

COPROPHILOUS FUNGI FROM CONFINED DEERS IN PAVIA (LOMBARDIA, ITALY)

(Hongos coprófilos en ciervos confinados en Pavia (Lombardía, Italia))

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Key words: Coprophilous fungi, deer, *Zygomycetes*, *Ascomycetes*, *Hyphomycetes*.

Palabras clave: Hongos coprófilos, ciervo, *Zygomycetes*, *Ascomycetes*, *Hyphomycetes*.

SUMMARY

Eighteen samples of fresh dungs were collected in spring time in order to establish the presence of coprophilous fungi in a group of deers confined in a private park located near Pavia (Lombardia, Italy). A total of 40 genera and 57 species distributed in: 65 *Zygomycetes*, 56 *Ascomycetes*, 100 *Hyphomycetes* and 2 *Basidiomycetes* (n= 223) was detected.

Dominant species were: *Mucor hiemalis*, *Graphium penicillioides*, *Fusarium verticillioides*, *Mucor racemosus*, *Saccobolus versicolor*, *Chaetomium bostrychodes* and *Doratomyces columnaris*.

It is also noted the diversity and density of some species in this kind of dungs and new records of some *Ascomycetes* in this geographic area, such as: *Ascodesmis sphaerospora*, *Coprotus disculus*, *Sphaerodes fimicola*, *Zopfiella leucotricha*, *Kernia nitida* and *Thelebolus crustaceus* are also mentioned.

INTRODUCTION

Coprophilous fungi play several roles in soil and grassland ecosystem, specially in the decomposition and mineralization of herbivore feces (Angel & Wicklow, 1975). The quantification, distribution and the structure of coprophilous community are related to different physiological, ecological and biogeographical features which are closely linked to the kind and composition of the substratum as well as to the surrounding terrestrial habitat (Sinh & Webster, 1973; Safar & Cooke, 1988 a,b; Kuthubutheen & Webster, 1986).

The complexity of fecal substrata is similar to soil systems and exhibits a high degree of spatial and temporary

RESUMEN

Con el fin de determinar la presencia de hongos coprófilos en un grupo de ciervos confinados en un parque privado en las cercanías de Pavia (Lombardia, Italia), se colectaron 18 muestras de excrementos frescos en un periodo primaveral.

Se detectaron un total de 40 géneros y 57 especies distribuidas en: 65 *Zygomycetes*, 56 *Ascomycetes*, 100 *Hyphomycetes* y 2 *Basidiomycetes* (n=223).

Las especies dominantes fueron *Mucor hiemalis*, *Graphium penicillioides*, *Fusarium verticillioides*, *Mucor racemosus*, *Saccobolus versicolor*, *Chaetomium bostrychodes* y *Doratomyces columnaris*.

Se comenta la diversidad y densidad de algunas especies en este tipo de excrementos y se mencionan nuevos recod de algunos *Ascomycetes* en ésta área geográfica, tales como: *Ascodesmis sphaerospora*, *Coprotus disculus*, *Sphaerodes fimicola*, *Zopfiella leucotricha*, *Kernia nitida* y *Thelebolus crustaceus*.

heterogeneity in biotic and abiotic components, thus making difficult to measure in situ (Miller, 1995). Moreover, changes in species composition during succession affect measurement of fungal diversity (Wicklow & Moore, 1974, Kuthubutheen & Webster, 1986).

Italian contributions to the occurrence and distribution of coprophilous fungi in animal dung remain scarce and limited to certain group of animals. Data covering this country are still rare and referred to the mycota of rabbit dung from Piemonte (Vesco dal et al., 1967) and the fungal population colonizing the horse, goat and sheep dung in Lombardia (Caretta et al., 1994).

The present survey was therefore undertaken to acquire further taxonomic informations about the occurrence and diversity of coprophilous fungi from Roe deer dung confined to a same kind of food in a 3.000 m² park located in the suburbs of Pavia (Lombardia). Anecological event which we considered interesting because of the unfrequent association of this animals with a purely antropophilic habitat.

MATERIALS AND METHODS

The dungs of 18 deers were collected in 1995 in the private park of S. Martino Siccomario a suburb of Pavia. Samples were collected in spring. These animals had been fed with hay, grass and cereals, in particular maize, all year long.

Eighteen samples of fresh dung were singly collected in sterile containers in different park areas. Each sample was divided into three sub samples of approximately equal size, and placed on moist blotting-paper in individual sterile Petri dishes.

Samples were incubated for 60 days at room temperature (19-22 °C) and daily exposed to natural light. Substratum moisture was maintained by periodic additions of sterile distilled water for up to 30 days.

Dung was inspected for fungal fructifications at regular intervals during this incubation period.

Isolations were usually attempted in 2 media, PDA and agar with decoction of the same deer feces. Moreover in most cases macroscopic and microscopic observations were carried out directly in living material of the dung.

No attempt was made to count the total number of colonies of each species in a single Petri dish. Species occurring more than once over the three replicate dishes were counted as one occurrence. Portions of dung samples and slides of some fungal species are retained at the Institute of Medical Mycology of Pavia. The microscopic preparation of fungi were mounted in cotton-blue and lactophenol or simply lactophenol.

