The coprophilous succession

Michael J. Richardson

165 Braid Road, Edinburgh EH10 6JE, UK; e-mail: mjrichardson@clara.net

Richardson, M.J. (2002). The coprophilous succession. In: *Fungal Succession* (eds. K.D. Hyde and E.B.G. Jones). Fungal Diversity 10: 101-111.

This paper reviews the background to studies of the coprophilous succession, presents some data from observations made on samples collected for a study of the occurrence and diversity of coprophilous fungi, and suggests where further studies might help to elucidate the functional aspects of the succession.

Key words: ascomycetes, basidiomycetes, dung, fungal ecology, zygomycetes.

Introduction

The sequential fruiting of coprophilous fungi on animal dung after deposition, particularly that of herbivores, has long been known and used as a good and easily demonstrable example of succession. In this context, succession is understood as the sequence of observation of fungi, as identified by their fruiting, rather than an understanding of succession as the sequential replacement of one organism by another. It is an example of diversity in time, with a sequential occurrence of different species of fungi appearing on dung, in the field or on incubation in controlled conditions. It is relatively easily quantifiable, in terms of time of fruiting, if not in terms of productivity, and open to experimental manipulation, but there are few extensive and in-depth studies. Webster (1970), in a Presidential Address to the British Mycological Society, reviewed many aspects of the sequence in reviewing the biology of coprophilous fungi, and Wicklow's (1992) review is the most recent and comprehensive. Harper and Webster (1964) reported on what is probably the most detailed experimental study of the succession, on rabbit pellets from three locations in England. They concluded that the succession was related more to the time needed for the different species to reach maturity and fruit, rather than to any effects of latency of germination or growth rate, and that under experimental conditions the sequence of fruiting was quite consistent, and corresponded to that observed in nature. In mixed cultures they showed that competition does not affect time of appearance, which is satisfactorily explained by the minimum time to fruiting of the particular species. Larsen (1971) studied

the succession of fungi on dung removed from the large intestines of 16 fallow deer killed by hunting in a Danish deer park. In contrast to Harper and Webster (1964), she found that the time to fruiting was variable for any particular species, but that abundant *Pilobolus* and other zygomycetes were observed on days 2-4, most discomycetes had fruited within the second week of incubation, and the pyrenomycetes and basidiomycetes appeared about one week later. She suggested that part of this variation might be attributed to the fact that the passage of food through [the larger red] deer can take from 1-10 days, which could have a bearing on the time taken to fruit in dung when it is freshly collected and incubated. Wicklow and Moore (1974) found that the fruiting sequence on pellets from laboratory fed rabbits was greatly influenced by temperature, and agreed with Larsen in that they were unable to distinguish any specific discomycete / pyrenomycete successional pattern, and different species were favoured by the incubation temperatures used (10, 24 and 37.5 C). There are few recent studies of the sequence of fruiting in any detail.

The well-known sequence of phycomycetes ("sugar fungi"), ascomycetes and basidiomycetes has often been explained by the ability of these different fungi to use successively more complex substrates. Lodha (1974) suggests, however, that it has less to do with the "run-down" of nutrients, and can be better explained by the time taken for each fungus to produce its fruiting structures from a simultaneous start to growth when the dung is deposited.

Observations made during the collection of data for a study on the distribution and occurrence of coprophilous fungi (Richardson, 2001) have provided information from a large number of field-collected samples on the sequence of fruit body appearance, which are reported here and further document the detail of the succession as identified by fruiting.

Materials and methods

Samples that appeared to be relatively recent and unweathered were collected, into new paper envelopes if dry or clean plastic pots if wet, and usually set to incubate within a day or two of collection. If samples could not be incubated shortly after collection, they were gently air dried and stored in paper envelopes until incubation. Samples were incubated on moist paper towelling in plastic boxes with lightly fitting transparent lids, under ambient light and at room temperature (~ 15-18 C). Care was taken to ensure that cultures were not too wet. Samples were examined frequently at intervals of a few days, with a 7-45 × stereomicroscope. Fruiting bodies were removed and mounted in water for examination at higher magnification. Samples were normally kept for 4-12 weeks, with observations continuing for as long as new fungi continued to be observed. The nature of the records means that the

analysis is not ideal for fully quantifying successional details since, although dates of first occurrence of fungi were recorded, dates of the last noted record would be an unreliable indicator of the date of last occurrence, since once the occurrence of a species was noted and confirmed its subsequent presence (or more importantly in this respect, absence) was not necessarily noted. Any observations on the length of fruiting are, therefore, imprecise. Data for the most frequent taxa occurring on 301 samples of the commonest types of dung collected (rabbit (113), sheep (64), deer (54), hare (37), cattle (12) and grouse (21)) have been used, from samples collected in the UK (272) and France (29) in 1998-2001 have been used.

