium in nests of Circus pygargus*. Acta Mycol. 45 (1): 97–114, 2010.

A total of 45 species of cellulolytic fungi and ten *Fusarium* species were identified in seven nests of Montagu's Harrier. Three genera (*Chaetomium, Trichoderma, Fusarium*) represented 80% of the frequency of cellulolytic fungi. Of them, *Chaetomium globosum, Trichoderma viride* and *T. koningii* were some of the most frequent species. A high differentiation of the richness and frequency of species of cellulolytic fungi depending on the nest and its individual layers was observed. Reasons for the differences in the frequency and species composition of the fungi were discussed.

Key words: fungi, species richnes, cellulolytic, abilities, genus *Fusarium*, nest material, wild-living birds

INTRODUCTION

Nests of the Montagu's Harrier *Circus pygargus* contain high amounts of plant material rich in cellulose. In favourable humidity conditions, a high cellulose content in the nest material provides a good substrate for the growth of cellulolytic fungi producing extracellular cellulolytic enzymes with endoglucanase and exoglucanase activity (cellobiohydrolase and β-glucosidase), hydrolysing cellulose to monosaccharides. These enzymes are produced by numerous fungi occupying the soil and plant remains occurring in the soil and its surface or colonising tissues of living plants. Many of such fungi are represented by saprobes, e.g., *Chaetomium* or *Trichoderma*, while others, e.g., *Fusarium*, are phytopathogens or potential phytopathogens (Domsch et al. 1980; Ghos, Ghos 1992; Kornilłowicz-Kowalska et al. 2003). Fungi of

the genus *Fusarium* are causative agents of plant diseases such as wilt, seedling blight or head blight (Vesonder, Golinski 1989; Kwaśna et al. 1991) and produce mycotoxins, e.g. trichotecenes, zearalenone or fumonisins, responsible for mycotoxicoses in humans and animals.

The current knowledge of the occurrence of cellololytic fungi and *Fusarium* representatives in birds' nests is relatively poor (Hubalek et al. 1973; Hubalek 1974; Hubalek, Balat 1974). Little is also known on the influence of physico-chemical properties such as pH and humidity on the frequency and species composition of these macromycetes in birds' nests. Studies have usually been conducted on nests of *Passeriformes* (Hubalek et al. 1973; Hubalek 1974; Hubalek, Balat 1974) in which conditions differ from those in nests of water birds and wetland birds due to biotope types occupied by the birds. Nests of Montagu's Harrier in wetland habitats are therefore interesting objects of mycological studies.

Montagu's Harrier is a diurnal raptor which nests in different habitat types: steppes, open marshes as well as corn fields or young plantations of coniferous trees in Europe and on other continents where it occurs (Cramp, Simmons 1980; Clarke 1996). The bird populates entire Poland, largely lowland regions avoiding distinctly mountainous ones. Its present population size is estimated at 1 300-1 500 breeding pairs (Tomiałojć, Stawarczyk 2003). It occupies a broad spectrum of habitats in Poland: wet open habitats such as marshes associated with wide river valleys, neglected fish ponds, willow (*Salix* spp.) bushes in river valleys. The species nests in corn fields in many areas in Poland; the trend has intensified in the last few years (Tomiałojć, Stawarczyk 2003).

Korniłłowicz-Kowalska and Kitowski (2009) investigated nests of Montagu's Harrier in wetland habitats concentrating on the so called total frequency and species composition of saprotrophic fungi and fungi potentially pathogenic to humans and animals. It was shown that populations of ubiquistic species with a broad spectrum of substrates were some of the most frequent species, including thermotolerant species belonging to opportunistic pathogens, e.g., *Scopulariopsis brevicaulis* and *Aspergillus fumigatus*. The frequency of the latter, which also constitutes a high risk to birds, was high in some nests. Among saptrotrophic fungi, populations of a few fungi known for cellulolytic abilities, mostly *Trichoderma* spp., had a high occurrence frequency.

The aim of this study was to examine the frequency and species composition of cellulolytic fungi and *Fusarium* representatives in nests of Montagu's Harrier with regard to the influence of some ecological factors.

MATERIAL AND METHODS

Nests and nest material preparation. Seven nests of Montagu's Harrier from the Błota Serebryskie Fens collected in 2004 (two nests: I and II) and 2005 (five nests: III,IV,V,VI and VII) were examined. The Błota Serebryskie Fens are some of peat bogs of calcareous fens near Chełm (Torfowiska Węglanowe koło Chełma) (East Poland: 51° 07'–51° 11' N, 23° 30'–23° 42' E).

The Błota Serebryskie Fens are lowland bogs lying on $CaCO_3$ beds. Bogs are dominated by the *Cladietum marisci* community. Water table levels range from 40 to 10 cm (spring) and from 20 to 0 (summer); water pH=7.7-8.6 (Buczek 2005). The fens are protected by environmental law as an area important for bird occurrence. 14-42 pairs of Montagu's Harrier nest here annually only in saw sedge (*Cladium mariscus*) fields (Krogulec 1994; Kitowski 2002; Kitowski unpublished data).

At the time of laying, the nest is oval- or circle-like with a diameter of 20-56 cm (Krogulec 1994; Kitowski unpublished data). Willow (*Salix* sp.) or birch (*Betula* sp.) twigs and dry stems of reed (*Phragmites australis*) 10-15 cm in length as well as other plants, e.g., common broom (*Sarothamnus scoparius*) and goosefoots (*Chenopodium* sp.), are the base and the edge of the nest. Its interior is lined almost exclusively with saw sedge leaves (*Cladium mariscus*). However, the centre of the nest where the eggs are laid is lined with grass blades, rhizomes of *Agropyron* sp. and rootlets. At the end of laying, the nest is a circle with a diameter of ca. 40 cm (22-51 cm) 10-15 cm of which is taken up by the lining (Kitowski unpublished data). During breeding the nest lining is supplied by feathers from moulting, pellets and down shed by growing chicks, prey remains (hair, feathers, bones) of rodents (*Rodentia*) and small birds as well as chitinous body parts of insects.

Entire nests or the material shaken out from one nest (nest VII) were collected after ca. 7-10 days after chick fledging when the nests no longer played important ecological and behavioural roles (Kitowski 2002).

Nest material was sampled for mycological examinations from the lining (inner layer), nest edge (outer layer) and the intermediate layer between them (middle layer) (Pugh 1966). Samples were collected from a few places in each layer, a total of 100-200 g, were averaged and homogenised. The entire nest was shaken out from nest VII with a poorly developed lining and the material was used to prepare the initial sample similarly to individual layers. A total of 19 nest material samples were prepared.