RESULTS AND DISCUSSION

A total of 223 isolates representative of : 65 *Zygomycetes*, 56 *Ascomycetes*, 100 *Hyphomycetes* and two *Basidiomycetes* was obtained from 18 deer fecal samples. Fifty six species belonging to 41 genera were recorded (Table. 1). The dominant species occurring over 50 % in 18 samples, were *Chaetomium bostrychodes*, *Doratomyces columnaris*, *Fusarium verticillioides*, *Graphium penicillioides*, *Mucor hiemalis*, *M. racemosus* and *Saccobolus versicolor* (Table. 1, Figure A).

Frequent species occurring between 30-49% were *Botryotrichum piluliferum*, *Piptocephalis lepidula*, *Scopulariopsis brevicaulis*, *S. brumptii*, *Sordaria fimicola* and *Penicillium spp.* (Table. 1).

The rare species isolates only once (8%), represented several groups of fungi mostly cosmopolitan coprophilous *Ascomycetes* (10/18), which are common in different kinds of feces collected from herbivorous animals such as : *Ascobolus perplexans*, *Ascodesmis sphaerospora*, *Chaetomium globosum*, *Ch. murorum*, *Ch. piluliferum*, *Coprotus disculus*, *Podospora miniglutinans*, *Sphaerodes fimicola*, *Sporormiella minima*, and *Zopfiella leucotricha*. (Table. 1). Their scarce propagules density in Petri dishes, may not reflect their occurrence in the habitat and this may be due to the kind of food eaten by these animals (pellets), a slower time of fructification, a clear interspecific competition between the fecal microbiota (bacteria and fungi), or to the conditions of incubations (Wicklow & Moore, 1974; Safar & Cooke, 1988 b).

The total number of species developing on these feces was higher than in other study in the same geographical locality with different types of dung (Caretta et al, 1994)

Most of the fungal groups exhibited fructifications usually about the 5 (*Zygomycetes*) to 10-25 days (*Asco-Deuteromycetes* and *Basidiomycetes*). Later on (30-60 days), only a few of them (*Asco* and *Deuteromycetes*) appeared when the moisture of the substratum fell due to the fact that the Petri dishes were not added sterile water. Main taxa were some : *Penicillium spp.*, *Coprotus disculus*, *Kernia nitida*, *Thelebolus crustaceus*, *Doratomyces stemonitis* and *Scopulariopsis brevicaulis*. However many of them made their appearance from 10 to 20 days. This coincides with the influence of the activity of water in the succession of coprophilous fungi described by Kuthubutheen & Webster (1986).

The mucoraceous and *Hyphomycetes* species are common of herbivore excrements of most animals, and may also occur on other types of decaying organic matter.

The presence of some of these fungi in deer dung, can be probably attributed to their ability to colonize the maize stored, one of the ingredients in the food for deers.

M. hiemalis the most dominant fungi isolated, is common in different herbivorous feces (Harper & Webster, 1964, Dal Vesco et al, 1967), usually appear after 3-5 days incubation with their parasites, specially *Chaetocladium brefeldii* and in our case with *Piptocephalis lepidula*. It is also one of the most common and representative soil fungi and its competitive ability, especially in agricultural soil, is due to the fact that they germinate and grow rapidly when stimulated by soluble nutrients, being even able to exclude *Trichoderma harzianum* from specific habitat (Wardle et al., 1993). Yet as Shearer (1995), states it this situations is surprising because the *Mucorales* are considered *r*-selected and poor competitors. The scarce presence of *T. harzia-*

Table 1. Relative frequency of total coprophilous fungi on fecal deer substrata

TAXA	Number of samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	n	%
<i>Acremonium</i> sp.							+											+		2	0,89
<i>Acremonium strictum</i> W.Gams															+					1	0,44
<i>Alternaria</i> sp.																+				1	0,44
<i>Ascobolus furfuraceus</i> Pers & Hook																+	+			2	0,89
<i>A. perplexans</i> Masee & Sal.												+								1	0,44
<i>Ascodesmis sphaerospora</i> Obrist															+					1	0,44
<i>Aspergillus clavatus</i> Desmaz.														+				+	+	3	1,34
<i>Botryotrichum piluliferum</i> Sacc. & March						+								+	+	+	+		+	6	2,69
<i>Chaetocladium brefeldii</i> v.Tiegh & Le Mond															+	+		+	+	4	1,79
<i>Chaetomium bostrychodes</i> Zopf	+			+				+			+		+	+	+	+	+	+	+	11	4,93
<i>C.globosum</i> Kunze						+														1	0,44
<i>C.murorum</i> Corda																			+	1	0,44
<i>C.piluliferum</i> Daniels																	+			1	0,44
<i>Cladorrhinum foecundissimum</i> Sacc.&March				+									+							2	0,89
<i>Coprinus</i> sp.						+										+				2	0,89
<i>Coprotus disculus</i> Kimbr.,Luck-Allen & Cain											+									1	0,44
<i>Doratomyces columnaris</i> Swart	+		+		+	+	+	+			+	+	+	+				+		11	4,93
<i>D.stemonitis</i> (Pers:Fr.) Morton & Smith						+												+	+	3	1,34
<i>Fusarium verticillioides</i> (Sacc.) Nirenb.	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+		14	6,27
<i>F.solani</i> (Mart.) Sacc.					+														+	2	0,89
<i>Fusarium</i> sp.															+				+	2	0,89
<i>Gilmaniella humicola</i> Barron																			+	1	0,44
<i>Gliocladium roseum</i> Bainier																		+	+	2	0,89
<i>Graphium penicillioides</i> Corda	+	+	+	+	+	+	+	+	+	+	+	+	+			+		+		15	6,72
<i>G.putredinis</i> (Corda) Hughes																		+		1	0,44
<i>Iodophanus carneus</i> (Pers.) Korf									+				+							2	0,89
<i>Kernia nitida</i> (Sacc.) Nieuwl.	+										+									2	0,89
<i>Mortierella reticulata</i> V.Tiegh.& Le Mond													+							1	0,44
<i>Mucor bainieri</i> Mehrotra & Baijal						+								+	+	+			+	5	2,24
<i>M.hiemalis</i> Wehmer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17	7,62
<i>M.plumbeus</i> Bonord			+			+									+		+			4	1,79