Results and discussion

Although based on observation rather than experiment, the results in Table 1 and Figs. 1-3 demonstrate and confirm the accepted sequence of fruiting of coprophils on dung collected in the field and incubated in moist chambers. Although attempts were made to collect samples that were freshly deposited, it is quite clear that that aim was not always achieved, since most taxa were observed on some samples either on collection, when set to incubate, or very shortly afterwards. Nevertheless, there are clear differences in the mean time of first observation. Contrary to the conclusions of Larsen (1971) and Wicklow and Moore (1974), who were unable to distinguish any specific discomycete / pyrenomycete successional pattern, there does seem to be a clear sequence of onset of fruiting in general in the main taxonomic groups (excluding *Viennotidia fimicola* and *Phomatospora* spp., which are taxonomically, and so perhaps ecologically, unrelated to the typical coprophils), when considered as a whole (Fig. 1).

When individual taxa or groups of species are considered, statistical analysis (1-way ANOVA of $\log_{10}(n+1)$ transformed data) showed that there were highly significant differences ($p<0.05$) between the mean time taken for them to appear. *Pilaira* and *Pilobolus* spp. were the earliest, with most *Pilaira* being observed within the first 4 days, and *Pilobolus* taking on average about 3 days longer to develop. This is perhaps not surprising, given the more complex nature of the *Pilobolus* sporangiophore and the possibility that more investment is needed for its production in comparison to the less sophisticated *Pilaira* sporangiophore. Wood and Cooke (1987) showed that metabolic activity of *Pilaira anomala* (Ces.) J. Schröt. was highest in the first six days of incubation, which coincides with when it is most often observed fruiting. There was no difference between the mean time for *Chaetocladium* and *Piptocephalis* spp. to appear. Since they are zygomycetes and pathogens of Mucorales, they were usually observed during the first week of incubation, at the same time as

Table 1. Mean time and range of first observation of selected taxa of coprophilous fungi after start of incubation.

	Time of first record (days of incubation)			No. of observations
	Min.*	Mean	Max.	
Pilaira spp.	0	3.5	10	84
Lasiobolus spp.	0	4.3	52	68
Piptocephalis and *Chaetocladium* spp.	0	4.7	18	63
Thelebolus nanus Heimerl and *T. stercoreus* Tode	0	6.4	33	136
Viennotidia fimicola (Marchal) P.F. Cannon & D. Hawksw.	0	6.4	43	69
Pilobolus spp	0	6.6	35	191
Ascozonus spp.	1	7.0	17	17
Saccobolus spp.	0	8.1	66	140
Sporormiella spp.	0	8.8	94	357
Sordaria spp.	0	8.9	58	90
Schizothecium tetrasporum (G. Winter) N. Lundq.	0	9.0	58	128
Ascobolus spp.	0	10.7	40	241
Thelebolus microsporus (Berk. & Broome) Kimbr.	0	13.6	37	26
Coprinus heptemerus M. Lange & A.H. Sm.	3	14.1	86	24
Delitschia spp.	0	14.6	69	40
Podospora section *Rhyophila*	0	16.4	72	140
Schizothecium vesticola (Berk. & Broome) N. Lundq.	0	16.7	72	154
Coniochaeta spp.	0	18.3	108	166
Anopodium ampullaceum N. Lundq.	1	19.7	47	16
Podosordaria tulasnei (Nitschke) Dennis	4	19.8	68	38
Schizothecium conicum (Fuckel) N. Lundq.	0	20.3	82	83
Trichodelitschia spp.	0	21.1	108	58
Podospora spp. (exc. sect. *Rhyophila*)	1	22.9	114	67
Iodophanus carneus (Pers. ex Fr.) Korf *apud* Kimbr. & Korf	1	23.0	96	78
Coprinus stercoreus Bull.: Fr. and *C. miser* (P. Karst.) P. Karst.	1	23.5	108	244
All *Coprinus* spp.	1	24.4	108	408
Hypocopra spp.	1	28.8	78	25
Arnium spp.	1	31.7	99	53
Coprotus sexdecemsporus (P. Crouan & H. Crouan) Kimbr.	4	37.7	94	29
Phomatospora spp.	29	51.6	116	32