Isolation and identification of fungi. Cellulolytic fungi were isolated with the dilution method and by laying fine fragments (no longer than 0.5 cm in length) of the nest material on a cellulose substrate (Whatman 1 filter paper) as the only source of C and energy containing ($g \times dcm^{-3}$) NH_4NO_3 – 20, KNO_3 – 1.0, KH_2PO_4 – 1.0, KCl – 0.5, $MgSO_4 \times 7H_2O$ – 0.5, $FeSO_4$ – traces, NaCl – traces, agar – 20.0, streptomycin – 0.03%, chlortetracycline – 0.002%, pH – 5.5. Forty fragments were analysed for each layer. Fungi belonging to the genus *Fusarium* were isolated with the dilution method on the Nash and Snyder medium (1952) containing ($g \times dcm^{-3}$) peptone – 15.0, KH_2PO_4 – 1.0, $MgSO_4 \times 7H_2O$ – 0.5, agar – 20.0, PCNB – 10.0, streptomycin – 0.03%. Cellulolytic fungi and *Fusarium* were cultured at 26°C.

The frequency of fungi (nests collected in 2005) was determined as the mean values of cfu. g-1 of the dry weight of the nest material. The dry weight was determined with the weight method at 105°C. The rate of colonisation of the nest material by cellulolytic fungi was determined in %. Growing pure fungal cultures were transferred onto PDA slants (*Fusarium*) or cellulose slants (without antibiotics). Species identification of the isolates was based on macro- and microscopic observations conducted on plates and in microcultures using PDA, Czapek-Dox medium and selective nutrient agar (SNA) for *Fusarium*. A variety of systematic studies was used for

the final classification of the fungi (Domsch et al. 1980; Nelson et al. 1983; Kwaśna et al. 1991).

Determination of humidity and chemical properties of the nests. Water content was determined with the weight method at 105°C. The following chemical analyses were performed: pH in H_2O and KCl, organic C content (Tiurin method), total C and total S content (elemental analysis by combustion analysis; a thermal conductivity detector), total N, total P, K, Ca, Mg after sample mineralization using the wet assay method in a mixture of concentrated H_2SO_4 and perhydrol (N-total, P-total - flow spectrophotometry; K, Ca, Mg - atomic absorption spectroscopy method). The data are presented in Table 1.

The results giving the number of fungi belonging to the two groups (cellulolytic fungi and *Fusarium*) were analysed by the statistical method counting standard deviations.

The species diversity of cellulolytic fungi and *Fusarium* based on the number of isolates of fungi representing individual species was analysed by calculating Simpson's index (Krebs 1994) according to the formula:

$$D = 1 - \sum_{i=1}^{S} (p_i^2)$$

where p_i is the share of isolates (strains) of species „i" in a fungal community (an entire nest or a layer of it) and is the quotient of the number of strains of the species and the number of isolates of all fungi obtained on an isolation medium (for cellulolytic fungi isolated with the dilution method and for fungi of the genus *Fusarium*). Values of Simpson's index range from 0 to 1-1/S, where S is the number of species in a community of fungi.

Species domination (Trojan 1975) was determined using the formula: $D = 100 \cdot (Sa : S)$, where S – number or isolates of species "a", S – the sum of isolates of a group (cellulolytic fungi, *Fusarium*). The frequency scale of species and taxonomic groups of fungi was as follows: sporadically < 1%, rarely 1-10%, frequently 11-25%, numerously 26-50%, very numerously > 50%.

Table 1
Chemical content of Montagu's Harrier (*Cyrcus pygargus*) nests

Nest number	Content (% of dry weight)								pH		Humidity (%)
	C total	C org.	N total	S total	P	K	Ca	Mg	H_2O	KCl	
III	45.97	44.14	2.50	0.36	0.19	0.20	0.76	0.046	-	-	24.51
IV	26.86	25.74	1.31	0.16	0.08	0.22	1.95	0.144	-	-	49.64
V	44.42	41.60	1.52	0.19	0.14	0.19	1.08	0.059	7.43 (7.60; 7.36; 7.33)	7.46 (7.50; 7.47; 7.40)	70.65
VI	44.43	41.14	2.72	0.42	0.40	0.25	1.35	0.080	7.51 (7.26; 7.70; 7.58)	7.52 (7.35; 7.60; 7.60)	70.30
VII	-	-	-	-	-	-	-	-	6.70	7.10	52.50

RESULTS

The frequency and colonisation rate of the nest material by cellulolytic fungi. The frequency of cellulolytic fungi (mean values) in the nests (I–IV) ranged between 220 and 300 000 cfu in 1 g of dry weight. Over 1 mln cfu · g^{-1} of dry weight of the nest material was obtained for nest VII, where the material for analysis was shaken out. A higher frequency of fungi obtained for nest V results from the accumulation of fungal propagules following the shaking. The analysis of the frequency of fungi in individual layers of the nests showed that the lowest frequencies of cellulolytic fungi were recorded in the outer layer and the highest frequencies were recorded in the lining (internal layer) of the nests with the exception of nest III (Fig. 1A).

The colonisation rate of the nest material by cellulolytic fungi was very high and ranged from 92.5% to 100% (Fig. 2).

The frequency of *Fusarium* in the nests. The frequency of fungi isolated on the Nash and Snyder medium, preferential for the genus *Fusarium*, ranged from 210 to 730 000 cfu · g^{-1} d.w. of the nest material, of which *Fusarium* populations constituted between 10% (NI) and 50% (NII, NIII) (Fig.1B).

Other frequencies were consistent with those of other fungi growing on the Nash and Synder medium. The species composition of these fungi is given in Tab. 7. The most numerous colonisation by *Fusarium* representatives was observed in the lin-

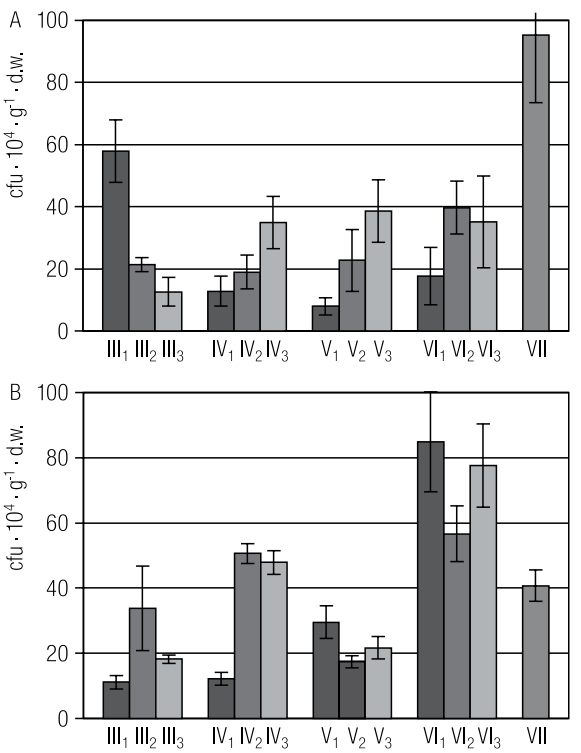

Fig. 1. The preavalence rate of fungi in the nests of Montagu's Harrier (cfu · 10^4 · g^{-1} d.w.): A – on the Waksman's medium for cellulolytic fungi, B – on the substrate for the genus *Fusarium.*

Explanations: I_1, II_1, III_1, IV_1 – the outer layer of the nest; I_2, II_2, III_2, IV_2 – the intermediate layer of the nest; I_3, II_3, III_3, IV_3 – the inner layer of the nest (the core).