Table 1. (Continuation)

TAXA	Number of samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	n	%
<i>M.racemosus</i> Fresen.		+	+	+		+	+	+	+	+		+	+	+		+		+	+	14	<u>6,27</u>
<i>Oedocephalum glomerulosum</i> (Buell.) Sacc.															+			+		2	<u>0,89</u>
<i>Penicillium</i> spp.		+				+			+	+				+	+			+		7	<u>3,13</u>
<i>Phycomyces blakesleanus</i> Burgeff															+					1	<u>0,44</u>
<i>Pilobolus kleinii</i> V. Tieghem															+				+	2	<u>0,89</u>
<i>Piptocephalis lepidula</i> (Marchal) Benjamin				+				+					+		+	+	+		+	7	<u>3,13</u>
<i>Podospora miniglutinans</i> Mirza & Cain																			+	1	<u>0,44</u>
<i>P.setosa</i> (Wint.) Niessl.		+														+			+	3	<u>1,34</u>
<i>Rhizopus stolonifer</i> (Ehremb.:Fr.) Vuill.															+					1	<u>0,44</u>
<i>Saccobolus citrinus</i> Boudier & Torrend				+		+		+			+									4	<u>1,79</u>
<i>S.versicolor</i> (P.Karst.) P.Karst.				+	+	+	+	+	+	+			+	+	+	+	+	+	+	14	<u>6,27</u>
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bain.		+	+			+		+			+	+		+					+	8	<u>3,58</u>
<i>S.brumptii</i> Salvanet-Duval					+	+	+						+					+	+	6	<u>2,69</u>
<i>Sepedonium hialinospinosum</i> Matsushima					+											+			+	3	<u>1,34</u>
<i>Sordaria fimicola</i> (Rob.) Cesati & De Notaris		+		+										+	+	+	+			6	<u>2,69</u>
<i>Sphaerodes fimicola</i> (Hans.) Cannon & Hawksw.										+										1	<u>0,44</u>
<i>Sporormiella minima</i> (Auersw) Ahmed & Cain															+					1	<u>0,44</u>
<i>Stilbella erythrocephala</i> (Ditmar) Lindau															+		+	+		3	<u>1,34</u>
<i>Syncephalis cornu</i> V. Tiegh. & Le Monn.										+		+								2	<u>0,89</u>
<i>S.reflexa</i> v. Tiegh.				+															+	2	<u>0,89</u>
<i>Thamnidium elegans</i> Link						+									+	+		+	+	5	<u>2,24</u>
<i>Thelebolus crustaceus</i> (Fuckel) Kimbr.												+						+		2	<u>0,89</u>
<i>Trichoderma harzianum</i> Rifai						+				+										2	<u>0,89</u>
<i>Trichurus spiralis</i> Hasselbr.																		+	+	2	<u>0,89</u>
<i>Volutella ciliata</i> Alb. & Schw. ex Fr.																		+		1	<u>0,44</u>
<i>Zopfiella leucotricha</i> (Speg.) Malloch & Cain												+								1	<u>0,44</u>

num, a species which is assumed to be not coprophilous can be explained by its appearance in the surrounding soil.

Dung is thus a mechanism for the dispersion of some soil fungi, like *F. verticillioides* (= *F. moniliforme*), one of the most prevalent field fungi occurring in a great variety of plant hosts. Their frequency in dung can be explained because it is one of the organisms associated with corn-based feeds and by the ability to adapt easily to this new ecological niche.

Fusarium verticillioides, anamorph of *Gibberella moniliformis*, was found on horse dung (Piontelli et al., 1981) and on cow dung (Dominik & Majchrowicz, 1970). It has been suspected of being involved in human and animal diseases and has been shown to be toxic for a variety of experimental animals. (Kriek et al., 1981 a, b). The new group of mycotoxins, called fumonisins, was characterized in 1988 by Bezuidenhout et al.