* 0 value indicates fungus present when set to incubate

s.d. of all data sets ranged between 1.4 and 3.4 days, when back-transformed from $\log_{10}(n+1)$

their hosts, as might be expected. Other early appearances were by apothecial fungi, especially *Lasiobolus* spp. and the very small theleboloid fungi, especially *Ryparobius polysporus* (P. Karst.) Speg., *Thelebolus nanus* and *T. stercoreus*. These were followed by *Ascobolus* and *Saccobolus*; there were no

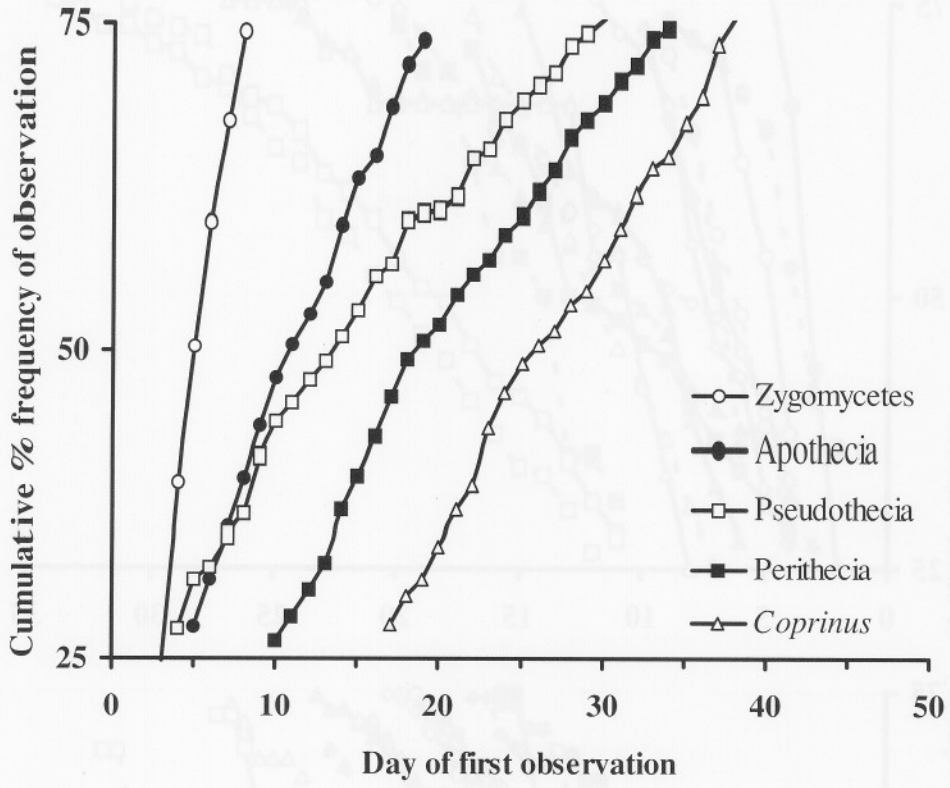

Fig. 1. Cumulative frequency curves (interquartile range) for the five main taxonomic groups studied, showing the main periods over which first fruiting is observed. The incubation time corresponding to the point where a curve crosses the 50% line from the *y*-axis is the mean time of first observation.

significant differences between the commonest species of these two genera but the latter were observed, on average, about 2-3 days earlier. This also is perhaps not surprising, as *Saccobolus* apothecia are often smaller and lighter in build than those of *Ascobolus*. *Iodophanus carneus*, *Thelebolus microsporus* and, particularly, *Coprotus sexdecemsporus* are unusual representatives of the apothecial mycota, often appearing at a relatively late stage in the succession.