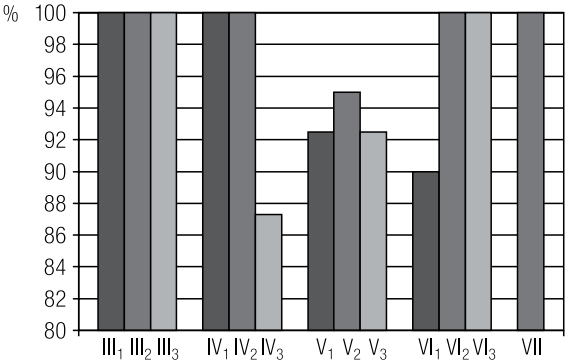

Fig. 2. The colonisation rate of the nest material by cellulolytic fungi (in %).

ing and the outer layer while the weakest growth of *Fusarium* was observed in the intermediate layer (Fig. 1B).

The species composition of cellulolytic fungi. Cellulolytic fungi occupying the nests were represented by 45 species of 20 genera (Tab. 2). A total of 1 276 pure cultures were differentiated; species of only five isolates of the genus *Penicillium* were not identified. The diversity of cellulolytic species obtained with the dilution method (40 species) was greater than that with the fragment method (20 species) (Tabs 3–4). Cellulolytic fungi were isolated using the latter method from their metabolically active forms (hyphae) overgrowing the nest material.

The most numerous colonisation of the nests was observed for cellulolytic fungi of the genera *Chaetomium, Trichoderma* and *Fusarium* (Tabs 2–3). Their share in the isolates of cellulolytic fungi isolated with the dilution method was 44%, 20% and 19%, respectively, that is in total 83% of all cellulolytic fungi isolated with this method. The share of the genera was 44%, 11% and 32%, respectively, for nest material fragments laid on the medium for cellulolytic fungi.

The species composition of the cellulolytic mycobiota differed depending on the nest. From 3 (V and VII) to 17 and 20 (II and I) species were recorded in individual nests when the dilution method was used and from 3 to 14 species when the fragment method was used (Tabs 3–4). Differences in the species composition of cellulolytic fungi also depended on the study year. Nests collected in 2004 were occupied by a community of cellulolytic fungi richer in species than those collected in 2005: 28 and 20 isolated species (dilution method), respectively – Tables 3–4.

The species richness of cellulolytic fungi in the nests, including frequencies of individual species, was analysed using Simpson's index (D) for four out of seven nests. The analysis indicated a considerable differentiation of species frequencies for both isolation techniques (Tabs 3–4).

The greatest differentiation of Simpson's indices was obtained for cellulolytic fungi isolated with the dilution method (0.289-NV to 0.758-NIII), that is three and ten species (Tabs 5–6). Smaller differences in Simpson's indices were recorded for frequencies of species isolated with the fragment method: (0.578-NV to 0.857-NVII) with the number of species 6 and 14, respectively (Tabs 5–7). Despite a considerable dispersion of the values of Simpson's indices among individual nests and depending on the isolation method, mean values of the index for the nests total were similar: 0.876 (fragment method) and 0.866 (dilution method). This shows a high frequency richness of species of cellulolytic fungi occupying the nests. This was also observed

Table 2
A list of species of cellulolytic fungi isolated from nests of Montagu's Harrier (*Circus pygargus*)

No.	Fungal species	Isolated with the dilution method	Isolated with the fragment method
1	*Acremonium strictum* W. Gams	+	-
2	*Alternaria alternata* (Fr.) Keissler	+	+
3	*Aspergillus fumigatus* Fres.	+	-
4	*Botryotrichum piluliferum* Sacc.& March.	-	+
5	*Chaetomium botrychodes* Zopf	+	+
6	*Ch. cochlioides* Pall.	+	+
7	*Ch. elatum* Kunze ex Steud.	+	+
8	*Ch. funicola* Cooke	-	+
9	*Ch. globosum* Kunze ex. Steud.	+	+
10	*Ch. indicum* Corda	+	+
11	*Ch. piluliferum* J. Daniels	-	+
12	*Chrysosporium pannorum* (Link) Hughes	+	+
13	*Cladosporium cladosporioides* (Fres.) de Vries	+	-
14	*Cl. sphaerospermum* Penz.	+	-
15	*Cylindrocarpon destructans* (Zinssm.) Scholten	-	+
16	*Doratomyces microsporus* (Sacc.) Morton & G. Sm.	+	-
17	*D. stemonites* (Pers. Ex Steud) Morton & G. Sm.	+	-
18	*Fusarium avenaceum* (Fr.) Sacc.	+	+
19	*F. culmorum* (W.G. Smith) Sacc.	+	+
20	*F. graminearum* Schwabe	+	-
21	*F. (Microdochium) nivale* (Fr.) Ces.	+	-
22	*F. oxysporum* Schlecht. emend. Sny & Hans	+	+
23	*F. poae* (Peck) Wollenw.	+	-
24	*F. sacchari* (Butler.) W.Gams	-	+
25	*F. sambucinum* Fuckel	+	-
26	*F. solani* (Mart.) Appel et Wollenw. Emend Sny et Hans	+	+
27	*F. sporotrichioides* Sherb.	-	+
28	*F. tricinctum* (Corda) Sacc.	+	-
29	*Gliocladium catenulatum* Gilm.&Abbott	-	+
30	*G. roseum* Bain.	+	+
31	*Paeciliomyces carneus* (Duche&Heim) A.H.S. Brown& G.Sm.	-	+
32	*Penicillium brevicompactum* Dierckx	+	-
33	*P. chrysogenum* Thom	+	-
34	*P. expansum* Link ex Gray	+	+
35	*P. purpurogenum* Stoll	+	-
36	*Penicillium* sp.	-	+
37	*Periconia atra* Corda	-	+
38	*Pithomyces chartarum* (Berk.&Curt.) M.B. Ellis	+	+
39	*Phoma herbarum Westend.*	+	-
40	*Scopulariopsis acremonium* (Delacr.) Vuill.	+	-
41	*S. brevicaulis* (Sacc.) Bain.	+	-
42	*Thermomyces lanuginosus* Tsiklinsky	+	-
43	*Thielavia heterotallica* Klopotek	+	-
44	*Trichoderma koningii* Oudem	+	+
45	*T. viride* Pers. ex Gray	+	+
46	*Verticillium albo-atrum* Reinke&Berthold	+	+
47	*V. tenerum* (Nees ex Pers.) Link	+	-

in relation to individual layers of the nests. Mean Simpson's indices for each layer (NIV – NVI) were 0.798 – 0.865 for the fragment method and 0.786 – 0.877 for the dilution method (Tab. 5).