Doratomyces columnaris, has been isolated by Swart (1967) on dung pellets of hares collected in the Melville Koppies Nature Reserve in Johannesburg. The main characteristic features on which the specific epithet is based, upon are the very short synnema tapped by a small almost flat head, from which the conidia arise in a column.

D. columnaris in our study was the species occurring with significantly higher frequency. In a previous study, this species was found on horse dung collected in the province of Pavia, Lombardia (Caretta et al., 1994).

Among the synnematosus fungi, *Graphium penicillioides* is another significant fungal species restricted to deer dung. *Graphium* species are commonly found on woody substrata or plant debris and animal dung. They are frequently found as anamorphs of *Ascomycetes*, *Ophiostoma*, *Petriella*, *Petriellidium*. *G. penicillioides* was reported by Ellis (1971), on wood of *Populus*; the host of this species is *Zea*.

Scopulariopsis species, anamorphs of *Microascus*, are commonly found on all types of decaying organic materials; they are quite frequently isolated from soils.

The species most frequently found on animal dung (pig, monkey, camel, horse, goat, cow and sheep) was *S. brevicaulis* (Udagawa & Takada, 1971; Dominik & Majchrowicz 1970, Piontelli et al., 1981; Caretta et al., 1994). It is a cellulolytic and proteolytic fungus associated sometime in humans onychomycosis, ulcerating granuloma and chronic granulomatous inflammation of tendon sheaths and muscle (Emmons et al., 1977). *S. brevicaulis* has the ability to attack arsenic compounds with the production of volatile gas identified as trime-thylarsine (Challenger, 1954).

Among the *Ascomycetes* on deer dung only some species were predominant: *C. bostrychodes*, *Saccobolus versicolor* and *S. citrinus*.

Chaetomium bostrychodes was present in abundance in a relatively high number of deer dung. This

species has a cosmopolitan distribution with the principal substrata being dung, seeds, soil or animal hairs.

This fungus is able to decompose cellulose (Vesco dal et al., 1967) and is of interest because of mycotoxin production.

Larvae of the sciarid fly *Licorella mali* avoided ascomata of *Ch. bostrychodes* that had growth on rabbit dung, but consumed the fruit bodies of other coexisting coprophilous fungi (Helsel & Wicklow, 1978). The presence of larvae of *L. mali* in rabbit dung decreased the number of sporulating species of coprophilous fungi, and increased the relative abundance of *Ch. bostrychodes* (Wicklow & Yocom, 1982). For Wicklow (1979, 1988), *Ch. bostrychodes* has effective mechanical and chemical defenses against fungus feeding arthropods; this fact is related to the toxicity of *Chaetomium* metabolites as chaetomin, chaetocins, chaetoglobosins and other metabolites (Udagawa et al., 1979, Udagawa, 1984).

Saccobolus versicolor is the most variable species of the genus *Saccobolus* (Brummelen, 1967). Apart from the variation in the shape and structure of the fruit-bodies, there is a considerable variation in pigmentation of these, ones the size of the ascospores and the variation of the episporium. For Brummelen (1967), it is impossible to distinguish more or less constant forms within the species.

With regard to other fungal taxa found on the deer dung, some are known only or prevalently from dung as *Oedocephalum glomerulosum*, *Stilbella erythrocephala* or *Syncephalis cornu* (Jeffries & Kirk, 1976), others are interesting species as *Mortierella reticulata* and *Phycomyces blakesleanus*. These rarely collected fungi tell us little about their habitat, but sometimes from these few findings, valuable ecological data can be extracted.

Another interesting species recorded on deer dung and native of Europe, was *Z. leucotricha* (= *Tipterospora leucotricha* (Speg) Lundq.). Originally this fungus was collected by Spegazzini (1878) on rotten branches of *Sambucus nigra* from Conegliano, Venezia, Italy and named *Sordaria leucotricha*. All the synonyms species examined by Lundqvist (1969), were found on various herbaceous material and seeds. On dung this species was not known. A synopsis of the genus *Zopfiella* and generic delimitation of *Zopfiella*, *Podospora* and *Tripterospora* was proposed by Guarro et al., 1991.

Many of coprophilous fungi found in the present study, were listed by various authors on deer dung and the feces of other animals (Lundqvist, 1972; Richardson, 1972; Parker, 1979; Bell, 1983).

Bell & Mahoney (1995), have observed that the dung from domesticated animals that receive regular intestinal drenches to rid them of internal parasites, shows a disappointing lack of fungi growing on it.