The "pyrenomycetes" show similar variations in the time taken to sporulate for many of their taxa. *Viennotidia fimicola* is often one of the earliest to be observed. It is not one of the typical coprophilous peri- or pseudothecial taxa (i.e. it does not belong to the Sordariales or Dothideales), and its biology is different from those fungi (Weber and Webster, 1997, 1998). Of the more typical coprophils, some *Sporormiella* species can be expected to appear in the early days of incubation, together with *Sordaria* spp. and *Schizothecium tetrasporum*. *Schizothecium tetrasporum*, with a mean time

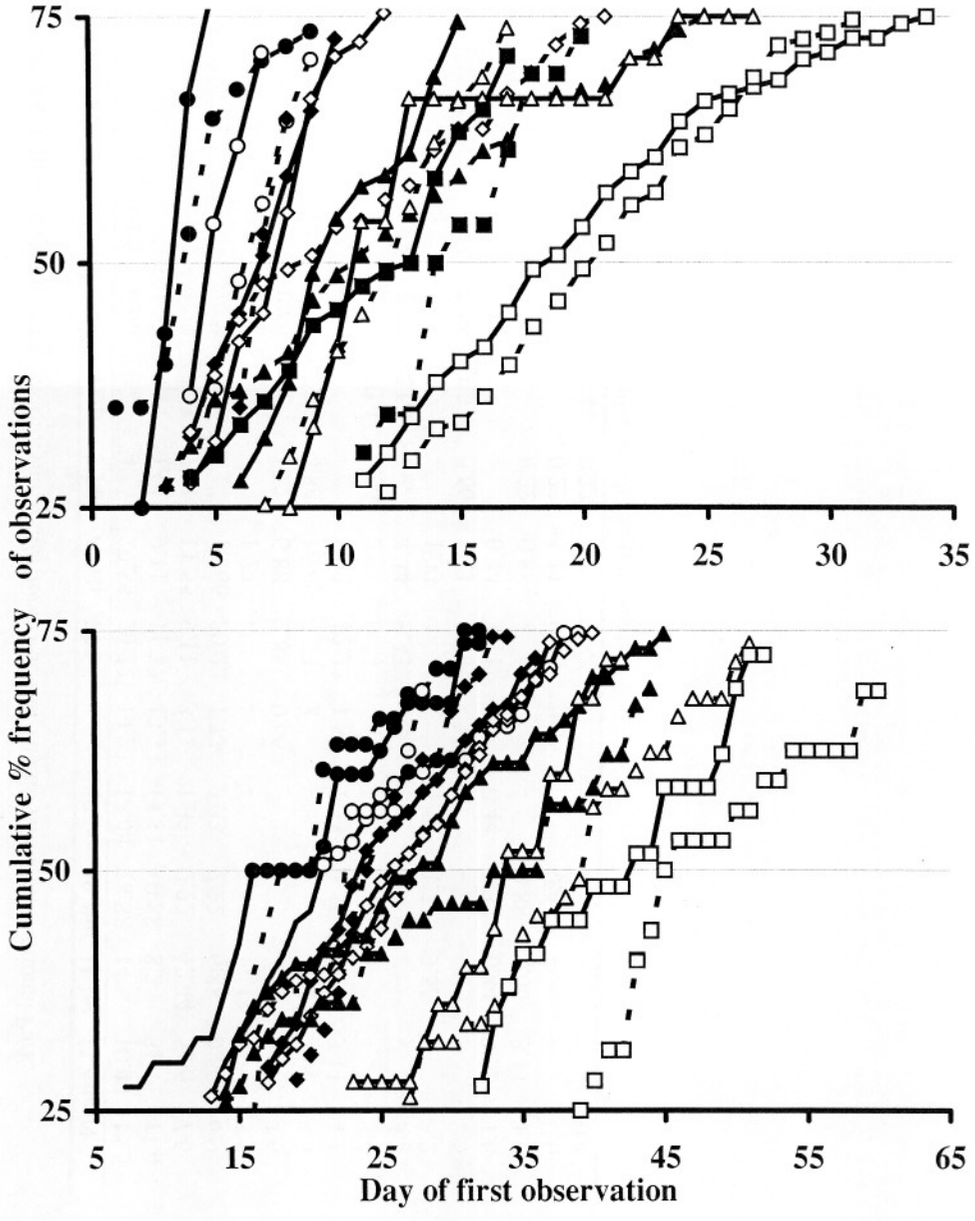

Fig. 2. Cumulative frequency curves (interquartile range) for different taxa, showing the main periods over which first fruiting is observed. The incubation time corresponding to the point where a curve crosses the 50% line from the *y*-axis is the mean time of first observation. The figure is presented in two parts to allow better discrimination of those taxa which appear early in the succession.