The analysis of the frequencies of individual species of cellulolytic fungi (Tabs 3–4) showed that the greatest numbers of three *Chaetomium* species: *Ch.*

Table 3
The composition and frequency of species of cellulolytic fungi isolated from nests of Montagu's Harrier (dilution method)

No.	Fungal species	I			II			III			IV			V			VI			VII	Total
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
1	*Acremonium strictum*	-	-	3*	-	-	-	-	-	-	1	-	-	-	-	-	2	-	-	-	6
2	*Alternaria alternata*	-	-	-	-	-	-	2	-	2	3	-	3	-	-	-	-	-	-	1	11
3	*Aspergillus fumigatus*	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	3
4	*Botryotrichum piluliferum*	2	1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
5	*Chaetomium bostrychodes*	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
6	*Ch. cochlioides*	-	-	-	-	10	10	14	-	-	-	-	-	-	-	-	-	-	-	-	34
7	*Ch. elatum*	3	3	7	3	-	4	-	-	-	-	-	-	-	-	-	-	-	6	-	26
8	*Ch. funicola*	14	4	3	-	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48
9	*Ch. globosum*	2	-	9	3	3	7	-	-	-	-	-	-	4	18	31	7	27	16	18	145
10	*Ch. indicum*	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
11	*Ch. piluliferum*	2	6	8	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	17
12	*Chrysosporium pannorum*	-	-	-	-	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	7
13	*Cladosporium cladosporoides*	-	-	-	-	-	-	-	3	8	-	-	-	-	-	-	-	-	-	-	11
14	*Cl. sphaerospermum*	1	-	3	-	-	-	-	1	4	1	-	-	-	-	-	-	-	-	-	10
15	*Doratomyces stemonites*	-	1	-	3	-	-	10	4	8	-	-	-	1	-	-	-	-	-	4	31
16	*Fusarium avenaceum*	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
17	*F. culmorum*	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
18	*F. graminearum*	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
19	*F. (Microdochium) nivale*	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	5
20	*F. oxysporum*	-	-	-	-	-	-	-	-	-	10	5	-	-	-	-	-	-	3	-	18
21	*F. poae*	2	-	-	-	-	-	2	5	4	-	-	-	-	-	-	-	-	-	-	13
22	*F. sambucinum*	-	2	-	-	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-	6
23	*F. solani*	-	2	1	-	6	-	-	-	-	-	9	-	-	-	-	-	-	-	-	18
24	*F. sporotrichioides*	2	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
25	*F. tricinctum*	-	-	2	-	-	-	-	-	-	13	9	25	-	-	-	-	-	-	-	49
26	*Gliocladium catenulatum*	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
27	*G. roseum*	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	3
28	*Penicillium brevicompactum*	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
29	*P. chrysogenum*	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
30	*P. expansum*	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
31	*P. purpurogenum*	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
32	*Phoma hibernica*	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	10
33	*Scopulariopsis acremonium*	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
34	*S. brevicaulis*	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
35	*Thermomyces lanuginosus*	1	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
36	*Thielavia heterotallica*	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1

37	*Trichoderma koningii*	-	-	-	-	-	-	27	32	-	-	-	-	-	-	-	-	-	-	59
38	*T. viride*	-	-	-	-	8	22	-	-	-	-	-	-	5	1	4	3	12	10	5	70
39	*Verticillium albo-atrum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2
40	*V. tenerum*	1	-	1	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	10
	Total	35	21	56	13	61	57	60	45	31	31	24	39	10	19	35	12	39	37	28	653
		112			131			136			94			64			88				

Legend: III, IV, V, VI, VII – nest number; 1.2.3 – nest layer; 1-outer. 2-middle. 3-lining; * number of isolates

Table 4

The colonisation frequency of the material in nests of Montagu Harrier by individual species of cellulolytic fungi (fragment method)

No.	Fungal species	III			IV			V			VI			VII	Total
		1	2	3	1	2	3	1	2	3	1	2	3		
1	*Alternaria alternata*	5*	-	-	-	-	-	-	-	-	-	-	-	-	5
2	*Botryotrichum piluliferum*	-	-	-	-	-	-	2	6	-	3	-	-	-	11
3	*Chaetomium cochlioides*	3	6	12	-	5	5	-	-	-	-	-	-	-	31
4	*Ch. elatum*	-	-	-	-	-	-	-	-	3	-	-	-	-	3
5	*Ch. funicola*	3	-	-	-	-	7	12	4	2	-	-	-	-	28
6	*Ch. globosum*	-	-	-	-	-	-	24	31	35	11	26	40	20	187
7	*Ch. piluliferum*	-	-	-	-	-	-	-	-	-	6	5	4	-	15
8	*Cylindrocarpon destructans*	-	1	-	-	-	-	-	-	-	-	-	-	-	1
9	*Fusarium oxysporum*	-	-	-	-	8	-	-	-	-	-	-	-	-	8
10	*F. sacchari*	2	1	5	-	5	-	-	-	-	-	-	-	-	13
11	*F. solani*	3	-	-	7	5	-	-	-	-	5	-	-	-	20
12	*F. sporotrichioides*	-	5	9	1	-	3	-	-	-	-	-	-	8	26
13	*Gliocladium catenulatum)*	-	-	-	16	3	6	-	-	-	-	-	-	-	25
14	*G. roseum*	-	-	-	5	13	12	1	-	-	-	-	-	-	31
15	*Paeciliomyces carneus*	-	-	7	1	-	-	-	-	-	-	-	-	-	8
16	*Penicillium expansum*	1	-	-	-	-	-	-	-	-	-	-	-	-	1
17	*Penicillium* sp.	5	-	-	-	-	-	-	-	-	-	-	-	-	5
18	*Periconia atra*	-	-	-	-	-	-	4	-	-	-	-	-	-	4
19	*Pithomyces chartarum*	-	-	6	-	-	-	-	-	-	-	-	-	-	6
20	*Trichoderma koningii*	44	24	11	13	12	2	-	-	-	-	-	-	-	106
21	*T. viride*	2	12	9	8	15	11	2	-	11	11	-	-	14	95
22	*Verticillium albo-atrum*	3	-	-	-	-	-	-	-	-	-	-	-	-	3
Total		71	49	59	51	66	46	45	41	51	36	31	44	42	632
		179			163			137			111				

Legend as in Table 3

globosum, *Ch. funicola* and *Ch. cochlioides*, as well as two *Trichoderma* species*: T. koningii* and *T. viride*, colonised the nests. Populations of *Fusarium*: *F. avenaceum, F. oxysporum, F. poae* and *F. tricinctum*, *Gliocladium: G. roseum* and *G. catenulatum*, as well as *Doratomyces stemonites* were less numerous. The frequency distribution of individual cellulomycete species given above (as well as a few others) was uneven (Tabs 3–4). The frequency of a species population was high in some nests while it was low in others or the species was altogether absent. *Chaetomium globosum* was highly numerous in some nests and even its mass occurrence was observed. The frequency of this typical cellulolytic fungus was 66%, 69% and 48% of the total frequency of cellulolytic fungi when isolated with the dilution method in nests V, VI and VII, respectively, while its population constituted only 10% in nests I and II and the fungus was not recorded in nests III and IV. *Ch. cochlioides*, *Ch. funicola* and *Ch. elatum* were more frequently recorded *Chaetomium* representatives, although they were less numerous than *Ch. globosum* (Tabs 3–4). *Ch. cochlioides* occupied four out of seven nests. The contribution of the species to the cellulomycete community ranged from 6% (NIV) to 12% (NIII) for the fragment method and from 10 to 16%, respectively, for the dilution method. *Ch. funicola* was isolated mostly from nests collected in 2004 (NI, NII) and its frequency was ca. 20% of total cellulolytic fungi in these nests. *C. piluliferum* and its anamorph *Botryotrichum piluliferum* which represented even 21% of the frequency of cellulolytic fungi in NI, and *Ch. elatum*, which represented 5% and 12% of it in NI and NII, respectively, were also isolated from these nests. *Ch. indicum*, ca. 2% of the population of cellulolytic fungi in NI, was one of the least frequently recorded representatives of *Chaetomium*.