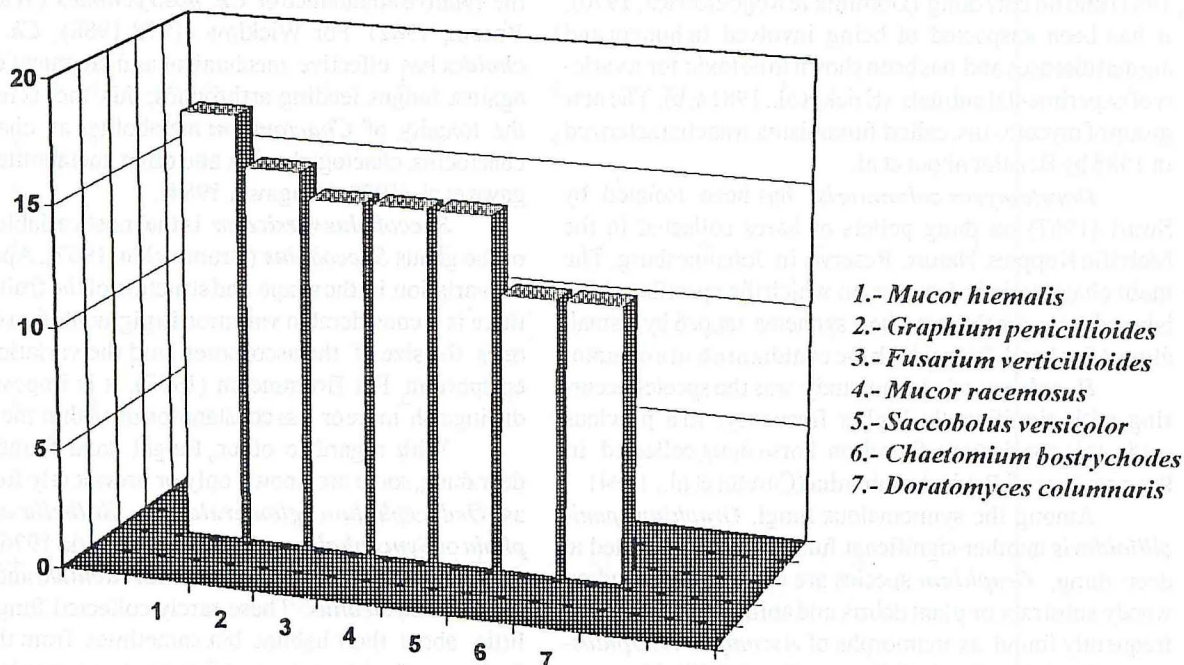
Most fungi occurring on deer dung are anamorphs

of *Ascomycota*, particularly of genera belonging to the formerly so called "*Discomycetes*" and to the "*Pyrenomycetes*", some of which are coprophilous and typical components of the fungal succession on dung (Seifert et al., 1983).

Despite the complexity of the fungal population within alimentary canal on deer, the gross composition of the mycota of a particular animals is remarkably stable in

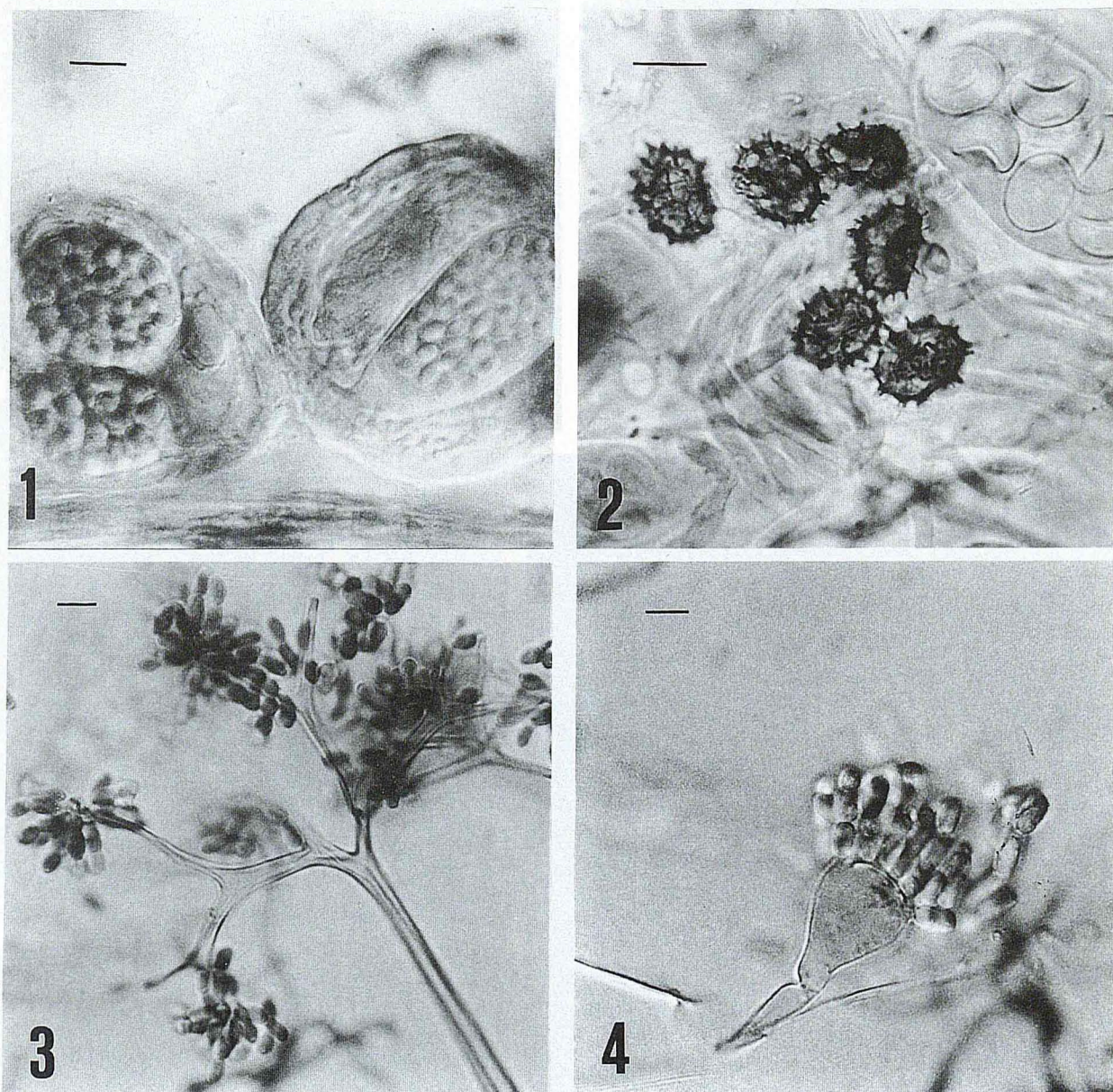
their ecotope and tends to be characteristic for these animals. The main differences in fungal composition from animal to animal is difficult to be related to a specific cause, but it is mainly influenced to the kind of food, like grasses, forages and grains, the size of animal, the geographic localization, and interspecific competitive interactions by compounds which inhibit or stimulate the growth of some fungi and other biotic and abiotic factors.