to first observation of 9 days, appears significantly earlier than two other common *Schizothecium* spp., *S. vesticola* and *S. conicum,* which on average are first observed after 16-20 days. *Podospora* spp. tend to appear somewhat later than the more lightly built *Schizothecium* spp.; whether or not there is an association between these two features is open to investigation. Within *Podospora*, species in section *Rhyophila* were first observed significantly earlier (mean time 16-17 days) than species from other sections of the genus (23 days). The most frequent species of this section is *P. decipiens* (G. Winter ex Fuckel) Niessl, which is one of the most common and easily recognised temperate *Podospora* species and accounted for over 70% of the records of section *Rhyophila* in this study. Other "pyrenomycetes" most frequently observed in mid-succession are *Anopodium, Coniochaeta*, *Delitschia* and *Trichodelitschia* spp. *Podosordaria tulasnei* stromata are also usually evident by mid-succession, although they do not produce perithecia under ordinary damp chamber incubation conditions (Webster and Weber, 2000). The tendency of the pseudothecial species, particularly *Sporormiella,* to appear earlier than perithecial ones is highly significant, and may be related to the ability of some species to "get a good start" by being able to live under near-anaerobic conditions in the gut (Brewer *et al.*, 1972). Late succession pyrenomycetes include species of *Hypocopra* and *Arnium*, mostly maturing after 1 month's incubation, and *Phomatospora* spp., which were never found before at least one month's incubation and which had a mean time to first observation of over 7 weeks. Like *Viennotidia* at the start of the succession, *Phomatospora* does not belong to a typically coprophilous group of taxa, and this may explain its unusual position in the fruiting sequence.

Of the basidiomycetes, *Coprinus heptemerus* (≡ *Coprinellus heptemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson) was the first to appear, on average after about 2 weeks, while other *Coprinus* species, including the common *C. stercoreus* (≡ *Coprinopsis stercorea* (Bull.: Fr.) Redhead, Vilgalys & Moncalvo) and *C. miser* (≡ *Parasola miser* (P. Karst.) Redhead, Vilgalys & Hopple) appeared on average after about 3.5 weeks. *Coprinus heptemerus* has an ecology different from what is normally considered to be typical of saprotrophic coprophils, being antagonistic towards many other coprophilous fungi, especially early succession species (Harper and Webster, 1964; Ikediugwu and Webster, 1970), which may explain its early fruiting.

Although the detailed observations presented here relate to the most frequent taxa from some European collections, some comments can be made about others which have occurred, either with insufficient frequency from those samples or in samples from other regions. Although not represented

sufficiently for analysis in the samples providing data for this study, *Thecotheus* spp. are also relatively late-appearing, species being recorded from 7 samples, but not before at least 5 weeks incubation and with an average time to first appearance of 6.5 weeks. *Podospora gigantea* J.H. Mirza & Cain and *Sporormiella herculea* (Ellis & Everh.) S.I. Ahmed & Cain produce relatively massive perithecia, and spores (those of *P. gigantea* are *ca.* 120-130 × 54 µm, and of *S. herculea* are 100-140 × 16 µm), and tend to be observed after long periods of incubation, e.g. *P. gigantea* (62 and 68 days), *Sp. herculea* (73 days). It is possible that such fungi, appearing late in the succession, do not compete with the earlier fungi for simple nutrients, but are able to exploit the more intractable components of the substrate, which remain available for much longer periods. Subjectively, they also fruit for a much longer period than the early-succession fungi. They may also need additional substances which appear in dung as it decomposes.

Many members of the coprophilous mycota are adapted for passage through the gut, which is necessary for some species before spores will germinate, and it is considered that mycelium of most species begins to develop when the dung is deposited, and those species which are observed to fruit later simply take longer to mature, and use more complex substrates, e.g. lignocellulose, rather than the readily accessible simple carbohydrate and nitrogenous components (Lodha, 1974). Some species, however, e.g. *Sporormiella minima*, and possibly other coprophils, can be isolated from the rumen of sheep and will grow under near-anaerobic conditions (Brewer *et al.*, 1972). That *S. minima* may already be growing when the dung is deposited would help to explain why it can be found fruiting on relatively fresh dung in the field. It is, however, also able to continue fruiting for long periods of incubation. Fungi adapted to grow under such conditions might be expected to appear early in any succession.