The data (Tabs 3–4) also show that the nests collected in 2004 (NI, NII) were characterised by a diversity of *Chaetomium* species greater than that recorded in 2005 (NIII-VII).

Of the two *Trichoderma* species recorded in the nests, *T. koningii* had a slightly greater frequency than *T. viride*: 17%-44% and 10%-33%, respectively. *T. koningii* was, however, less widespread (two nests) than *T. viride*, which colonised six out of seven nests (Tabs 3–4).

Ten *Fusarium* species were recorded within cellulolytic fungi in the nests. They mostly occurred in nests III and IV. *Fusarium tricinctum* had the greatest frequency, however, only in nest IV. The cellulolytic population of this species represented 50% of total cellulolytic fungi in the nest. *F. oxysporum* was also recorded more frequently.

Table 5
Simpson's indices (D) for cellulolytic fungi in entire nests and individual layers of the nests of Montagu's Harrier (*Circus pygargus*)

Nest		Isolation method	
		Culture from dilutions	Fragment laying
III		0.758	0.761
IV		0.698	0.857
V		0.289	0.732
VI		0.590	0.578
III-VI total		0.866	0.876
Nest layer	outer	0.877	0.865
	middle	0.786	0.861
	lining	0.831	0.798

Table 6
The composition and frequency of *Fusarium* populations (Nash and Snyder medium) in nests of Montagu's Harrier

No.	Fungal species	III			IV			V			VI			VII	Total
		1	2	3	1	2	3	1	2	3	1	2	3		
1	*Fusarium aqueductum*	-	-	-	-	-	-	-	-	-	5	5	5	-	15
2	*F. avenaceum*	-	-	3	10	8	27	-	2	-	-	-	-	-	50
3	*F. equiseti*	-	-	-	-	-	7	-	-	-	-	-	-	-	7
4	*F. oxysporum*	-	-	-	22	6	-	-	-	-	11	1	-	-	40
5	*F. poae*	4*	4	-	8	3	-	13	3	8	-	-	-	10	53
6	*F. sacchari*	-	-	-	-	-	-	-	-	-	-	8	-	-	8
7	*F. semitectum*	2	-	-	-	-	-	-	-	-	-	-	-	-	2
8	*F. solani*	-	-	-	-	-	-	-	-	3	-	-	-	-	3
9	*F. sporotrichioides*	-	-	4	1	2	8	-	-	-	-	-	-	-	15
10	*F. tricinctum*	-	-	2	2	4	9	-	-	-	-	-	-	-	17
Total		6	4	9	43	23	51	13	5	11	16	14	5	10	210
		19			117			29			35				

Legend as in Table 3

Table 7
The frequency of other (non-*Fusarium*) fungal species isolated from nests of Montagu's Harrier on the Nash and Synder medium

No.	Fungal species	III			IV			V			VI			VII	Total
			1	2	3	1	1	2	3	1	1	2	3	1	
1	*Acremonium kiliense*	2	14	13	-	-	-	-	-	-	17	5	42	12	105
2	*A. strictum*	8	15	64	24	14	11	10	11	9	-	-	-	-	166
3	*Acremonium* sp.	4	-	-	-	-	-	-	-	-	-	-	-	-	4
4	*Botrytis cinerea*	-	5	-	-	-	-	-	-	-	-	-	-	-	5
5	*Mortierella* sp.	-	-	-	-	-	-	-	-	-	-	-	-	6	6
6	*Mucor hiemalis*	-	-	-	-	-	-	-	3	-	-	-	-	-	3
7	*Rhizoctonia solani*	-	23	-	-	12	-	-	-	-	-	-	-	-	35
8	*Trichoderma koningii*	-	-	-	-	-	-	-	-	-	9	7	16	16	48
9	*T. viride*	-	-	-	-	-	-	-	3	-	-	3	-	17	23
10	*Verticillium albo-atrum*	-	-	-	-	-	-	-	-	7	15	-	-	-	22
11	*V. dahliae*	-	-	12	-	-	-	-	-	-	-	-	-	-	12
12	*V. lecani*	-	-	-	-	-	-	4	-	-	-	-	-	-	4
13	Non sporulating (dark mycelium)	22	14	10	6	19	-	4	3	6	16	-	23	-	123
Total		36	71	99	30	45	11	18	20	22	57	15	81	51	556
		206			86			60			153				

Legend as in Table 3

Its frequency was 16% of cellulomycetes in nest IV (Tab. 3). A greater frequency of individual *Fusarium* species was obtained when the selective isolation method for the genus was used (Tab. 6). *F. poae, F. avenaceum* and *F. oxysporum*, 25%, 24% and 19% of the total *Fusarium* frequency were the most frequently recorded species in the five nests collected in 2005. A smaller share of ca. 8% of *Fusarium* populations on average was observed for *F. aqueductum, F. sporotrichioides* and *F. tricinctum*. Low frequency of *F. tricinctum* isolation on the preferential medium for *Fusarium* (Nash and Snyder medium with PCNB) in comparison with the medium used to isolate cellulolytic fungi indicates intrageneric competition in which *F. tricinctum* is a poorer competitor. On the other hand, this indicates a more frequent incidence of cellulolitic abilities within *F. tricinctum* than in other *Fusarium* populations in the study environment (Tabs 3–5). A frequency analysis of *Fusarium* isolated on the Nash and Snyder medium confirmed that the greatest frequency and richness of *Fusarium* species (six species) was observed in nest IV, similarly to the cellulose medium (Tab. 3 and 5). The total frequency of these species in nest IV was as high as 56% of the total *Fusarium* representatives in the nests (NIV–VII). The lowest frequency (5%) and only one species, *F. poae,* was isolated on the Nash and Snyder medium from nest VII (the material was shaken out). *Fusarium poae* was additionally the most widespread *Fusarium* species in the nests isolated on the Nash and Snyder medium: it colonised four out of the five nests examined (Tab. 6).

Both *Fusarium* species and other species, loosely called accompanying species, were isolated on the Nash and Snyder medium: nine species altogether and two taxa identified only to the genus level. Due to the fact that 14% isolates represented potentially phytopathogenic species in this group of fungi, their frequency was also analysed (Tab. 7). These were in particular *Botrytis cinerea, Rhizoctonia solani, Verticillium albo-atrum, V. dahliae*. *Acremonium*, a genus related to *Fusarium*, was isolated in this group. The fungus produces wettable 1-celled conidia, resembling microconidia in *Fusarium*. The genus was very numerous and represented 36% of all isolates isolated on the Nash and Snydera medium (a total of 766) – Table 7.