Figure A. Frequency (%) of dominant species isolated in deer dung

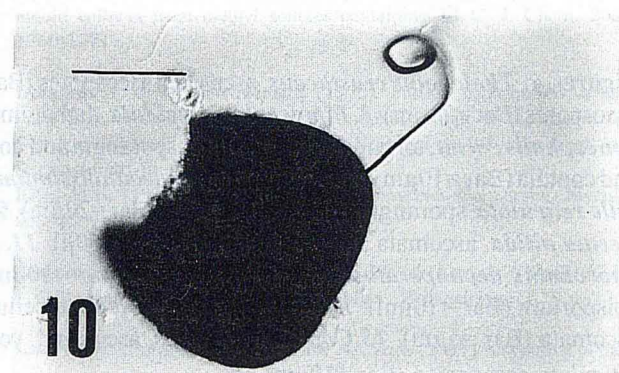
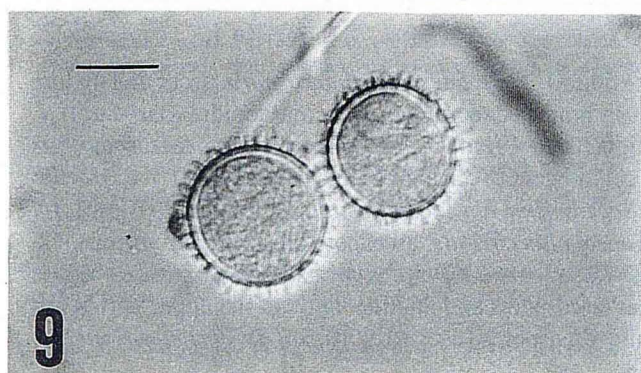