Sufficient is known about the general ecological aspects of the timing of fruiting sequence, but virtually nothing about the cultural and chemical conditions which drive it. Some species from different stages of the succession are available in pure culture (Anon., 2001, and other culture collections), and others could be obtained. It would be useful to examine how these behave in experimental studies of single and mixed cultures of different species, inoculated for example into the controlled conditions of the copromes of Wood and Cooke (1984, 1987). Copromes are standardised "faecal pellets", made by drying, powdering and mixing a bulk of dung and then reforming it into pellets of uniform size and composition using a tableting machine. They are bagged and sterilised by γ-irradiation and stored until required. Inoculum, incubation and nutrient conditions can then be varied and controlled as required, and

chemical analysis of the pellets over the duration of the incubation can show how the various components are being utilised. As far as I am aware the only studies using copromes, made from rabbit faeces, are those of Wood and Cooke (1984, 1987), and Safar and Cooke (1988a,b). Safar and Cooke (1988a,b), studying the interaction of ascomycetes, a *Coprinus* and bacteria, showed that bacteria greatly influenced the development of ascomycetes on dung. They also concluded that time to fruiting was determined by the time at which resources could be allocated to reproduction, rather than by growth rate. These studies could be extended by comparing aspects of the succession by inoculating copromes made of dung from different animals with single and groups of species.

Copromes could also be used to follow the changes in the chemical composition of the substrate with time. Information on the detailed organic composition of dung of five temperate herbivores (sheep, cattle, rabbit, hare and deer) was summarised by Richardson (2001). There are some significant differences between species in the detailed composition but, to generalise, it contains *ca.* 1-3% nitrogen, 1.5-3.5% starch and soluble carbohydrate, 40-60% cellulose and 10-45% lignin. It is possibly the low amount of simple N and C compounds that support the early zygomycetes. The cellulose and lignin decomposers are then responsible for the breakdown and utilisation of the insoluble, physical substrate. Although it is established that many coprophilous fungi can utilise cellulose (Fries, 1955; Denison and Koehn, 1977; Wicklow *et al.*, 1980; Taj-Aldeen *et al.*, 1990; Pardo, Sivori and Ranalli, 1997; Magnelli and Forchiassin, 1999), it is not clear which of the fungi are responsible for lignin breakdown. Fries (1955), using an indirect method, rather than quantifying lignin directly, reported that some coprophilous *Coprinus* species could utilize lignin, but noted that enzyme activity indicated by polyphenoloxidase production was not necessarily a reliable indicator of the ability to decompose lignin in nature. Wicklow *et al.* (1980) found that an unidentified basidiomycete caused significant loss of lignin, but a *Coprinus* sp. did not. Sequential analysis of copromes, inoculated with single species or groups of species, and subsampled over a period of time, with direct assay of lignin, could be used to elucidate which, if any, of the coprophilous fungi are involved in recycling the relatively high level of lignin in some dungs. Another area in which copromes may be of use is in reconciling what is observed experimentally with what actually happens in the field, where dung is often exposed to periods of extreme heat and dryness alternating with periods of hydration when fungal growth can occur. How does such a fluctuating environment affect the succession and community composition? Further, with the possibility of being able to detect and identify mycelium of individual

species with modern molecular techniques, it may be possible to make a distinction between the "succession" of mycelia in the dung habitat and the "sequence of fruiting" as observed. This distinction may be subtle, since it is conceivable that the ecological succession of fungi in dung may be evidenced by the sequence of fruiting. In the later stages of the "succession" mycelia of early-sporing fungi may well be absent, having been consumed by other members of the community after sporulation, but propagules may well remain, while in the early stages of incubation the late-sporers will be present. This seems little different from the analogy of, for example, a woodland succession where, at maturity early colonisers remain as a seed bank under the climax vegetation, while in the early stages young, non-fruiting saplings of the climax species are growing amongst the pioneer vegetation.

References

Anon. (2001). *Catalogue of the UK National Culture Collection (UKNCC): List of Fungi*. 1st edn. The UK National Culture Collection (UKNCC), Bakeham Lane, Egham, Surrey, U.K.