DISCUSSION

Nutrient-specialised saprotrophic micromycetes, such as keratinolytic or cellulolytic fungi, in natural environments, e.g. in the soil, are distributed non-uniformly (Korniłłowicz-Kowalska, Bohacz 2002a; Korniłłowicz-Kowalska et al. 2003). The uneven distribution of various physiological groups of fungal saprotrophs is conditioned by the dispersion of organic matter which is the source of food for the microorganisms. Therefore, the role of examinations on the organic matter occurring in the environment is greater than that of examinations of the entire environment. Nests of birds living in different biotopes are such microhabitats. They are usually agglomerations of different plant fragments (twigs, rootlets, grass blades, wooden pulp) and animal fragments (hair, feathers, bird faeces, chick down, prey remains, pellets). The nest mycobiota which comprises fungi that use simple organic compounds, e.g. representatives of Mucorales and ubiquistic fungi (polyphages), is differentiated by the presence of compounds that use less accessible organic matter

fractions such as ligninocellulose or keratine. Both typically saprotrophic species and potentially pathogenic species, opportunistic pathogens causing diseases in humans and animals as well as toxicogenic fungi are found in fungal communities in birds' nests (Hubalek 1974; Hubalek, Balat 1974; Pinowski et al. 1999; Korniłłowicz-Kowalska, Kitowski 2009).

As observed in a previous study by the authors (Korniłłowicz-Kowalska, Kitowski 2009), a high total frequency of saprotrophic micromycetes, a high richness and frequency of species with a broad spectrum of substrates (polyphages), sugar-loving fungi and species belonging to opportunistic pathogens in humans and animals were recorded in the nests of Montagu's Harrier. The present study shows that nests of Montagu's Harrier are characterised by a high frequency of fungi specialised in cellulose biodegradation. The number of propagule units of these fungi corresponded to the frequencies of cellulolytic fungi in composts consisting of plant material and hen feathers (Korniłłowicz-Kowalska, Bohacz 2002b). They exceeded, however, the frequencies of these microorganisms in soils fertilised with natural fertiliser (manure) and were considered to be rich in these micro-organisms (Korniłłowicz 1989). The high rate of colonisation of the nest material by cellulolytic fungi recorded here also indicates a high enzymatic activity of these micro-organisms and intensive processes of degradation of cellulose and other polysaccharides occurring in nests abandoned after breeding. Identification examinations and lists of total Simpson's coefficients show a great differentiation of the composition and frequency of cellulolytic species of fungi, both in relation to entire nests and their individual layers. The high values of Simpson's coefficient show that the communities of cellulolytic fungi in this microhabitat are dominated by a relatively small number of populations of these fungi. This was also observed for the total mycobiota in nests of Montagu's Harrier (Korniłłowicz-Kowalska, Kitowski 2009).

Chaetomium spp. (Ascomycetes), *Trichoderma* spp. and *Fusarium* spp. (mitosporic fungi) were the most numerous populations of cellulose-decomposing fungi. They constituted over 80% of total cellolytic fungi. Representatives of these genera were often recorded among fungal colonisers of wild-living birds' nests and plumage (Pugh 1965; Apinis, Pugh 1966; Mishra, Tiwari 1970; Hubalek 1974; Hubalek et al. 1973; Takatori, Hasegawa 1981; Kaul, Sumbali 1999). However, those were mostly species belonging to *Passeriformes*. *Chaetomium*, representing so called soft rot fungi causing the destruction of the surface wood layer resulting form cellulose biodegradation, other polysaccharides and lignin structure injuries, is one of the most effective cellulose destruent (Domsch et al. 1980). Of the seven *Chaetomium* species isolated from the nests, *Chaetomium globosum* had the highest frequency and constituted 70-80% of the total cellulolytic micromycetes in some nests. Populations of *Ch. cochlioides, Ch. funicola* and *Ch. elatum* were less numerous (10-20%) in individual nests. The four *Chaetomium* species are thought to be widespread, both in nests and on feathers of different bird species, mostly *Passeriformes* (Hubalek 1974).

The analysis shows that the genus *Trichoderma* was represented in the nests by *T. viride* and *T. koningii*, which reach 33% and 44% of total cellulolytic fungi in some nests. The fungi were previously infrequently isolated from birds' nests. Hubalek (1974) recorded *T. viride* in nests of song thrush (*Turdus philomelos*) containing the lining of wooden pulp (lignocellulose – author's note). *T. viride* was more often isolated from the plumage of *Passeriformes*, also including *Turdus philomelos* (Pugh

1965; Mishra, Tiwari 1970). *Trichoderma koningii* was recorded in nests of *Passer domesticus* and a few other *Passeriformes* birds (Apinis, Pugh 1967).

Fungi belonging to the genus *Fusarium* were recorded by Pugh (1986) as well as by Takatori and Hasegawa (1971) in nests and plumage of different species of terrestrial birds: *Passeriformes* and *Columbiformes*. According to Hubalek (1974), however, *Fusarium* mostly colonises nests of water birds. The genus was also recorded on feathers of water birds (Pugh 1966). Of *Fusarium* species recorded in birds' nests, only *F. oxysporum, F. sporotrichioides* and *F. moniliforme* have been listed (Hubalek 1974). Present examinations show that ten *Fusarium* species with cellulolytic abilities occurred in the nests with different frequencies. Examinations of the composition and frequency of *Fusarium* species on the selective medium for the genus (Nash and Snyder medium) show that almost 70% of the fungi were constituted by populations of *F. poae, F. avenaceum* and *F. oxysporum*.

Cellulolytic *Gliocladium* spp., mostly *G. roseum*, and darkly pigmenting fungi *Cladosporium herbarum, Cl. cladosporoides, Cl. sphaerospermum, Doratomyces stemonites, D. microsporus, Alternaria alternata*, occurred less frequently in the nests (less than 10% of total records). The species were previously isolated from nests and (or) plumage of many songbirds (*Passeriformes*) and water birds (Pugh 1965; 1966; Hubalek et al 1973; Hubalek 1974; Hubalek, Balat 1974; Takatori, Hasegawa 1981). Among them, *Doratomyces* (*Stysanus*) *stemonites* is believed to be closely related to water birds (nest and plumage) (Hubalek 1974).

Cellulolytic fungi such as *Penicillium* spp., *Aspergillus*, including *A. fumigatus*, *Scopulariopsis* spp. excluding *S. acremonium* and *S. brevicaulis*, and a few others were recorded sporadically in the nests. As a previous study by Korniłłowicz-Kowalska, Kitowski (2009) shows, *A. fumigatus* and *S. brevicaulis* were recorded as frequent within so-called total fungi (Martin's medium) and potentially zoopathogenic fungi (Sabouraud medium). In the light of the new data, a very low number of records of cellulolytic isolates of *S. brevicaulis* and especially *A. fumigatus*, which causes opportunistic infections of raptors (Joseph 2000), shows the absence of a correlation between these fungi and cellulose substrates in the nests. This observation supports the suggestion proposed in a previous study (Korniłłowicz-Kowalska, Kitowski 2009) that *A. fumigatus* is nutritionally related to keratin waste found in nests.