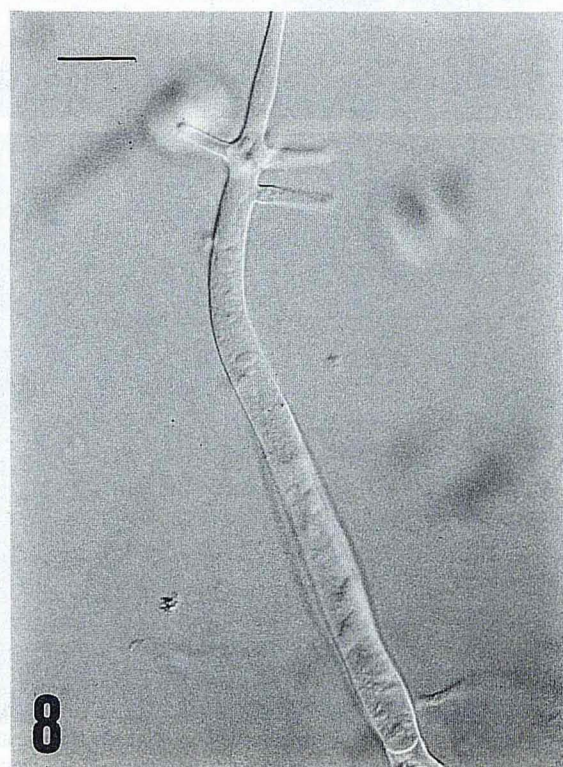
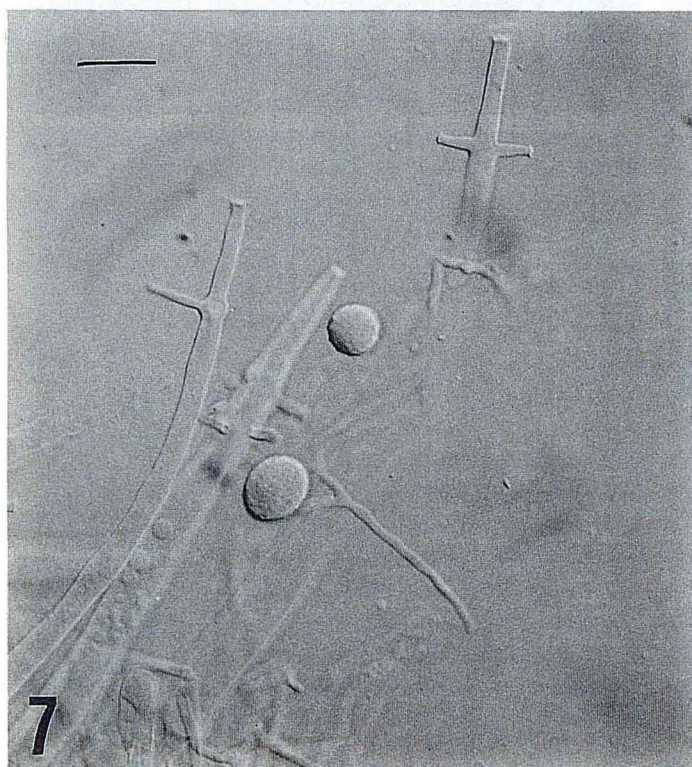
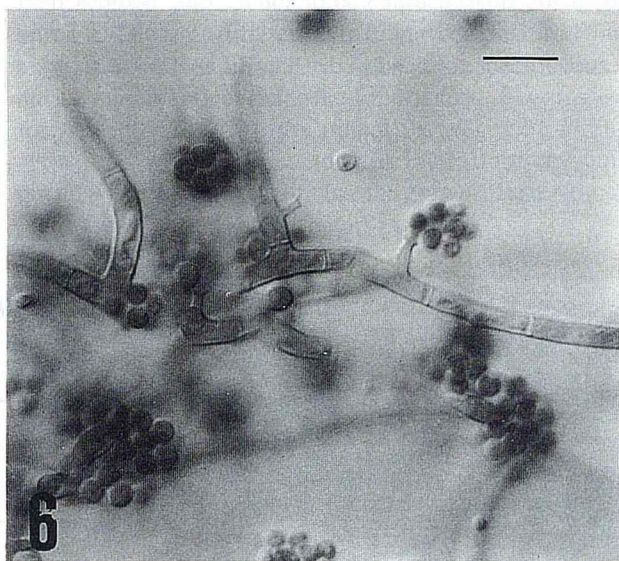
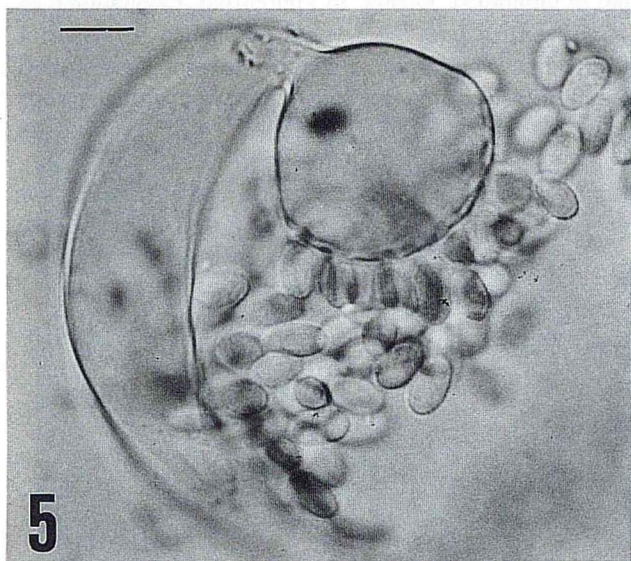