Brewer, D., Duncan, J.M., Safe, S. and Taylor, A. (1972). Ovine ill-thrift in Nova Scotia. IV. The survival at low oxygen partial pressure of fungi isolated from the contents of the ovine rumen. Canadian Journal of Microbiology 18: 119-128.

Denison, D.A. and Koehn, R.D. (1977). Cellulase activity of *Poronia oedipus*. Mycologia 69: 592-603.

Fries, L. (1955). Studies on the physiology of *Coprinus*. Svensk Botanisk Tidskrift 49: 475-535.

Harper, J.E. and Webster, J. (1964). An experimental analysis of the coprophilous fungus succession. Transactions of the British Mycological Society 47: 511-530.

Ikediugwu, F.E.O. and Webster, J. (1970). Antagonism between *Coprinus heptemerus* and other coprophilous fungi. Transactions of the British Mycological Society 54: 181-204.

Larsen, K. (1971). Danish endocoprophilous fungi, and their sequence of occurrence. Botanisk Tidsskrift 66: 1-32.

Lodha, B.C. (1974). Decomposition of digested litter. In: *Biology of Plant Litter Decomposition, Vol. 1* (eds. C.H. Dickinson and G.J.F. Pugh). Academic Press, London: 213-241.

Magnelli, P. and Forchiassin, F. (1999). Regulation of the cellulase complex production by *Saccobolus saccoboloides*: induction and repression by carbohydrates. Mycologia 91: 359-364.

Pardo, A.G., Sivori, A.S. and Ranalli, M.E. (1997). Comparative study of cellulolytic enzyme zymograms of species of *Thecotheus* and *Iodophanus* (Pezizales-Ascomycetes). Mycotaxon 63: 269-286.

Richardson, M.J. (2001). Diversity and occurrence of coprophilous fungi. Mycological Research 105: 387-402.

Safar, H.M. and Cooke, R.C. (1988a). Exploitation of faecal resource units by coprophilous Ascomycotina. Transactions of the British Mycological Society 90: 593-599.

Safar, H.M. and Cooke, R.C. (1988b). Interactions between bacteria and coprophilous Ascomycotina and a *Coprinus* species on agar and in copromes. Transactions of the British Mycological Society 91: 73-80.

Taj-Aldeen, S.A., Al-Habbeb, E.K. and Abdullah, S.K. (1990). Cellulytic activity of coprophilous fungi. Cryptogamic Botany 2: 25-29.

Weber, R.W.S. and Webster, J. (1997). The coprophilous fungus *Sphaeronaemella fimicola* – a facultative mycoparasite. Mycologist 11: 50-51.

Weber, R.W.S. and Webster, J. (1998). Stimulation of growth and reproduction of *Sphaeronaemella fimicola* by other coprophilous fungi. Mycological Research 102: 1055-1061.

Webster, J. (1970). Presidential Address. Coprophilous Fungi. Transactions of the British Mycological Society 54: 161-180.

Webster, J. and Weber, R.W.S. (2000). Rhizomorphs and perithecial stromata of *Podosordaria tulasnei* (Xylariaceae). Mycologist 14: 41-44.

Wicklow, D.T. (1992). The coprophilous fungal community: An experimental system. In: *The Fungal Community: Its Organization and Role in the Ecosystem* (eds. D.T. Wicklow and G.C. Carroll). 2nd edn. Marcel Dekker Press, New York: 715-728.

Wicklow, D.T. and Moore, V. (1974). Effect of incubation temperature on the coprophilous fungus succession. Transactions of the British Mycological Society 62: 411-415.

Wicklow, D.T., Detroy, R.W. and Adams, S. (1980). Differential modification of the lignin and cellulose components in wheat straw by fungal colonists of ruminant dung: ecological implications. Mycologia 72: 1065-1076.

Wood, S.N. and Cooke, R.C. (1984). Use of semi-natural resource units in experimental studies on coprophilous succession. Transactions of the British Mycological Society 82: 337-339.

Wood, S.N. and Cooke, R.C. (1987). Nutritional competence of *Pilaira anomala* in relation to exploitation of faecal resource. Transactions of the British Mycological Society 88: 247-255.

(Received 10 September 2001, accepted 21 September 2001)