Of the total 20 genera and 45 species of fungi isolated, the majority are cosmopolitan species, widespread in soil, colonising the phyllosphere, dead plant remains or, like some, occurring in the air. They are mostly saprotrophs, e.g., *Chaetomium* spp., *Trichoderma* spp., *Gliocladium* spp., *Doratomyces* spp., *Penicillium* spp., or potential phytopathogens: *Fusarium* spp., *Verticillium* spp., *Alternaria* spp., *Cladosporium* spp., *Rhizoctonia* spp. (Domsch et al. 1980). As noted above, many of these genera were isolated from the plumage and pellets, faeces and other animal remains (Hubalek 1974; Domsch et al. 1980). *Doratomyces stemonites*, isolated with a very high frequency (over 50%) from pellets of Montagu's Harrier in one of the nests (Korniłłowicz-Kowalska, Kitowski 2009) as well as *Fusarium tricinctum*, less widespread in the natural environment in comparison with other *Fusarium* species, are noteworthy (Domsch et al. 1980). Of rarely recorded species, darkly pigmenting micromycetes such as *Periconia atra* and *Pithomyces chartarum* should be mentioned. The former was isolated together with many other species (e.g., *Fusarium oxysporum*, *F. sacchari*, *Cladosporium* spp., *Alternaria alternata, Trichoderma viride*) by Mazurkiewicz-Zapałowicz, Wróbel and Buczek

(2008) from the saw sedge phyllosphere. As saw sedge leaves are used as the lining in nests of Montagu's Harrier, we think that this plant material was a source of a considerable amount of cellulolytic fungi and *Fusarium*.

The results allow us to claim that humidity and thermal conditions recorded in the nests during the incubation and chick rearing led to the selection of certain populations of cellulolytic micromycetes. As Table 1 shows, despite differences, nest humidity was high (23%–71%) and the pH ranged from neutral to slightly alkaline (pH_{KCl} 7.10–7.60). As indicated by the frequency of their occurrence, mostly fungi with a high water activity coefficient (wa) as well as thermotolerant and alkalotolerant fungi had favourable conditions in the microhabitat. Similar observations were made in relation to nutritionally unspecialised fungi colonising nests of Montagu's Harrier (Kornifłowicz-Kowalska, Kitowski 2009). It was also observed that the frequency of cellulolytic fungi was greater in nests with a higher (NV–NVII) rather than a lower (NIII–NIV) humidity. This was caused by a frequency increase in *Chaetomium*, *Trichoderma* and *Fusarium* populations considered to be so called tertiary colonisers which require at least wa = 0.9–0.95 for growth (Grant et al. 1989). *Chaetomium globusom* and *Trichoderma viride* were especially hydrophilous (Apinis, Pugh 1966; Hubalek 1974; Domsch et al. 1980). The species did not occur in nest III (with the lowest humidity, 23%–25%). *Doratomyces stemonites* or *Trichoderma koningii*, which had lower water condition requirements, had good growth conditions in this environment (Domsch et al. 1980). *T. koningii* as well as *Ch. globosum, Ch. cochlioides* and *Ch. funicola* are also thermotolerant (Apinis, Pugh 1966) and grow at 37°–38°C or even higher temperatures. It may be supposed that the selection of these fungi occurred as early as during incubation when the nest temperature can reach even 40–41°C (Pinowski et al. 1999).

The high nest humidity and temperature did not encourage the development of species such as *Penicillium* which prefer environments with lower wa values (Griffin 1972, 1994). The majority of species of this genus are also psychrophiles or psychrotrophs and acidophilous fungi (Apinis, Pugh 1966; Griffin 1972; 1993). *Cladosporium* spp. and *Alternaria* spp. are also considered to be relatively psychrophilous or psychrotolerant (Apinis, Pugh 1966), which explains their low frequency in the nests within a short period after they were abandoned by the birds.

The examinations show that over 40% of the species composition, that is 20 fungal species, of the nests are potential phytopathogens, mostly *Fusarium* spp. Species such as *Verticillium albo-atrum, V. dahliae, Rhizoctonia solani, Botrytis cinerea, Alternaria alternata*, *Cladosporium* spp. were also recorded. A high contribution of potentially phytopathogenic fungi in the mycobiota of the nests was conditioned by the plant origin of the nest material, rich in cellulose and other polysaccharides. The development of these fungi as well as exclusively saprotrophic fungi was also encouraged by a high nitrogen and phosphorus content (Tab.1) from bird faeces, feathers and other animal substrates. Previous studies by Kornifłowicz (1989); Kornifłowicz-Kowalska et al. (2003) showed that the development of *Fusarium* and cellulolytic fungi is stimulated by the presence of nitrogen and phosphorus in the soil.

The present findings allow us to think that birds' nests, including nests of Montagu's Harrier, may be some of the links in the circulation of phytopathogenic fungi, such as *Fusarium* spp., *Verticillium albo-atrum* or *V. dahliae*. These fungi may survive, reproduce and, consequently, spread aerogenically, also by birds. In Montagu's

Harrier, the process is undoubtedly encouraged by high breeding densities that reach even 10.6 nest/100 hectares (Krogulec 1992).

CONCLUSIONS

- Nests of Montagu's Harrier are characterised by a high frequency, colonisation rate and species richness of cellulolytic fungi and *Fusarium.*
- The number and frequency of fungal species differed depending on the nest and the layer (outer, middle, lining).
- The highest frequency was recorded for fungal species with high water condition requirements, often thermotolerant, of the genera: *Chaetomium, Trichoderma, Fusarium.*
- Species with lower water requirements, psychrophilous or psychrotolerant species, such as *Penicillium* spp., *Cladosporium* spp., were recorded rarely and their frequency was lower.
- As observed, a neutral or slightly alkaline reaction of the nests encourages growth of alkalotolerant species.

Acknowledgement. This work was supported by the by the Committee of Scientific Researches, a grant no. 2P04G03330.

REFERENCES

Apinis A.E., Pugh G.J.F. 1967. Thermophilous fungi of birds' nests. Mycopathologia 33: 1–9.

Buczek T. 2004. Błotniak łąkowy *Circus pygargus* (In:) M. Gramadzki (ed.).Poradnik ochrony siedlisk i gatunków. Natura 2000 – podręcznik metodyczny. Ministerstwo Środowiska, Warszawa: 226–230.

Clarke R. 1996. Montagu's harrier. Arlequin. Chelmsford.

Cramp, Simmons K.E.L. 1980. The Birds of the Western Paleartic. Volume 2. Oxford University Press, Oxford, UK.

Domsch K.H., Gams W., Anderson T.H. 1980. Compendium of Soil Fungi. I Acad. Press. London.