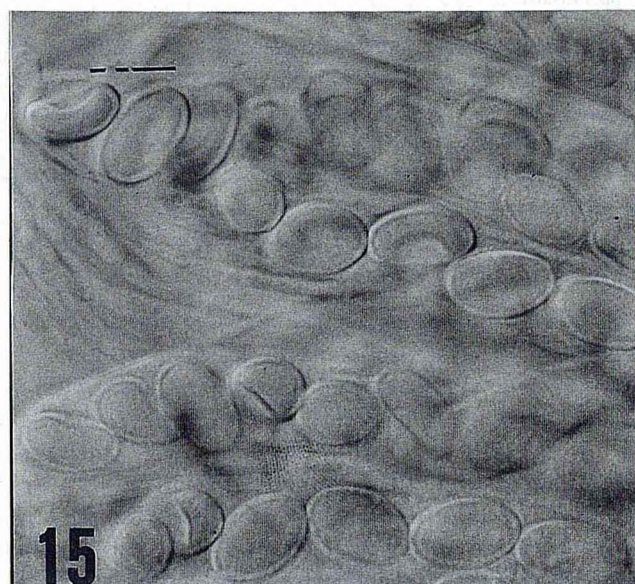
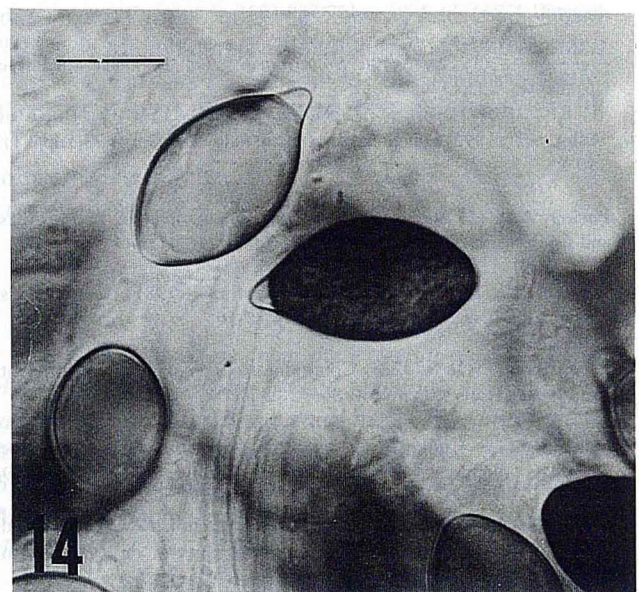
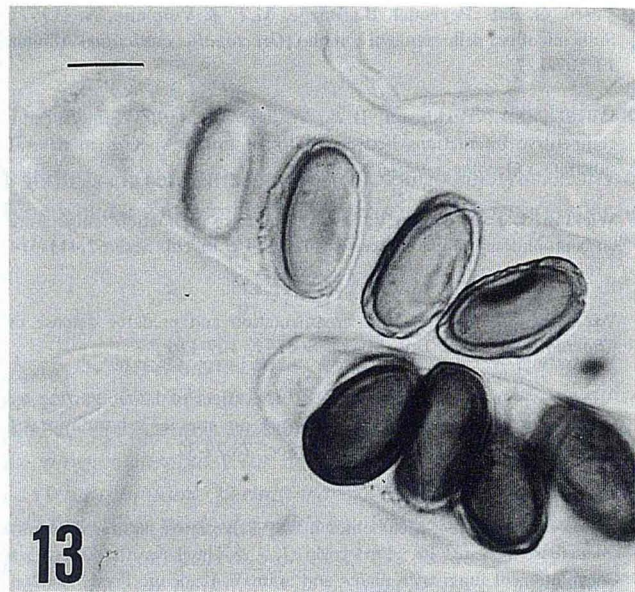
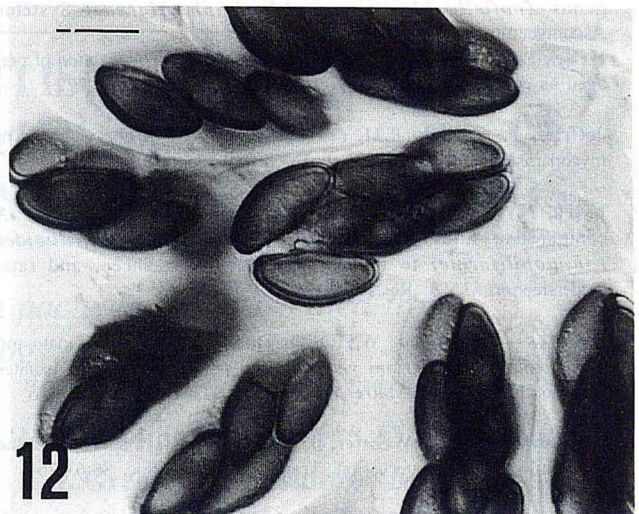
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Figures. 1. *Thelebolus crustaceus*, ascus and ascospores (Bar=50µm). 2. *Ascodesmis sphaerospora*, ascus and mature ascospores (Bar=20µm). 3. *Piptocephalis lepidula*, dichotomously branched sporangiosphore and conidia (Bar=8µm). 4. *Syncephalis cornu*, terminal swelling merosporangia and conidia (Bar= 10µm). 5. *Syncephalis reflexa*, merosporangia and conidia (Bar=10µm). 6. *Cladorrhinum foecundissimum*, conidiogenous cell and conidia (Bar=10µm). 7-8. *Mortierella reticulata*, sporangiophores and conidia (Bar=20µm). 9. *Sepedonium hialinospinosum*, conidia (Bar=10µm). 10. *Kernia nitida*, ascomata and circinate hair (Bar= 50µ). 11. *Saccobolus citrinus*, ascospore clusters (Bar=10µm). 12. *Saccobolus depauperatus*, ascospore clusters (Bar=10µm). 13. *Ascobolus perplexans*, ascospores with a loose episporium (Bar=10µm). 14. *Zopfiella leucotricha*, bicellular ascospores and hialine hairs of the upper part of the ascomata (Bar=5µm). 15. *Coprotus disculus*, ascus and young ascospores (Bar=10µm). 16. *Sphaerodes fimicola*, reticulate ascospores (Bar=10µm).





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