Ellis H.B. Dematiaceaus Hyphomycetes. 1971. Commonwealth, Mycological Institute Kew Surrey, England.

Ghosh B., Ghosh A. 1992. Degradation of cellulose by fungal cellulase. (In:) G. Winkelmann (ed.). Microbial Degradation of Natural Products VCH Weinheim 83–125.

Grant C., Hunter C.A., Flannigan B., Bravery A.F. 1989. The moisture requirements of moulds isolated from domestic dwelling. Int. Biodet. 25: 259–284.

Griffin D.M. 1972. Ecology of soil fungi. Chapman and Hall. England.

Griffin D.M. 1994. Fungal Physiology. I. Willey – Liss. New York USA.

Hubalek Z. 1974. Fungi associated with free–living birds in Czechoslovakia and Yugoslavia. Acta Sc. Nat. Brno 8 (3): 1–62.

Hubalek Z., Balat F. 1974. The survival of microfungi in the nests of tree sparrow (*Passer montagus* L.) in the nest-boxes over the winter season. Mycopathologia 54: 517–530.

Hubalek Z., Balat F., Touškova I., Vlk T. 1973. Mycoflora of birds nests in nest-boxes. Mycopath. Mycol. Appl. 49: 1–12.

Joseph V. 2000. Aspergillosis in Raptors. Seminars in Avian and Exotic. Pet Medicine 9: 66–74.

Kaul S., Sumbali G. 1999. Impact of some ecological factors on the occurrence of poultry soil-inhabiting keratinophiles. Mycopathologia 143: 155–159.

Kitowski I. 2002. Behaviour of Montagu's Harrier juveniles *Circus pygargus* during the post-fledging dependency period in southeast Poland. Berkut 11: 201–207.

Korniłłowicz-Kowalska T. 1989. Wpływ intensywnego nawożenia obornikiem and granulatem keratynokoro-mocznikowym na wybrane zespoły mikroflory glebowej. Zesz. Probl. Post. Nauk Rol. 370: 85–96.
Korniłłowicz-Kowalska T., Bohacz J. 2002a. Some correlations between the occurrence frequency of keratinophilic fungi and selected soil properties. Acta Mycol. 37 (1/2): 101–116.
Korniłłowicz-Kowalska T., Bohacz J. 2002b. Próba kompostowania odpadów pierza z zastosowaniem inokulum grzybowego. II. Dynamika rozwoju drobnoustrojów. Acta Agrophys. 73: 189–197.
Korniłłowicz-Kowalska T., Iglik H., Wojdyło B. 2003. Correlation between the abundance of cellulolitic fungi and selected soil properties. Acta Mycol. 38: 161–172.
Korniłłowicz-Kowalska T., Kitowski I. 2009. Diversity of fungi in nests and pellets of Montagu's Harrier (*Circus pygargus*) from east Poland – importance of hemical and ecological factors. Ecol. Chem. Enngineer. S. (in press)
Krebs C.J. 1994. Ecology. The Experimental Analysis of Distribution and Abundance, Fourth Edition. Harper Collins, New York, USA
Krogulec J. 1994. Breeding ecology of Montagu's Harrier *Circus pygargus* on calcareous marshes near Chelm. Unpublished Ph.D. Thesis. University of Maria Curie Sklodowska. Lublin. (in Polish).
Kwaśna H., Chełkowski I., Zajkowski P. 1991. Flora polska. Grzyby (Mycota) 22: (Deuteromycetes), (Hyphomycetales), *Fusarium*. PWN. Warszawa.
Mazurkiewicz-Zapałowicz K., Buczek T. 2008. Grzyby mikroskopowe związane z kłocią wiechowatą (*Cladium mariscus* L.) Pohl. Chrońmy Przyrodę Ojczystą 64 (1): 45–57.
Mishra R.R., Tewari R.P. 1970. Fungal species associated with certain common birds. Sci. Cult. 36: 350–352.
Nash S.M., Snyder W.C. 1962. Quantitative estimation by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytophology 52: 567–572.
Nelson P.E., Toussoun T.A., Morasas W.F.O. 1983. *Fusarium* species: An illustrated manual for identification. The Pensylvania state, University Press.
Pinowski J., Pinowska B., Haman A. 1999. Fungi in birds' plumage and nets. Intern. Stud. Sparrows 26: 3–28.
Pugh G.J.E. 1965. Cellulolytic and keratinophilic fungi recorded on birds. Sabouraudia 4: 85–91.
Pugh G.J.E. 1966. Associations between birds, nests, their pH and keratinophilic fungi. Sabouraudia 5: 43–53.
Takatori K., Hasegawa A. 1981. Isolation of keratinophilic and non-keratinophilic fungi from birds nests. Trans. Mycol. Soc. (Japan) 22: 347–352.
Tomiałojć L., Stawarczyk T. 2003. The avifauna of Poland. Distribution, numbers and trends. Wroclaw, PTPP "pro Natura" (in Polish).
Trojan P. 1975. General ecology. Wyd. PWN, Warsaw (in Polish).
Vesonder R.F., Golinski P. 1989. Metabolites of *Fusarium* (In:) J. Chełkowski (ed.). *Fusarium*, Mycotoxins, Taxonomy and Pathogenicity. Elsevier, Amsterdam: 1–40.

Występowanie grzybów celulolitycznych oraz *Fusarium* w gniazdach *Circus pygargus*

Streszczenie

Celem przedstawionej pracy było zbadanie liczebności oraz składu gatunkowego grzybów celulolitycznych i *Fusarium* w gniazdach błotniaka łąkowego (*Circus pygargus, Falconiformes*). Stosując metodę rozcieńczeń oraz wykładania fragmentów materiału gniazdowego na podłoża selektywne dla tych grzybów, zbadano 19 próbek z 7 gniazd zlokalizowanych na obszarze torfowisk węglanowych koło Chełma w płd.-wsch. Polsce (Rezerwat Błota Serebryskie). Próbki do badań pobierano z 3 warstw gniazda: wyściółki, warstwy zewnętrznej oraz środkowej leżącej pomiędzy nimi.

Stwierdzono, że gniazda błotniaka łąkowego cechuje wysoka ogólna liczebność grzybów celulolitycznych i *Fusarium*, duże bogactwo oraz zróżnicowanie frekwencji ich gatunków. Wykazano, że 80% zbiorowiska grzybów celulolitycznych reprezentowały 3 rodzaje: *Chaetomium, Trichoderma* i *Fusarium.* Spośród 45 zanotowanych gatunków najwyższą liczebnością wyróżniały się: *Chaetomium globosum*, *Trichoderma koningii* i *T. viride*. W obrębie *Fusarium* najczęściej notowano: *F. poae, F. avenaceum* i *F. oxysporum*. Odnotowane dominanty gatunkowe należały do grzybów hydrofilnych, na ogół termotolerancyjnych. Niską frekwencją odznaczały się natomiast gatunki preferujące środowisko o niskiej aktywności wodnej, psychrofilne i acidofilne takie jak: *Cladosporium* spp. i *Penicillium* spp.